Update on retinal vessel structure measurement with spectral-domain optical coherence tomography

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A B S T R A C T

This study was conducted to demonstrate a new scan method for retinal vessel structure measurement in a specific region of fundus (zone B) using spectral-domain optical coherence tomography (SD-OCT), and to assess its reliability. One temporal superior retinal vessel pair passing through a concentric ring (zone B), which was defined between half and one disc distance from the optic disc border, was chosen for the measurement using a volume scan in SD-OCT. On the SD-OCT image, retinal arteriolar outer diameter (RAOD), retinal arteriolar lumen diameter (RALD), retinal venular outer diameter (RVOD) and retinal venular lumen diameter (RVLD) were measured. Retinal vessel diameters on color fundus photographs were also analyzed. Fifty-five healthy individuals were recruited to evaluate intraobserver and interobserver reproducibility between the two examiners. The intraobserver intraclass correlation coefficient (ICC) ranged from 0.972 to 0.981, and the interobserver ICC ranged from 0.968 to 0.980. In the Bland–Altman plot, the 95% limits of interobserver agreement for the RAOD, RALD, RVOD and RVLD were —5.60 to 4.84 μm, —5.78 to 5.05 μm, —7.52 to 6.62 μm and —7.10 to 5.63 μm, respectively. The retinal arteriolar and venular lumen diameters on the SD-OCT image were close to the mean arteriolar and venular diameters obtained from the color fundus photographs. Volume scan method produced better images of retinal vessels showing the fine structures of the vessel wall, and provided reliable retinal vessel structure measurement in zone B with good repeatability and reproducibility.

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Introduction

The retinal vasculature is the only part of the central circulation system that can be directly viewed non-invasively in vivo and studied in detail. Abnormalities of the retinal vessels have been prospectively associated with hypertension, coronary heart disease and subclinical cerebral infarction (Cooper et al., 2006; Kawasaki et al., 2009; Wang et al., 2006). Retinal vascular caliber is considered an important indicator of both cardiovascular and cerebrovascular diseases (Sun et al., 2009) and needs to be accurately assessed. With the development of fundus imaging techniques, several commercial ophthalmic devices have been explored for retinal vessel diameter measurement, such as fundus photography (FP), Dynamic Vessel Analyzer, retinal oximeter and scanning laser flowmetry (Blondal et al., 2011; Cooper et al., 2002; Guan et al., 2003; Polak et al., 2000). Among these instruments, a computer-based program for measuring retinal vessel caliber based on digital FP pictures was widely used in many epidemiological studies (Chew et al., 2013; Davies-Tuck et al., 2012; Gopinath et al., 2010; Kifley et al., 2007). In FP methodology, retinal vessels within a specific area (zone B) between 0.5 and 1 disc diameter (DD) from the optic margin were chosen for measurement, and researchers thought that these vessels in zone B are some distance away from the disc and become unambiguously arteriolar, whereas the vessels near the disc (zone A, within 0.5 DD from the margin) may be of an arterial configuration (Hubbard et al., 1999; Parr, 1974).

Optical coherence tomography (OCT) is a biological tissue-imaging device based on the measurement of optical reflections. This cross-sectional imaging technology has allowed investigators to study the pathogenesis and morphological alteration of retinal structures in various retinal diseases. Spectral-domain OCT (SD-OCT) has been used for quantitative analysis of retinal tomographic architecture, such as retinal nerve fiber layer thickness, choroidal and optic disc morphometry (Regatieri et al., 2012; Sakata et al., 2009). Recently, SD-OCT has been explored for retinal vessel diameter measurement. Goldenberg et al. (2013) used a horizontal cube scan at the optic disc margin for retinal vessel diameter measurement with SD-OCT, although retinal vessel wall thickness was not reported in their study. Muraoka et al. (2013)
also have described a circular scan to detect cross section image of retinal vessel, and measured retinal vessel outer diameter, lumen diameter and wall thickness in a hypertension population, but the retinal vessels that they measured were located at zone A. To the best of our knowl-
edge, no study has attempted to assess the vessel diameter and wall thickness of retinal vessels in the zone B using SD-OCT. So we used a vol-
ume scan, which is built in the OCT system, to evaluate the vessel diam-
ter and wall thickness in the zone B, to assess the intraobserver repeatability and interobserver reproducibility, and to compare the findings with the outcomes from FP measurement.

