CANCER DIAGNOSIS & PROGNOSIS
2: 330-335 (2022) doi: 10.21873/cdp.10113

Direct Observation of Retinal Microvessels in Cancer Patients After Systemic Administration of Bevacizumab and Oxaliplatin

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Abstract. *Background/Aim: Antiangiogenic chemotherapy is the backbone of the various anticancer therapies. To date no practical biomarker predicting their antitumor effects and toxicity has been reported. We aimed to determine the feasibility of direct retinal observation as a practical biomarker in antiangiogenic chemotherapy. Patients and Methods: By direct retinal observation using a nonmydriatic retinal camera, we measured retinal microvessel diameters in 10 patients with colorectal cancer before and after intravenous infusion of bevacizumab and oxaliplatin. All patients also received oral capecitabine during their therapy. Results: Retinal microvessel diameters were decreased from baseline temporarily by 14.5±6.5% after infusion of bevacizumab and oxaliplatin in five patients who responded to treatment and 8.8±6.2% in the other five patients (p=0.008). Conclusion: Measurement of retinal microvessel diameters by direct observation appears to be feasible in patients receiving systemic chemotherapy. The decrease of retinal microvessel diameters might indicate improved tumor response to treatment with bevacizumab-containing systemic chemotherapy.*

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Key Words: Bevacizumab, retinal vessels, colorectal cancer, biomarker.

Antiangiogenic therapy is the basis of various cancer therapies. Bevacizumab, a recombinant humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has been widely employed in the standard chemotherapy of many solid malignancies such as colorectal cancer (1). Despite extensive research for the detection of biomarkers such as angiogenesis-related biochemical factors, genetic polymorphisms, gene and/or protein expression, and radiographical images, no practical biomarker predicting antitumor efficacy and toxicity has been identified yet (2).

Age-related macular degeneration (AMD) is a common eye disease that usually occurs in people aged 50 and older causing irreversible central vision loss. Diagnostic retinal examination using optical coherence tomography or fluorescein angiography reveals choroidal neovascularization that develop under the retina scarring of the macula. Direct intravitreal injection of anti-VEGF agents, such as ranibizumab, aflibercept, and brolucizumab, is a treatment option for AMD (3). Bevacizumab, commonly administered at a dose of 1.25 mg, is an alternative option causing similar effects on both vision and retinal thickness, despite its off-label use. According to a prospective study of 11 patients with AMD, the retinal arteriolar diameter was decreased after each intravitreal injection of ranibizumab, a smaller fragment (Fab) of bevacizumab probably due to vasoconstriction (4).

Retinal observation is routine practice in ophthalmology. Besides its use in the diagnosis of ophthalmic diseases, it is also known that aberrant alternation of the retinal microvessels reflects systemic disorders, such as atherosclerosis (5), hypertension (6), and diabetes (diabetic retinopathy) (7, 8). We, thus, hypothesized that retinal microvessel diameters might decrease after systemic administration of bevacizumab in cancer patients receiving systemic chemotherapy, indicating tumor response to systemic treatment. Direct observation of patient's retinal microvessels could thus be a biomarker predicting antitumor efficacy and toxicity of angiogenesis inhibitors.

Table I. *Patient characteristics*.

	Age	Sex	Courses	Microvessel diameter* (%)	Tumor shrinkage**	Histopathological regression***	Treatment-related hypertension
1	54	M	2, 3, 4	7.6	PR	Grade 2	
2	67	M	2, 3, 4	12.0	PR	Grade 2	Grade 2
3	43	M	2, 3, 4	8.1	PR	Grade 1a	
4	59	M	3, 4	9.8	PR	Grade 1b	Grade 2
5	45	F	3, 4	16.8	PR	Grade 1b	
6	49	M	2, 3, 4	9.9	SD	Grade 1a	Grade 2
7	49	M	3, 4	8.4	SD	Grade 2	
8	67	M	2, 3, 4	8.9	SD	Grade 1a	
9	36	M	2, 3, 4	9.2	SD	Grade 1b	
10	29	M	2, 3, 4	8.5	SD	Grade 2	

*The average decrease of retinal microvessel diameters. **PR (partial response) and SD (stable disease) are comparable to PR and SD, respectively, defined with RECIST criteria. ***Grades of histopathological regression were assessed according to the Japanese Society for Cancer of the Colon and Rectum (13): Grade 1a, minimal effect (necrosis less than one-third of the lesion); Grade 1b, mild effect (necrosis less than two-third but one-third or more of the lesion); Grade 2, moderate effect (necrosis more than two-third of the lesion). There were no Grade 0, no regression or Grade 3, no tumor cells (pathological complete response) patients.

