Choroidal Structure in Normal Eyes and After Photodynamic Therapy Determined by Binarization of Optical Coherence Tomographic Images

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Submitted: March 26, 2014
Accepted: May 19, 2014

Citation: Sonoda S, Sakamoto T, Yamashita T, et al. Choroidal structure in normal eyes and after photodynamic therapy determined by binarization of optical coherence tomographic images. *Invest Ophthalmol Vis Sci*. 2014;55:3893–3898. DOI:10.1167/iovs.14-14447

Purpose. To determine changes in choroidal structure by binarization of optical coherence tomographic (OCT) images.

Methods. Choroidal images were recorded by enhanced depth imaging OCT. The subfoveal choroidal images were analyzed, and the luminal and interstitial areas were converted to binary images by the Niblack method. The interrater, intrarater, and intersession agreements of the binary images were determined for healthy eyes. In eyes with age-related macular degeneration (AMD), the binary images of the choroid before photodynamic therapy (PDT) were compared to those after PDT. The untreated fellow eyes were studied as controls.

Results. In healthy eyes, the average ratio of the luminal to choroidal area was 65.4%. The interrater agreement rate was high, with intraclass correlation coefficient (ICC) 0.985 and 0.988 for the choroid and luminal areas, respectively. The intrarater ICC was 0.996 for the choroid and 0.997 for the luminal areas. The intersession ICC was 0.993 for the choroid and 0.984 for the luminal areas. In eyes with AMD, the subfoveal choroidal area, the luminal area, and the interstitial areas were thinner 6 months after PDT (all $P < 0.01$, Wilcoxon signed-rank sum test). The ratio of the luminal to choroidal area was significantly decreased to 62.8% ($P < 0.01$, Wilcoxon signed-rank sum test). The ratio for the fellow eyes was not significantly changed.

Conclusions. The Niblack binarization method can be used to analyze the luminal area of choroid in an OCT image with good repeatability and reproducibility. The change in the subfoveal choroidal area after PDT is due mainly to a decrease in the luminal areas.

Keywords: choroid, age-related macular degeneration, vessel, EDI-OCT

The choroid is a thin membranous tissue composed mostly of blood vessels and interstitial stroma with melanocytes and connective tissue.[1] The choroid plays an important role not only in choroidal inflammation, but also in the more common retinochoroidal diseases such as diabetic retinopathy and age-related macular degeneration (AMD).[1–7]

Histologic analyses have shown that changes of the choroidal interstitial stroma can occur via edema, fibrosis, and inflammation with cellular infiltration as observed in eyes with uveitis, diabetic retinopathy, and AMD.[2,3,8] However, fixation of the tissues during histologic processing induces artifacts including shrinkage, which makes it difficult to evaluate changes in the choroid caused by disease processes, especially the vascular tone and structure.[9,10]

Optical coherence tomography (OCT) is a noninvasive imaging method in which in situ cross sections of the retina and choroid can be obtained with micrometer resolution.[11,12] Using this method, several studies have reported that the choroidal thickness changes significantly under various pathologic conditions.[13–15] The question then arises as to not only how the choroidal thickness changes but also what structures change. To determine this, it is necessary to perform morphometric analysis of the choroid. However, because the choroid is essentially composed of blood vessels without uniform or elaborate architecture like the retina, doing morphometric analyses is quite difficult.

Several studies have assessed the vascular structures of the choroid by OCT.[10,16] Although the methods would be valuable, the techniques used required customized software that prevented their widespread use.

Thus, the purpose of this study was to convert the luminal and interstitial areas of the choroid obtained by enhanced depth imaging (EDI)-OCT into binary images. Open access software, ImageJ, was used for the conversion. We present the method used and show its high reproducibility and repeatability. In addition, we used this method to measure the luminal and interstitial areas of the choroid before and after photodynamic therapy (PDT) on eyes with AMD.