Material and methods

Study population

For this prospective study, healthy volunteers were consecutively recruited from Quzhou People’s Hospital between January 2013 and May 2013. All of the subjects had good cardiovascular health, no ocular surgery history, and no ocular diseases other than low myopia (less than −3.00 diopters). The study was approved by the research ethics com-
mittee of Quzhou People’s Hospital and was conducted in adherence to the tenets of the Declaration of Helsinki. The procedure and its conse-
quences were described to all of the volunteers, and written informed consent was obtained before examination. All of the subjects were ex-
amined by a trained ophthalmologist (YH Tong) to exclude ocular pathology. A single eye was chosen randomly for inclusion in the study. Where the image quality was not of sufficient quality for measurement, the other eye was included.

Optical coherence tomography imaging

Retinal vessel measurement was performed using the commercial SD-OCT (Heidelberg Engineering, Inc., Heidelberg Germany). The fol-
lowing protocol was used as standard operation procedures in the study. After pupil dilation with 1% tropicamide and 2.5% phenylephrine eye drops, each subject was seated in front of the OCT scanner, and the subject’s head was stabilized by a chin rest. An internal fixation target was used to avoid eye movement during scanning and to obtain the highest reproducibility (Schuman et al., 1996). The focus of the camera was centered on the optic disc, and the fundus was visualized on the display. For scanning of the retinal vessels in a specified area, a sheet of transparent film with four painted concentric circles was used to overlay the computer monitor screen (Fig. 1 A). According to the width of the scale bar in the OCT (5 mm in scale bar stand for 200 μm in fundus), we calculated the standard optic disc diameter of 1800 μm in the film as 45 mm. The first inner circle represented the optic disc. The second and fourth circles were equivalent to 1.5 and 2 DDs and represented zone B. The dotted circle indicated the middle of zone B. The position of the cir-
cle center was adjusted by the operator until it was located on the optic disc center. We measured the main temporal superior arteriole and ve-
nule in the region between the second and fourth circles (zone B). A vol-
ume scan with 384 A-scans and 11 μm interscan interval was performed across the vessel in zone B, and the volume scan was aligned manually as perpendicular as possible to the vessel running direction (Fig. 1 B). Sections within zone B were selected for measurement. When the vessel branched in zone B, sections should be chosen before vessel branching. After obtaining the OCT images, the outer diameter and lumen diameter of the scanned vessels were measured using the ImageJ software ver-
gov/ij). All of the images were exported to the software and vessel outer diameter and lumen diameter were manually measured using the “straight” tool. The scale factor was set according to the scale bar on the image, which is 200 μm per 70 pixels. In addition, vessel wall thickness was calculated as the difference between vessel diameter and lumen diameter divided by two.

Assessment of reproducibility

To investigate the intraobserver repeatability, the first examiner (TP Zhu) obtained independent test results using the same OCT scan-
ing protocol on the same subject. Volume scans were consecutively performed two times in each subject. The subject position and focus were randomly disrupted and the alignment parameters were newly adjusted between scan acquisitions.

Concerning interobserver reproducibility, a second examiner (YH Tong) performed only one volume scan in each subject. The first examination performed by the first examiner was investigated to establish agreement between observers. The order of the mea-
urements performed by each examiner was random and performed within the shortest time to prevent any effect of fatigue bias.

Fundus photography imaging

According to the Fundus Photograph Reading Center procedure, optic disc centered fundus color photographs with a stereoscopic 35-
degree field were collected with a Topcon TRC-50DX camera (Topcon Corporation, Tokyo, Japan). Fundus photographs were analyzed with the IVAN vascular measurement software from the Fundus Photograph Reading Center of the University of Wisconsin. The grading procedures have been described in detail elsewhere (Lundberg et al., 2013). In the present study, the widths of the main temporal superior arteriole and venule that pass through the zone B region were recorded in each individual.

Statistical analysis

Descriptive statistical data were described as the mean with stan-
dard deviation. To assess measurement error, the within-subject stand-
dard deviation (Sw) was calculated by taking the square root of the mean within-subject variance. The repeatability coefficient of the reti-

nal arteriolar and venular diameter measurements was calculated using the following equation: $\sqrt{2 \times 1.96S_w}$ or $2.775S_w$, meaning that the difference between any two future measurements is estimated to be no greater than the repeatability coefficient on 95% of measure-
ments. Intraobserver repeatability of the measurement method and interobserver reproducibility were also described by interclass correlation coefficients (ICCs). The ICC interpretation that we defined was slight reliability (for values between 0 and 0.2), fair reliability (from 0.21 to 0.4), moderate reliability (from 0.41 to 0.6), substantial reliability (from 0.61 to 0.8), and almost perfect reliability (for values higher than 0.81). Bland and Altman plots were used to assess intraobserver repeatability and interobserver reproducibility. The