Patients and Methods

This research was performed in compliance with the Declaration of Helsinki and was approved from the institutional Review Board (No. 1050). Each patient provided written informed consent for participating in the study.

Patients. A total of 70 patients with colorectal cancer that received chemotherapy with or without bevacizumab in an outpatient setting between April 2011 and August 2012 were evaluated. Among these patients, 10 patients received scheduled preoperative chemotherapy consisting of intravenous bevacizumab 7.5 mg/kg and oxaliplatin 130 mg/m^2 on day one in combination with oral capecitabine 1,000 mg/m^2 twice daily for two weeks, followed by a one week break, repeated every three weeks.

As a routine practice, patients who had a record of thrombotic diseases, interstitial pneumonia, peptic ulcer, hemoptysis, or other bleeding or coagulation disorders, as well as patients receiving anticoagulants or aspirin were excluded from the bevacizumab receiving arm.

Measurement of retinal microvessel diameters. Retinal photographs, left or right, were obtained using a nonmydriatic retinal camera (AFC-230, Nidek, Gamagori, Japan) before (baseline) and after each intravenous infusion of bevacizumab and oxaliplatin, throughout the therapy period in each patient. Measurements were commonly collected with a total of three treatments from the second to the fourth preoperative courses as they were enrolled before the second course. Retinal photographs were usually collected within one hour after the end of infusion. Based on our preliminary study of six healthy volunteer subjects (three males and three females), there were no significant modifications in retinal microvessel diameters due to daily activities such as walking and eating.

As retinal arteries and veins demonstrated similar decrease in diameters in another preliminary study, retinal veins were examined for the following analyses: diameters of retinal veins were measured at any eight locations within ×1.5-fold of the diameter of the optic disc from the outer edge of the optic disc on retinal photograph using Adobe Photoshop™ version 9 (Adobe Systems Incorporated, San Jose, CA, USA). The retinal vessel diameter on each photograph was standardized based on the diameter of the optic disc. The measurement was repeated by five different investigators to minimize inter-investigator variation.

R software, version 2.15.0 (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis.

Results

No significant difference was observed in the decrease of microvessel diameters between right and left fundi in five patients who received chemotherapy with bevacizumab (15.1±7.2% in right fundus; 16.0±5.7% in left fundus, mean± standard deviation, total 24 records). It was thus decided for each patient whether the left or the right fundus would be examined, but the same fundi were examined before and after chemotherapy. Furthermore, a decrease of retinal microvessel diameter persisted for several hours after the end of bevacizumab and oxaliplatin infusions, and it usually recovered by the initiation of the next chemotherapy.

Retinal microvessels diameters of 10 patients (nine males and one female) with a median age of 49 years (range=29-67 years) decreased 9.9±2.6% (total 54 records) from baseline after the end of bevacizumab and oxaliplatin infusions, and then recovered by the initiation of the subsequent chemotherapy three weeks later, as presented in the preliminary studies (Table I, Figure 1). Among the 10 patients, five achieved a tumor shrinkage of the primary lesion of more than 30% on radiological images, equivalent to partial response defined with RECIST criteria, while the other five did not. Retinal microvessel diameters of the five responders