Methods

This study was designed to determine a valid and reproducible method to differentiate the luminal area and the interstitial area of the choroid in spectral-domain OCT (SD-OCT) images of normal eyes. All of the investigative procedures conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects after an explanation of the procedures to be used and possible complications. The study was approved by the Ethics Committee of Kagoshima University Hospital (Kagoshima, Japan) and registered with the University Hospital Medical Network (UMIN) Clinical Trials

Registry (Trial No.: UMIN000012310; Title: Choroidal structure on OCT images). A detailed protocol is available at http://www.kufm.kagoshima-u.ac.jp/~op/gairai/RCstructurestudy.html.

Participants

Volunteers with no known eye disease were used to determine the repeatability and reproducibility of the binarization method for subfoveal choroidal SD-OCT images. Prior to the measurements, all eyes received ocular examinations, which included slit-lamp examination of the anterior segment of the eye and ophthalmoscopic examination of the fundus. The intraocular pressure was measured with a pneumotonometer (CT-80; Topcon, Tokyo, Japan). The best-corrected visual acuity (BCVA) was measured after determining the refractive error with an Auto Kerato-Refractometer (RM8900; Topcon). Only the right eye of each volunteer was examined.

The eligibility criteria were age between 18 and 50 years and eyes normal by ophthalmoscopy and OCT. The exclusion criteria included eyes with known ocular diseases such as glaucoma, diabetic retinopathy, presence of systemic diseases such as hypertension and diabetes, high myopia of more than –6.0 diopters (D), history of intraocular surgery or injections, and eyes in which the ocular fundus could not be observed due to media opacities. No eye was excluded due to poor OCT image quality because of poor fixation.

The second experiment was a retrospective study. Eyes with exudative AMD were selected from the medical records of Kagoshima University Hospital examined from November 2011 to January 2013. The diagnosis of AMD was made based on the fundus examination, fluorescein angiography, indocyanine green angiography, and OCT.[17]

Photodynamic therapy was performed according to the guidelines of the Japan Ophthalmic Society.[17] Patients with unilateral wet AMD requiring PDT based upon the guidelines were analyzed, but only those who received PDT unilaterally.[17] The fellow eyes were used as controls. The pre- and post-PDT thickness of the subfoveal choroid was compared. Pre-PDT was defined as the period within 1 week of the PDT, and the average post-PDT time was 6 months ± 2 weeks. All of the OCT images were taken from 9:00 hours to 11:00 hours during our AMD outpatient clinic time to minimize diurnal variations in the thickness of the choroid.

Measurement of Subfoveal Choroid by Spectral-Domain Optical Coherence Tomography

The subfoveal choroidal images were obtained with the EDI-OCT technique, and the scans were seven horizontal lines of 30 × 10° through the center of the fovea. The Heidelberg Spectralis OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) instrument was used, and it was pushed as close to the eye as possible to obtain an inverted image. Each image was obtained using the eye tracking system, and 100 scans were averaged to improve the signal-to-noise ratio. The subfoveal choroidal thickness (SFCT) was defined as the distance between the outer border of the hyperreflective retinal pigment epithelium (RPE) and the outer border of the choroid beneath the center of the fovea. Measurements of the SFCT were done in a masked fashion as we have reported in detail.[18,19] All eyes were examined without mydriasis.

The interrater agreements, intrarater agreements, and intersession agreements were determined for 20 healthy volunteers as we have reported in detail.[18–20] For interrater agreement, the measurements were done by two independent examiners (SS and MT). For intrarater agreement, the measurements were made by a single examiner (MT). Because there are significant diurnal or daily fluctuations of the choroidal thickness, all examinations on a single patient were done within 1 hour on the same day.

Binarization of Choroid of EDI-SD-OCT Images

After recording the EDI-SD-OCT images, the best image was displayed on a computer screen and evaluated by three masked graders independently (MS, HT, and MT). When two or more graders determined that the subfoveal choroidal image was clearly distinguishable, the image was deemed acceptable and used for the following analyses.