![Fig. 1. Retinal vessel scanning method and orientation of the zone B region. Zone B area was defined as between 0.5 and 1 DD from the optic margin. Temporal superior retinal vessels passing through zone B were detected on OCT scans. A sheet of transparent film with four painted concentric circles (A) was used to overlay the computer monitor screen (B) and to help the observer locate zone B for scanning. Sections within the zone B area were chosen for measurement. White arrows show the film edges.](image-url)
Results

The study evaluated 58 eyes of 58 volunteers. Three participants were excluded from the study because of poor quality OCT images. The mean age of the 23 men and 32 women was 29.5 ± 7 years (range: from 20 to 38 years). The mean refractive error was −0.78 ± 0.94 diopters (range: from −2.375 to 0.875 diopters) of the spherical equivalent.

The retinal arteriolar and venular structure could be very clearly demonstrated in the OCT images when the scan line was parallel to the vessel (Fig. 2A and B). Of note, the vessel walls were consecutive and hyper-reflective in the images, especially the retinal arteriolar walls. When the scan line was adjusted perpendicular to the retinal vessel, the cross section image shows that the retinal vessel lies in the nerve fiber layer and the ganglion cell layer (Fig. 2C). The vessel walls in the vertical direction were imaged by OCT; however, it failed to show the vessel walls in the horizontal direction. If the scan line was not perpendicular to the targeted vessel, the cross section image of the vessel structure becomes indistinct, and the vessel wall was occasionally not located in the images (Fig. 3). In addition, some retinal vessels were not very clear on the images acquired using the circular scan method, whereas the line scan method produced better images of these retinal vessels showing the fine structures of the vessel wall (Fig. 4). We also performed both two methods on 20 young healthy subjects, the line scan had a higher rate of 89.9 ± 8.3% to show clear cross section structure in all main retinal vessels in the zone B, while the mean rate acquired by the circular scan was only 54.3 ± 11.7% (P < 0.01, chi-square test).

Tables 1 and 2 show the intraobserver repeatability and interobserver reproducibility of the retinal arteriolar outer diameter (RAOD), retinal arteriolar lumen diameter (RALD), retinal venular outer diameter (RVOD) and retinal venular lumen diameter (RVLD) measurements by OCT. The within-subject SD was within 1–3% and repeatability was within 5–6% of the mean value for all four parameters. The intraobserver ICCs for the RAOD, RALD, RVOD and RVLD measurements obtained using the OCT system were between 0.972 and 0.981. The interobserver ICCs for the RAOD, RALD, RVOD and RVLD measurements were between 0.968 and 0.980. These ICCs represent very good reliability of measurements. As a whole, interobserver reproducibility of all measurements was slightly lower than intraobserver repeatability. The Blan–Altman plots show the good agreement of both the intraobserver and interobserver measurements of the RAOD, RALD, RVOD and RVLD parameters (Figs. 5 and 6). The 95% limits of agreement for the RAOD, RALD, RVOD and RVLD measurements obtained by the two examiners were −5.60 to 4.88 μm, −5.78 to 5.05 μm, −7.52 to 5.62 μm and −7.10 to 5.63 μm, respectively. The mean difference between the two examiners ranged from −0.95 to −0.37 μm.

Table 3 shows the mean temporal superior RAOD, RVOD, RALD and RVLD measured by OCT. The results of the same retinal arteriolar and venular diameters obtain from the IVAN software were similar for RALD and RVLD. Based on the results of the vessel outer diameter and lumen diameter, the mean temporal superior arteriolar wall was slightly thicker than the venular wall (P < 0.01, t-test).

Discussion

A recent study reported very good reproducibility of retinal vessel diameter measurements using the OCT technique. Muraoka et al. (2013), using the same SD-OCT as that used in our study (Spectral HRA + OCT), reported intervisit, interexaminer, and interevaluator ICCs ranging from 0.944 to 0.982 using an optic disc centered circle scan method. In this study, the Bland–Altman plot supported substantial intra- and inter- observer agreement in the measurements of retinal arterioles and venules located in the zone B region. Additionally, when estimating the reliability of measurement made by two observers, we found that interobserver repeatability coefficients and intraclass correlation coefficients were slightly better in the retinal arteriolar measurements. Moreover, the plot shows that the interclass 95% limits of agreement width for RVOD and RVLD are wider than that for RAOD and RALD. The higher variability of the venular measurements between different observers may be due to the lower venular vessel wall reflectivity on the OCT image, which could make it difficult for the observers to recognize the inner and outer borders of the vessel wall.