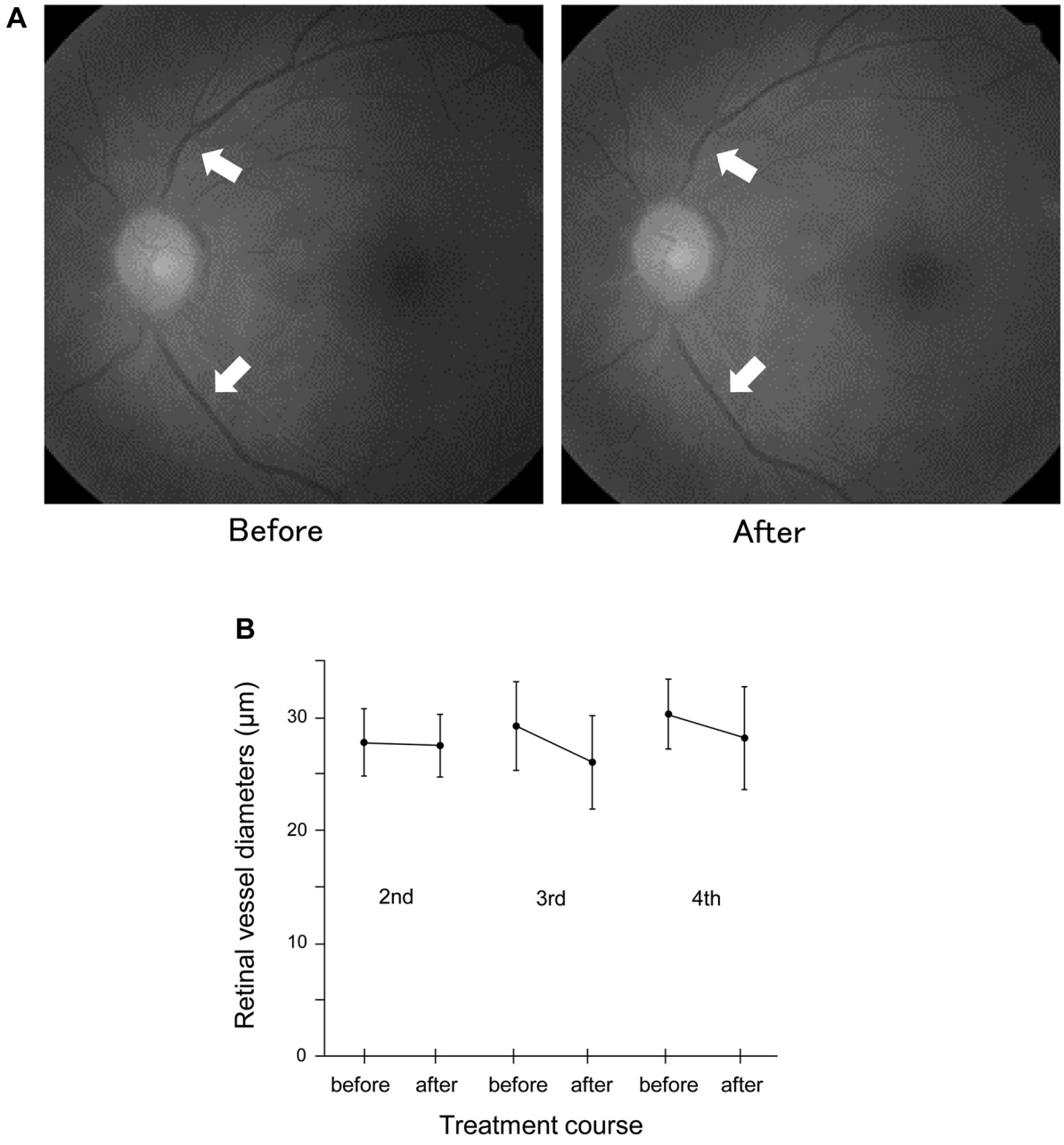

Figure 1. *Left retinal photographs of a representative case. (A) Retinal photographs of a 54-year-old male before (left) and after (right) infusions of bevacizumab and oxaliplatin in the third course. The diameter of retinal microvessels (veins, arrows) appears to be shorter after the treatment. (B) Serial changes of retinal microvessel diameters before and after treatment in the second, third, and fourth courses. The diameters (mean±standard error) decreased from baseline temporarily after the infusions, and then recovered by the initiation of the next treatment.*

decreased temporarily from baseline by 12.3±5.4% (in a total of 26 records), while that of the five nonresponders decreased by 8.2±5.6% (in a total 28 records, p=0.008, Wilcoxon rank sum test, Figure 2). In contrast, the decrease of retinal microvessel diameters of four patients who obtained moderate histopathological regression (Grade 2, necrosis more than two-third of the lesion, 11) did not differ from that of the other nonresponders (9.1±1.7%, total 22 records *vs*. 10.4±2.9%, in a total of 32 records). Among the 10 patients, three experienced bevacizumab-associated grade 2 hypertension (increased blood pressure) according to CTCAE version 4.0 during treatment. No significant difference in the change of