Binarization of the subfoveal choroidal area in the OCT image was done by a modified Niblack method. Briefly, the OCT image was analyzed by ImageJ (version 1.47; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA; http://imagej.nih.gov/ij/).

The examined area was selected to be 1500 μm wide, with margins 750 μm nasal and 750 μm temporal to the fovea. It extended vertically from the RPE to the chorioscleral border, and the choroidal area was set with the ImageJ ROI Manager. Then three choroidal vessels with lumens larger than 100 μm were randomly selected by the Oval Selection Tool on the ImageJ tool bar, and the average reflectivity of these areas was determined. The average brightness was set at the minimum value to minimize the noise in the OCT image. Then the image was converted to 8 bits and adjusted by the Niblack Auto Local Threshold. The binarized image was converted to RGB (red, green, blue) image again, and the luminal area was determined using the Threshold Tool. After adding the data of the distance of each pixel, the choroidal area, luminal area, and interstitial area were automatically calculated. The light pixels were defined as the interstitial choroid or choroidal stroma, and the dark pixels were defined as the luminal area. A more detailed protocol is presented in Supplementary Material S1.

Statistical Analyses

All statistical analyses were performed with a commercial analytical package (SPSS Statistics 19 for Windows; SPSS, Inc., IBM, Somers, NY, USA). The intrarater and interrater correlation coefficients were calculated using a two-way mixed-effects model for measurements of absolute agreement. Intersession agreement was computed with the same method. The area of the subfoveal choroid including the interstitial area and the luminal area before and after PDT was computed by Wilcoxon signed-rank test. A P value < 0.05 was considered to be statistically significant.

Results

Healthy Volunteers

Twenty volunteers agreed to participate in the study, and the demographic characteristics of the volunteers are shown in Table 1. The average age was 31.7 ± 7.8 years, and refractive error was low myopia.

Binarization of Subfoveal Choroid of Healthy Eyes of Volunteers

A representative EDI-OCT image and its converted binary image are shown in Figure 1. The vascular area of the choroid is represented by black areas and the interstitial areas by white areas.

The interrater agreement was very high, with an intraclass correlation coefficient (ICC) of 0.985. The ICCs were also high for the luminal and interstitial areas (Table 2).

Table 1. Demographic Data of Healthy Volunteers

	Mean	Range
Age, y	31.7	22 to 47
Refractive error, spherical equivalent	−3.4 D	−5.5 to −0.25 D
BCVA, logMAR	−0.16	0 to −0.30
IOP, mm Hg	14.15	11 to 19
Axial length, mm	25.03	22.67 to 27.68

Nine men, 11 women. IOP, intraocular pressure.

The intrarater agreement was also high, with an ICC of 0.996 (confidence interval [CI] 0.989–0.998) for the choroidal area, 0.997 (CI 0.994–0.999) for the luminal area, and 0.986 (CI 0.965–0.994) for the interstitial area.

The intersession agreement was very high, with an ICC of 0.993 (CI 0.975–0.998) for the choroidal area, 0.984 (CI 0.888–0.992) for the luminal area, and 0.969 (CI 0.942–0.996) for the interstitial area.

The subfoveal choroidal area was 675,526.4 ± 189,260.1 μm^2 (average ± SD); the luminal area was 445,562.0 ± 138,782.8 μm^2; and the interstitial area was 229,964.4 ± 56,538.2 μm^2. The average ratio of the luminal area to the choroidal area was 65.4%; that is, approximately 65% of the choroid is made up of blood vessels.

Subfoveal Choroid in AMD Eyes

The baseline demographic data of the AMD patients are shown in Table 3. All treated eyes had one or more subfoveal active lesions, and all of the foveal lesions received PDT treatment. In terms of fellow eyes, one eye had an old AMD scar, which did not require treatment. The other 14 eyes did not have any apparent sign of wet AMD requiring treatment.