**Fig. 2.** Representative retinal vessel images obtained using spectral-domain optical coherence tomography (SD-OCT). SD-OCT B scan image shows longitudinal sections of (A) retinal arteriole and (B) retinal venule, and (C) cross sections of retinal vessels. White asterisk: arteriolar lumen; black asterisk: venular lumen; red arrows: retinal arterioles; blue arrows: retinal venules; white arrowheads: arteriolar wall; black arrowheads: venular wall.
Muraoka et al. (2013) reported a circular scan method for retinal vessel measurement, the advantage of this method was that the examiner could detect all the main retinal vessels by a single scan. However, their measurement was performed in the zone A region. As we know, retinal vessel measurement in the zone A has limitations. First, strong vessel pulsation near the optic disc can be seen in many people, and that will affect measured results. Second, in some cases, retinal vessel pair happens to arteriovenously intertwine when coming out from the optic disc, and it will be difficult to identify the vessel borders on the OCT image under this condition (Goldenberg et al., 2013). For these considerations, zone B, which has some distance away from the disc, was selected for measurement in our study. From the findings of this study, it is recommended that scan line should be adjusted as close to perpendicular as possible to the retinal vessel to obtain vessel cross section images showing the fine structure of the vessel wall. Therefore, it is reasonable that the circular scan method occasionally fails to show discriminable cross section images of some retinal vessels because not all of the vessels that emanate from the optic disc are perpendicular to the scan circle in every subject. Thus, one advantage of line scan and volume scan methods is that the examiner can rotate the scan line and scan frame in any angle and put them on any position in the fundus.

Previous studies were designed to determine which vessel components are measured on the color fundus images. In theory, the retinal vessel wall cannot be visualized by FP because it is transparent (Patton et al., 2006). Pakter et al. (2011) utilized fundus angiography as a gold standard for vessel lumen measurement and compared the results of vessel diameter measurement in FP, they found that the vessel diameters obtained from the two methods were very close and suggested that vessel diameter measured in FP actually was blood column diameter, as it was described in a previous study (Rassam et al., 1994). In our study, the vessels were measured in different directions from OCT and FP, vertically and horizontally, respectively. We assume that the cross section of retinal vessels may tend to be a horizontal ellipse shape due to intraocular pressure, and the horizontal diameter may be wider than the vertical diameter. Based on the results in Table 3, retinal vessel diameters in FP were slightly wider than the retinal vessel lumen diameters. Interestingly, we found that the difference between the two instruments was greater in the retinal arterioles. Another reason for the wider arteriolar diameter in the FP measurement is that the retinal arteriolar diameter may be overestimated. As Pakter et al. (2011) described, the retinal arteriole and venule presented different colors on the FP image; the retinal venule had a dark red color and good contrast to the fundus background, and the software automatically detected the venule vessel border accurately on the image. However, the retinal arteriole had a light red color, and the vessel border could be slightly blurred between the blood column and the background, thus the software may report an overestimated arteriolar diameter.

In our present study, the retinal arteriole had a significantly thicker vessel wall compared with the retinal venule, which was consistent with other studies. Previous work by Muraoka et al. (2013) reported a thickness of $14.3 \pm 2.5 \mu m$ and $11.7 \pm 2.0 \mu m$ for the mean retinal arteriolar and venular wall in a group of young healthy subjects. Moreover, in a retinal microvascular imaging study with adaptive optics scanning laser ophthalmoscopy, Chui et al. (2013) demonstrated that venular wall was relatively thinner compared with arterioles with similar lumen diameters due to their different structure. The authors thought the reason for the thinner venular wall was perhaps that the mural cells in the retinal venular wall tend to be elongated and flatter on the
images. However, the outcomes were different in the study by Michelson et al. (2007), who used scanning laser Doppler flowmetry to evaluate the age-related vessel wall changes in temporal superior retinal vessels in the juxtapapillary area. The calculated vessel wall thicknesses of the retinal venules were higher than that of the corresponding retinal arterioles in most age groups, including both young and older subjects. Possible reasons for these apparently discrepant results may be the difference in the determination of vessel wall border in