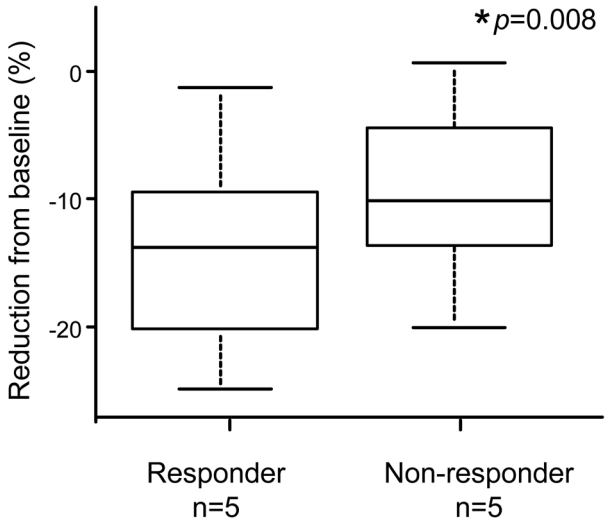

Figure 2. *Decrease of retinal microvessel diameters and tumor reduction (n=10). A decrease of retinal microvessel diameters was higher in responders than in nonresponders (p=0.008, Wilcoxon rank sum test). Box plots indicate the upper and lower quartiles and mean, with the whiskers showing 95% confidence intervals, and outliers.*

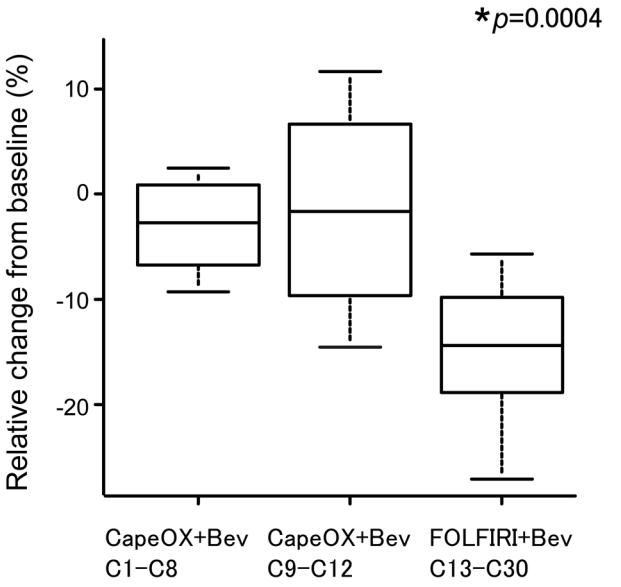

Figure 3. *Relative retinal microvessel diameters of a representative case. In a 68-year-old female patient, the decrease of retinal microvessel diameters was stable while the treatment was effective from the first to the eighth course (C1-C8). The change of the diameters was inconsistent while the patient continued the same treatment despite disease progression from the ninth to the 12th course (C9-C12). After switching to another chemotherapy (C13-C30), the decrease was more significant and consistent (p=0.0004, Kruskal-Wallis test). The box plots indicate the upper and lower quartiles and mean, with the whiskers showing 95% confidence intervals. CapeOX+Bev, Oxaliplatin, capecitabine, and bevacizumab; FOLFIRI+ Bev, fluorouracil, folinic acid, irinotecan, and bevacizumab.*

the retinal microvessel diameters was observed among the three patients (10.6±1.0%, in a total of 16 records) and the other seven patients (9.63±2.9%, total 38 records).

In addition, the findings from another 68-year-old female patient who continued bevacizumab-based chemotherapy for more than one year revealed an association between the decrease of retinal microvessel diameters and the efficacy of chemotherapy (Figure 3). The decrease of retinal microvessel diameters was relatively consistent (2.7±4.0%, mean±standard error, in a total of eight records) while the chemotherapy was effective. On the other hand, the decrease was inconsistent (1.6±9.6%, total four records) while the patient continued receiving the same chemotherapeutic scheme despite clinical disease progression. After switching to another chemotherapy, the decrease on microvessel diameters was superior and consistent (14.4±5.8%, in a total 18 records).

Discussion

With the direct observation of retina in patients who received bevacizumab-based chemotherapy, the retinal microvessel diameters were observed to be decreased from baseline temporarily, shortly after a systemic infusion of bevacizumab. Although the underlying mechanism of this interesting phenomenon remains unclear (9, 10), bevacizumab causes vasoconstriction by decreasing blood flow in the retinal microvessels, thus reducing the diameter of microvessels. As it is believed that angiogenesis inhibitors exert their therapeutic effects by reducing blood flow to the tumor, it is expected that a decrease of retinal microvessel diameter could reflect a response to systemic treatment with bevacizumab-based chemotherapy. In particular, in the present study it was observed that a decrease in microvessel diameters was associated with greater tumor reduction.