The SFCT was 278.8 ± 75.0 μm in eyes with AMD at the baseline, which was significantly reduced to 217.5 ± 70.0 μm at 6 months after PDT ($P = 0.01$, Wilcoxon signed-rank test). The BCVA at the baseline was 0.37 ± 0.29 logarithm of the minimum angle of resolution (logMAR) units, which improved to 0.33 ± 0.31 at 6 months after PDT; but the degree of improvement was not significant ($P = 0.15$, Wilcoxon signed-rank test).

The baseline choroidal area of eyes with AMD was 629,578.8 ± 163,165.5 μm^2; the luminal area was 414,443.9 ± 116,911.1 μm^2; and the interstitial area was 215,134.9 ± 49,091.3 μm^2. The ratio of the luminal area to the choroidal area was 65.4% of the choroidal area.

The baseline choroidal area of the fellow eyes with no AMD was 678,659.5 ± 164,127.4 μm^2; the luminal area was 447,690.0 ± 112,112.5 μm^2; and the interstitial area was 230,969.5 ± 53,349.1 μm^2. The ratio of the luminal area to the choroidal area was 65.8% of the choroidal area. The differences in the three measurements between the AMD and fellow eyes for the three corresponding areas were not significant ($P = 0.83$ for total subfoveal choroidal area, $P = 0.87$ for luminal area, and $P = 0.55$ for interstitial area; Fig. 2).

Six months after the PDT, the choroidal area was reduced to 500,778.2 ± 160,152.2 μm^2, which was significantly smaller than the baseline choroidal area. The ratio of the luminal area to interstitial area was 62.8% at 6 months after the PDT, which was significantly smaller than that at baseline ($P < 0.01$, Wilcoxon signed-rank test). Both the luminal area of 318,872.4 ± 116,395.8 μm^2 and the interstitial area of 181,905.8 ± 46,771.3 μm^2 were also decreased significantly in comparison to the baseline values (both $P < 0.01$, Wilcoxon signed-rank test; Fig. 3).

On the other hand, the choroidal area of the fellow eyes was 669,852.9 ± 171,895.6 μm^2, which was not significantly different from the baseline choroidal area. Both the luminal area, 44,506.8 ± 121,598.7 μm^2, and the interstitial area, 225,346.1 ± 52,079.1 μm^2, were not changed significantly relative to the baseline (65.8% vs. 66.1%, $P = 0.20$, Wilcoxon signed-rank test; Figs. 3, 4).