**Table 1**

<table>
<thead>
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<th>Sw (μm)</th>
<th>Repeatability coefficient (μm)</th>
<th>ICC (95% CI)</th>
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<tbody>
<tr>
<td>RAOD</td>
<td>1.89</td>
<td>5.22</td>
<td>0.981 (0.967,0.989)</td>
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<tr>
<td>RALD</td>
<td>1.86</td>
<td>5.14</td>
<td>0.977 (0.961,0.986)</td>
</tr>
<tr>
<td>RVOD</td>
<td>1.99</td>
<td>5.50</td>
<td>0.975 (0.957,0.985)</td>
</tr>
<tr>
<td>RVLD</td>
<td>1.81</td>
<td>5.02</td>
<td>0.972 (0.953,0.984)</td>
</tr>
</tbody>
</table>

Sw = within-subject standard deviation; ICC = intraclass correlation coefficients; RAOD = retinal arteriolar outer diameter; RALD = retinal arteriolar lumen diameter; RVOD = retinal venular outer diameter; RVLD = retinal venular lumen diameter.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Sw (μm)</th>
<th>Repeatability coefficient (μm)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAOD</td>
<td>1.88</td>
<td>5.21</td>
<td>0.980 (0.966,0.988)</td>
</tr>
<tr>
<td>RALD</td>
<td>1.95</td>
<td>5.41</td>
<td>0.973 (0.954,0.984)</td>
</tr>
<tr>
<td>RVOD</td>
<td>2.04</td>
<td>5.66</td>
<td>0.974 (0.957,0.985)</td>
</tr>
<tr>
<td>RVLD</td>
<td>1.96</td>
<td>5.42</td>
<td>0.968 (0.946,0.981)</td>
</tr>
</tbody>
</table>

Sw = within-subject standard deviation; ICC = intraclass correlation coefficients; RAOD = retinal arteriolar outer diameter; RALD = retinal arteriolar lumen diameter; RVOD = retinal venular outer diameter; RVLD = retinal venular lumen diameter.
the images. As mentioned by Michelson et al. (2007), their calculated vessel wall thickness comprised the plasma edge stream and the slow corpuscle flow near the border.

OCT imaging provided more information on retinal vessel structure, such as vessel lumen diameter, vessel wall thickness and wall/lumen ratio (WLR). The mean arteriolar WLR in this study was 0.36 ± 0.04,
which was calculated using the formula, (arteriolar outer diameter — lumen diameter) / lumen diameter (Michelson et al., 2007). Arteriolar WLR changes may reflect vascular structure remodeling (Ritt and Schmieder, 2009), and a previous study has shown that WLR was a more sensitive indicator than arteriole/venule ratio in the assessment of hypertensive cerebrovascular damage (Balauau et al., 2009). In addition, it was suggested that arteriolar WLR could be a potential marker of endothelial dysfunction in both the retinal and systemic vasculatures (Cuspidi and Sala, 2011). Another advance of our OCT method is that light rays from OCT can remarkably penetrate ocular media opacities such as mild cataract, mild posterior capsular opacification, and mild vitreous hemorrhage. For example, in the case of branch retinal vein occlusion, when the retinal vessels were covered by mild hemorrhage in the zone B region, the vessel structure with the hemorrhage could still be well demonstrated on the OCT image, while it could not be visualized by FP.

There are several limitations of the present study. First, only one temporal superior vessel pair in the zone B area was chosen in our study, and the other retinal vessels were not included for measurement. Assessment of retinal vessels in all four quadrants will increase the test time and cause the participant to be too fatigued to cooperate, which may then reduce the reliability of the measurement. Second, the sample size was small, and three participants were excluded because of inadequate signal strength. We found that in some large retinal venules, the hyper-reflectivity of the vessel wall becomes obscure and even disappear on the OCT image, particularly the lower venular wall. A potential explanation for the weak vessel wall signal in large vessels may be the interference of light rays by the large blood column flow. We lastly evaluated normal eyes only in this study; the accuracy of OCT measurement when applied to individuals with different types of ocular pathology should be examined in future studies.

Conclusions

Volume scan method produced better images of retinal vessels showing the fine structures of the vessel wall, and can be used to obtain high-quality retinal vessel images for vessel outer diameter, lumen diameter and wall thickness measurements. This technique has a high degree of repeatability and accuracy. SD-OCT appears to be a potentially valuable tool for assessment of retinal arteriolar and venular changes in ocular and systemic vascular diseases.

Contributions

The first two authors contributed equally to this study. Zhu T.P. designed and conducted the study, and wrote the manuscript. Tong Y.H. designed and conducted the study, and wrote the manuscript. Zhan H.J. performed the statistical analysis, and Ma J. designed and conducted the study.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgment

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