Apart from the limited number of patients, this preliminary study contains some further limitations, and the results should thus be interpreted with caution. Initially, the decrease of retinal vessel diameters was not related with histopathological tumor response or toxicity (hypertension) in the present study. If retinal microvessel diameter can accurately reflect response to systemic treatment, it should also be correlated with the histopathological tumor response as well as with toxicity. Moreover, the possibility that this phenomenon is specific to bevacizumab or other angiogenesis inhibitors should not be excluded as oxaliplatin in combination with bevacizumab may also decrease the retinal microvessel diameters. The present study did not examine a sufficient number of patients receiving monotherapy of oxaliplatin or bevacizumab. The ratio of oxaliplatin and bevacizumab contribution to vascular modifications thus remains unclear.

Nevertheless, this study demonstrated that direct observation of retinal microvessels is feasible in patients receiving systemic chemotherapy. As fundus examination is a noninvasive technique in ophthalmology and retinal microvessels are the only vessels in the body that can be directly observed, ophthalmologic assessment of retinal microvessels could be a potential biomarker candidate for the assessment of systemic treatment response upon angiogenesis inhibitors treatment like bevacizumab.

The UK Biobank, which includes 68,550 participants, uses fully-automated image analysis to assess non-mydriatic retinal imaging, blood pressure, and arteriosclerosis index (11). Recently retinal capillary density and caliber have been associated with the severity of diabetic retinopathy derived by optical coherent tomography angiography (OCTA) from a cross-sectional study involving about 600 eyes in the United States (12). Development of easy analytical systems enables assessment of blood pressure or diabetic retinopathy in large numbers in the real world clinics. Such technologies may be utilized to evaluate retinal microvessel diameters in large numbers of cancer patients, which may play an important role in detecting therapeutic effects and side effects of new angiogenesis inhibitors.

Conflicts of Interest

Dr. Ando reports grants and personal fees from Chugai Pharmaceutical Co., Ltd., grants and personal fees from Kyowa Kirin Co., Ltd., grants and personal fees from Nippon Kayaku Co., Ltd., grants and personal fees from Yakult Honsha Co., Ltd., grants and personal fees from Ono Pharmaceutical Co., Ltd., grants and personal fees from Taiho Pharmaceutical Co., Ltd., grants and personal fees from Daiichi-Sankyo Co., Ltd., grants and personal fees from Eisai Co., Ltd., personal fees from Eli Lilly Japan K.K., personal fees from Novartis Pharma K.K., personal fees from Bayer Holding Ltd., personal fees from Bristol-Myers Squibb, personal fees from Sawai Pharmaceutical Co., Ltd, personal fees from Roche Diagnostics K.K., personal fees from MSD K.K, personal fees from Astellas Pharma Inc., personal fees from Otsuka Holdings Co., Ltd., personal fees from Sanwa Kagaku Kenkyusho Co., Ltd., personal fees from Hisamitsu Pharmaceutical Co., Inc., and grants from Mochida Pharmaceutical Co., Ltd.

Dr. Nakayama reports personal fees from Chugai Pharmaceutical Co., Ltd., personal fees from Yakult Honsha Co., Ltd., personal fees from Taiho Pharmaceutical Co., Ltd., personal fees from Eli Lilly Japan K.K., personal fees from Miyarinsan Pharmaceutical Co., Ltd., personal fees from Takeda Pharmaceutical Co., Ltd., and research funding to his institution from Eli Lilly Japan K.K. as outside the submitted work. All remaining Authors have declared no conflicts of interest.

Authors' Contributions

Conceptualization, A.M., Y.I., Y.A.; Methodology, A.M., Y.I., T.S., Y.A.; Software, A.M., Y.I.; Validation, A.M., T.S., Y.I.; Formal Analysis, A.M.; Investigation, A.M., Y.I., T.S., C.I., K.U., G.N., Y.A.; Writing – Original Draft Preparation, A.M.; Writing – Review & Editing, A.M., Y.A.; Visualization, A.M., Y.I., Y.A.; Supervision, H.T., Y.A; Project Administration, A.M., Y.A.; Funding Acquisition, Y.A. All Authors have read and agreed to the published version of the manuscript.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number 23590638. We would like to thank Keiko Yoshida, Yukiko Kato, Kiyoko Miura, and Yuka Murasaki for their support of this study.