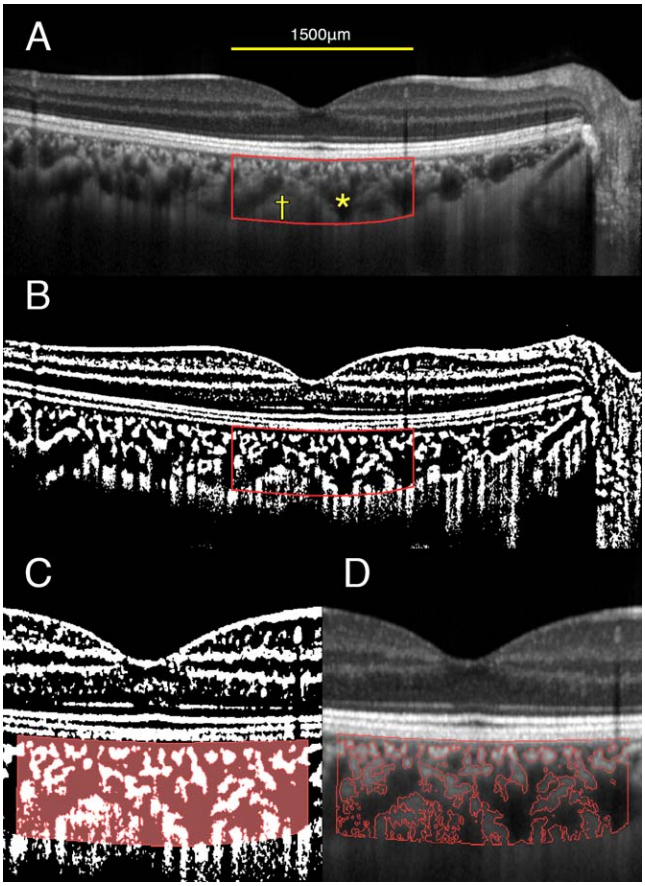

Figure 1. Enhanced depth imaging OCT image and converted binary image of a normal healthy eye. An EDI-OCT image of a healthy eye (**A**) was converted to a binary image (**B**) using the ImageJ software. The luminal area (dark area, *asterisk*) and the interstitial area (*cross*) are seen. The rectangle surrounded by a *red line* was excised, and the dark areas were traced by the Niblack method ([**C**], *brown area*). The binarized image (**B**) and the margin of traced area (**C**) were merged, and the merged image shows that the traced areas coincide with the dark choroidal areas of the OCT image (**D**).

Discussion

To differentiate the luminal area and the interstitial area of the choroid, a method that has been used consists of tracing the margins of each type of area manually. However, this method is subjective and strongly affected by the rater's experience and proficiency. It is also very time-consuming. To avoid bias, we applied an autolocal thresholding method, an image processing method of image segmentation. It can be used to convert a grayscale image to a binary image and is often used for personal identification in computer/networking technology and recovery of damaged or ancient documents and other indistinct images.[21,22]

Our results showed that the subfoveal choroidal structures in OCT images can be analyzed by converting them to binary images with the publicly accessible software ImageJ. The intrarater findings showed that this technique was highly

Table 2. Interrater Agreement

	Examiner 1	Examiner 2	ICC (95% CI)
Choroid, μm^2	667,668.2 ± 189,305.7	683,384.7 ± 190,327.5	0.985 (0.955–0.995)
Luminal area, μm^2	441,830.9 ± 138,814.7	449,293.1 ± 139,513.6	0.988 (0.97–0.995)
Interstitial area, μm^2	225,837.3 ± 56,890.9	234,091.6 ± 56,629.6	0.975 (0.863–0.992)

repeatable, and the interrater and intersession findings showed that it was highly reproducible.

But is the technique valid? Histologically, the choroid is composed of blood vessels and interstitial tissues. The interstitial tissues include pigment cells, smooth muscles, neurons, vascular walls, inflammatory cells, and connective tissue. Unfortunately, they cannot be differentiated even with the most advanced OCT. Therefore, we used the binarization technique to differentiate the vascular (luminal) from the interstitial areas. Initially we examined different methods such as the Berson, Mean, Median, Midgrey, Sauvola, and Niblack methods, and we found that the Niblack was the most suitable method for differentiating the luminal areas and the interstitial areas in the EDI-OCT images of the choroid. Although we do not have definitive evidence that the dark areas represented the vascular areas and the light areas the interstitial tissues, the findings of earlier studies and numerous empirical observations strongly suggest that the dark areas were the vascular components.[10,16] In addition, a comparison of the original EDI-OCT images to the binary images showed that the dark areas corresponded with the vascular components of the choroid, especially the larger choroidal vessels. Thus, our binarization technique is not only reproducible and repeatable but is also valid.

Previously, Branchini et al.[10] analyzed the choroidal structure in OCT images using custom-made software, and they reported that the ratio of the choroidal interstitial area to the vascular lumen was 0.27, which is equivalent to 78.7% (1/1.27) of the luminal area/choroidal area we used. Thus, their choroidal images had relatively more luminal areas than interstitial areas than did our findings of 65% to 66%. However, the OCT images they used were not obtained by EDI-OCT; thus, the choroid–scleral border was not as distinct as in EDI-OCT images. Actually, the deep choroid is frequently shown as a "dark area" without use of the EDI-OCT method. This might have made the luminal area too large. Sohrab et al.[16] also reported that the ratio of the area of vessel lumen to the choroidal area was 87.2%, which is much higher than our results. However, they measured cross sections of the choroid, exclusively measuring the large vessel layers. Importantly, they also used custom-made software for their analysis, which makes it difficult to compare their data to our data directly.[10,16] We used the software ImageJ, which is freely and easily accessible; thus our method can be confirmed or rejected by other researchers, which will be necessary to compare our results to those obtained in future studies. Indeed, Chen et al.[23] reported that ImageJ software was better for evaluating choroidal thickness of OCT images than the Heidelberg Eye Explorer software.

Table 3. Demographic Data of Photodynamic Therapy–Treated Patients

	Mean	Range
Age, y	68.1	55 to 81
Refractive error, spherical equivalent	−0.49 D	−2.5 to 1.5 D
BCVA, logMAR	0.37	0 to 1.15
IOP, mm Hg	13.7	8 to 17

Thirteen men, two women.

For a more clinical application, we compared the effect of PDT on the luminal and interstitial areas of the choroid. Our results showed that both areas decreased in size, but the luminal area decreased more than the interstitial area after PDT. Thus, the ratio of the luminal to choroidal area was 65.4% at baseline and 62.8% after PDT. It has been reported that choroidal thickness decreases after PDT,[24,25] but the structures involved have not been determined. The thickness of the choroid changes easily, and the change can be caused by at least four factors: changes of the osmolarity-active molecules (proteoglycans), vascular permeability, irrigation of fluid and ionic molecules through RPE layers, and changes of the nonvascular smooth muscles.[1] Because the luminal areas decreased, it is reasonable to assume that the number of vessels decreased and/or the diameter of vessels decreased. In humans, hypoperfusion is sometimes noted in PDT-eradicated areas,[26,27] and dropout or closure of choriocapillaris has been detected histologically.[28,29] Even though closure or apparent damage of large vessels in the choroid has been noted under clinically applied PDT, it is possible that some mid- to large-size vessels are closed after PDT.

Another possibility is a decrease of the vascular diameter caused by an increase of vascular tone. Vascular endothelial growth factor can dilate vessels by upregulating endothelial nitric oxide synthase (eNOS)-dependent pathways, and thus downregulation of VEGF can also explain our findings. Vascular endothelial growth factor plays an important role in maintaining homeostasis of the choroid, and it is secreted mainly on the basal side of the RPE.[30–33] The RPE is not apparently damaged after PDT at the light microscopic level, but there are some slight changes such as a mild degree of focal detachment from Bruch's membrane.[28,29] The amount of VEGF secreted by the RPE is markedly reduced even with minimal changes of the microenvironment in vitro, so it may be possible that VEGF secretion by RPE is reduced after PDT.[32] This issue needs further investigation.

Our results showed that the luminal and interstitial areas decreased after PDT. Vascular endothelial growth factor increases vascular permeability; this allows intravascular osmotically active molecules to move into the interstitial tissue, resulting in the interstitial tissue swelling. Thus, an inhibition of the release of VEGF might result in a decrease in the release of osmotically active molecules.

Importantly, the ratio of the luminal area to the interstitial area became smaller than that in healthy eyes or that in fellow eyes without AMD. The ratio of vascular area might decline for a long period after PDT, which is consistent with the hypoperfusion detected by indocyanine green angiography.[27,34] Although significant adverse events related to PDT in normal retinas and choroid have not been reported, careful follow-up is necessary to determine the long-term effects of PDT especially on the degeneration of retinochoroidal tissues. This issue should be considered with regard to determining the use of PDT.