References

1 Yamada Y, Denda T, Gamoh M, Iwanaga I, Yuki S, Shimodaira H, Nakamura M, Yamaguchi T, Ohori H, Kobayashi K, Tsuda M, Kobayashi Y, Miyamoto Y, Kotake M, Shimada K, Sato A, Morita S, Takahashi S, Komatsu Y and Ishioka C: S-1 and irinotecan plus bevacizumab versus mFOLFOX6 or CapeOX plus bevacizumab as first-line treatment in patients with metastatic colorectal cancer (TRICOLORE): a randomized, open-label, phase III, noninferiority trial. Ann Oncol *29(3)*: 624-631, 2018. PMID: 29293874. DOI: 10.1093/annonc/mdx816

2 Schneider BP, Shen F and Miller KD: Pharmacogenetic biomarkers for the prediction of response to antiangiogenic treatment. Lancet Oncol *13(10)*: e427-e436, 2012. PMID: 23026828. DOI: 10.1016/S1470-2045(12)70275-9

3 Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA and Giust MJ: Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology *113(3)*: 363-372.e5, 2006. PMID: 16458968. DOI: 10.1016/j.ophtha.2005.11.019

4 Papadopoulou DN, Mendrinos E, Mangioris G, Donati G and Pournaras CJ: Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. Ophthalmology *116(9)*: 1755-1761, 2009. PMID: 19560206. DOI: 10.1016/j.ophtha.2009.03.017

5 Liew G, Kaushik S, Rochtchina E, Tan AG, Mitchell P and Wang JJ: Retinal vessel signs and 10-year incident age-related maculopathy: the Blue Mountains Eye Study. Ophthalmology *113(9)*: 1481-1487, 2006. PMID: 16828507. DOI: 10.1016/j.ophtha.2006.03.051

6 Klein R, Klein BE, Tomany SC and Wong TY: The relation of retinal microvascular characteristics to age-related eye disease: the Beaver Dam eye study. Am J Ophthalmol *137(3)*: 435-444, 2004. PMID: 15013865. DOI: 10.1016/j.ajo.2003.10.020

7 Terai N, Haustein M, Siegel A, Stodtmeister R, Pillunat LE and Sandner D: Diameter of retinal vessels in patients with diabetic macular edema is not altered by intravitreal ranibizumab (lucentis). Retina *34(7)*: 1466-1472, 2014. PMID: 24457978. DOI: 10.1097/IAE.0000000000000095

8 Ikram MK, van Leeuwen R, Vingerling JR, Hofman A and de Jong PT: Retinal vessel diameters and the risk of incident age-related macular disease: the Rotterdam Study. Ophthalmology *112(4)*: 548-552, 2005. PMID: 15808242. DOI: 10.1016/j.ophtha.2004.10.038

9 Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, Elliott R, Scherer SJ and Chen DS: Predictive impact of circulating vascular endothelial growth factor in four phase III

trials evaluating bevacizumab. Clin Cancer Res *19(4)*: 929-937, 2013. PMID: 23169435. DOI: 10.1158/1078-0432.CCR-12-2535

10 Lambrechts D, Lenz HJ, de Haas S, Carmeliet P and Scherer SJ: Markers of response for the antiangiogenic agent bevacizumab. J Clin Oncol *31(9)*: 1219-1230, 2013. PMID: 23401453. DOI: 10.1200/JCO.2012.46.2762

11 Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, Strachan DP and Rudnicka AR: Associations of retinal microvascular diameters and tortuosity with blood pressure and arterial stiffness: United Kingdom Biobank. Hypertension *74(6)*: 1383-1390, 2019. PMID: 31661987. DOI: 10.1161/HYPERTENSIONAHA.119.13752

12 Kushner-Lenhoff S, Kogachi K, Mert M, Chu Z, Shahidzadeh A, Palejwala NV, Wolfe J, Itty S, Drenser KA, Capone A Jr, Dugel PU, Moshfeghi AA, Ameri H, Daskivich LP, Wang RK and Kashani AH: Capillary density and caliber as assessed by optical coherence tomography angiography may be significant predictors of diabetic retinopathy severity. PLoS One *17(1)*: e0262996, 2022. PMID: 35081154. DOI: 10.1371/journal.pone.0262996

13 Japanese Society for Cancer of the Colon and Rectum: Japanese classification of colorectal carcinoma, 2nd English edition. Kanehara, Tokyo, Japan, 2009.

Received January 31, 2022
Revised February 25, 2022
Accepted March 21, 2022