There are several limitations in this study. First, this was a retrospective study and could not be free of sampling bias. Although a significant decrease was found in the luminal area of

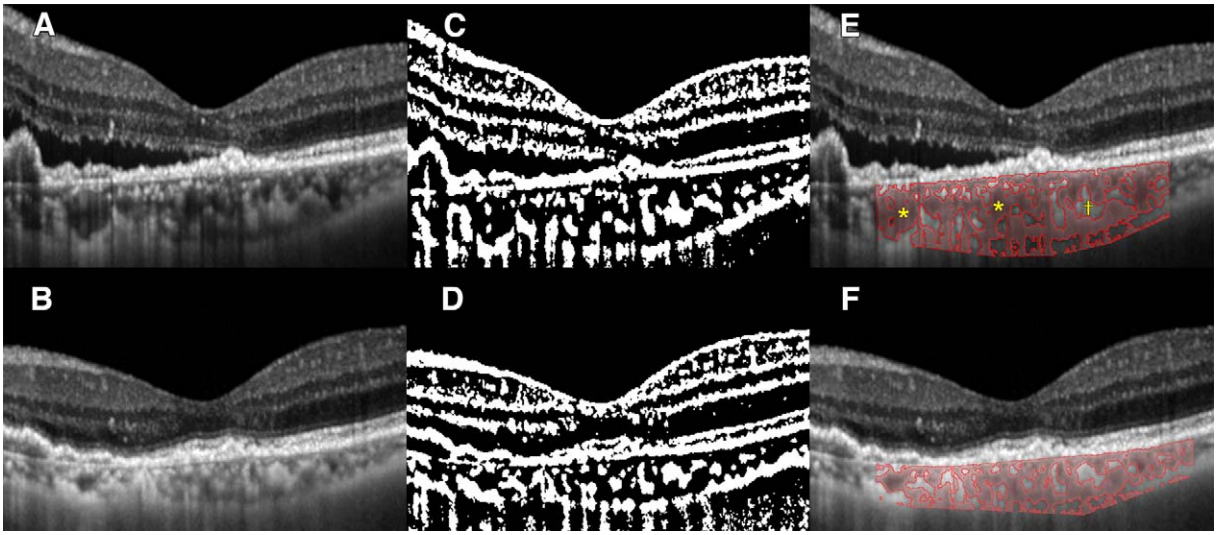

FIGURE 2. Representative eye with AMD before and after PDT. The choroidal area was reduced 6 months after photodynamic therapy (PDT) (**A**, **B**). Binarization analyses show that the ratio of luminal to choroidal area was reduced from 66.3% to 63.2% after PDT (**C**, **D**). (**E**, **F**) Measurement portion of the luminal area is surrounded by *red*. (**A**, **C**, **E**) Baseline; (**B**, **D**, **F**) 6 months after PDT. *Asterisks*: luminal area. *Cross*: interstitial area.

the choroid after PDT, 15 cases are too few to allow definitive conclusions. The choriocapillaris was always identified as a dark area in a binary EDI-OCT image; thus it was always categorized as a luminal area. Therefore, the present method cannot evaluate the effect of PDT on choriocapillaris because of limitations in the current OCT technology. Two-dimensional assessments do not always reflect the choroidal volume, although the width of the examined area was constant at 2 × 750 μm, and it is highly likely that the results represent the subfoveal choroidal volume. Lastly, we did not select the Niblack method among the several different binarization methods in an objective manner. There might be a more suitable binarization method to analyze choroidal images on OCT.

In conclusion, the conversion of EDI-OCT images to binary images with publicly accessible software can be used to quantify the luminal and interstitial areas of the choroid. The interclass, intraclass, and intersession agreements were high; and the procedures were valid so that this method can be widely used. Using this technique, we found that the luminal and interstitial areas decreased after PDT and that the luminal area decreased more. This method should provide a new means of studying the pathophysiology of human choroid in greater detail.

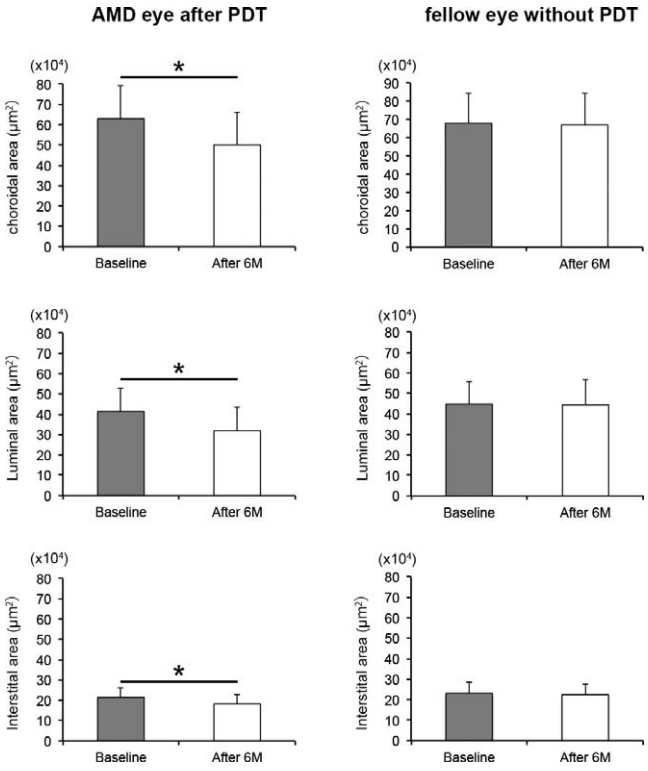

FIGURE 3. Subfoveal choroidal area of EDI-OCT image at baseline and 6 months after photodynamic therapy. The subfoveal choroidal area, luminal area, and interstitial area were significantly reduced 6 months after photodynamic therapy (*$P < 0.01$, Wilcoxon signed-rank test).

Acknowledgments

The authors thank Duco Hamasaki, PhD, for providing critical discussions and suggestions for our study and revision of the final manuscript.

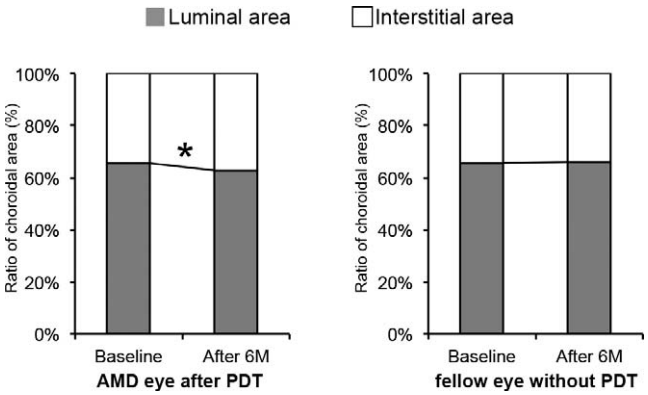

FIGURE 4. Ratio of luminal to choroidal area of OCT image after photodynamic therapy. In eyes with age-related macular degeneration (AMD), not only the choroidal area but also the ratio of the luminal area to choroidal area was significantly reduced 6 months after PDT (*$P < 0.05$, Wilcoxon signed-rank test). In the fellow eyes without PDT, neither the ratio of luminal to choroidal area nor the choroidal area was significantly altered after 6 months.

Supported by a grant from the Research Committee on Chorioretinal Degeneration and Optic Atrophy, Ministry of Health, Labor, and Welfare, Tokyo, Japan, and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of the Japanese Government. The authors alone are responsible for the content and writing of the paper.

Disclosure: **S. Sonoda**, None; **T. Sakamoto**, None; **T. Yamashita**, None; **M. Shirasawa**, None; **E. Uchino**, None; **H. Terasaki**, None; **M. Tomita**, None

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