

Smooth Border Between Inner Nuclear/Outer Plexiform Layers: Branch Retinal Vein Occlusion Biomarker of Fewer Macular Edema Recurrences

Hirofumi Sasajima (✉ hiro.sasa1228@icloud.com)

Aichi Medical University

Kotaro Tsuboi

Oregon Health & Science University

Rokuki Kiyosawa

Aichi Medical University

Akira Fukutomi

Aichi Medical University

Kenta Murotani

Kurume University

Motohiro Kamei

Aichi Medical University

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Abstract

Purpose: To determine if the status of the border between the inner nuclear layer (INL) and outer plexiform layer (OPL) is associated with the number of macular edema (ME) recurrences secondary to branch retinal vein occlusion (BRVO).

Methods: Thirty-seven consecutive eyes with BRVO treated with anti-vascular endothelial growth factor (VEGF) drugs were included. In optical coherence tomography (OCT) images at the initial visit, the border between the INL and OPL within the 1.5-mm vertical line from the fovea was manually traced using ImageJ software. The jagged ratio (JR), i.e., the border length divided by the spline curve length of the border was calculated using the “Spline fit” mode in the software.

Results: The mean JR was 1.1 ± 0.07 (range, 1.01-1.27). The JR was correlated significantly with the number of anti-VEGF injections/year ($P < 0.0001$, $r = 0.721$). Among 37 eyes, nine (24.3%) received two or fewer injections/year, and their JRs were less than 1.05. The multivariate linear regression analysis showed that only the JR was correlated significantly with the number of anti-VEGF injections/year ($P < 0.0001$).

Conclusions: A smooth border between the INL and the OPL on OCT images at the initial visit may be a biomarker for fewer ME recurrences in eyes with BRVO.

Introduction

Macular edema (ME) is the leading cause of visual loss related to branch retinal vein occlusion (BRVO).¹⁻
³ In the treatment of ME secondary to BRVO, the efficacy of anti-vascular endothelial growth factor (VEGF) agents has been reported in large randomized trials with anti-VEGF agents as first-line treatment.⁴⁻
⁷ However, considering the anti-VEGF injection regimen, the regimens of the large clinical trials, such as BRAVO and VIBRANT, included 6 monthly injections regardless of ME in the first 6 months.^{4,7} It still remains unclear if multiple anti-VEGF injections are necessary to initially treat ME secondary to BRVO. Generally, about 20% to 30% of patients with ME in BRVO require anti-VEGF injections a maximum of one to two times annually. The ZIPANGU trial reported that 24.1% of eyes treated with ranibizumab (Lucentis, Genentech Inc., South San Francisco, CA) monotherapy required two or fewer injections annually under the one followed by pro re nata (PRN) regimen.⁸ If eyes with possibly fewer recurrences of ME are identified, a loading dose of multiple anti-VEGF injections might be unnecessary to them. To date, however, the factors predictive factors of fewer recurrences of ME remain unclear.

Researchers recently reported the potential predictive value of imaging biomarkers on high-resolution optical coherence tomography (OCT), which enables quantitative assessments, including intraretinal cystoid spaces,^{9,10} intraretinal hyperreflective foci,^{9,10} a highly reflective line,¹¹ subretinal fluid,^{9,12} disorganization of the retinal inner layers,^{9,13,14} external limiting membrane disruption,^{9,15,16} ellipsoid zone disruption,^{9,11,15} and foveal bulge.¹⁶ The locations of abnormal fluid accumulation in the retina also

have been investigated in eyes with ME in BRVO; Shroff et al. reported three distinct anatomic patterns of structural changes on OCT, i.e., a serous retinal detachment in only 15%, cystoid ME in 40%, and a combination of the two in 45%¹⁷; Yamaike et al. reported that cystoid spaces were often seen in the inner nuclear layer (INL) (76%) and outer plexiform layer (OPL) (84%) on OCT images in eyes with BRVO¹⁸; Yiu et al. reported intraretinal fluid in the INL, OPL, and outer nuclear layer in 78.1%, in the OPL and outer nuclear layer (19.3%), and in the INL (2.6%).¹⁹

When reviewing OCT images of eyes with BRVO and ME in our clinic, we observed that all eyes had cystoid spaces in the OPL, some had them only in the OPL, and others had them in the INL and OPL; but no eyes had them only in the INL. Interestingly, we noticed that the eyes with a smooth border between the INL and the OPL seemed to have fewer recurrences of ME, while the eyes with a jagged border received more injections. We, therefore, conducted this study to identify a correlation between the shape of the border between the INL and OPL at the initial visit and the number of recurrences of ME secondary to BRVO. We also evaluated the correlation between the clinical characteristics including the jagged ratio (JR) at the initial visit and the visual acuity (VA) at the 12-month visit.

Methods

Patients and Examinations

This retrospective observational study included consecutive 65 eyes of 65 patients who visited the Department of Ophthalmology, Aichi Medical University Hospital, from May 25, 2016, to October 1, 2018, and had visual impairment associated with treatment-naïve BRVO. The institutional review board of Aichi Medical University approved the study protocol (reference number: 2019-082), which adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants included in the study. We reviewed the medical and ocular histories of these patients in detail.

The inclusion criteria included patients with ME secondary to BRVO within 6 months after onset and who were treated with anti-VEGF drugs (either 0.5 mg/0.05 mL ranibizumab or 2.0 mg/ 0.05 mL aflibercept (Eylea, Regeneron Pharmaceuticals, Inc., Tarrytown, NY) in one + PRN regimen for at least 12 months. According to our institutional protocol for treatment of ME secondary to BRVO, anti-VEGF drugs were strictly administered when the CST exceeded 300 microns. The exclusion criteria included the presence of hemicentral RVO, epiretinal membranes, macular holes, diabetic retinopathy, age-related macular degeneration, and poor OCT images because of significant cataract or retinal hemorrhage. Patients also were excluded who had an active ocular infection or intraocular inflammation; had undergone surgeries during the current study such as phacoemulsification, vitrectomy, and laser photocoagulation; or were treated with a sub-Tenon's capsule injection of triamcinolone acetonide.

During the study period, all patients underwent ophthalmic examinations including measurement of the BCVA and evaluations by indirect ophthalmoscopy, fundus photography (TRC-50LX, Topcon, Tokyo, Japan), and OCT (Cirrus-HD 5000, Carl Zeiss Meditec, Dublin, CA). All patients except one underwent

fluorescein angiography (FA) examination using ultra-widefield fundus camera (California, Optos PLC, Dunfermline, UK). The nonperfusion area (NPA) was measured on FA images obtained 30 to 40 seconds after dye injection, and eyes with a NPA smaller than five disc diameters were considered to be perfused.¹

Assessment of the Border JR

To evaluate the jaggedness of the border between the INL and the OPL, we obtained an OCT B-scan image vertically across the fovea at the initial visit and measured the border length on the affected side 1.5 mm from the fovea using ImageJ software version 1.53a (National Institutes of Health, Bethesda, MD, available at <http://imagej.nih.gov/ij>)²⁸ (Fig 4). First, we dragged and dropped an OCT image onto the application icon on the computer. Second, using “Set Scale” mode of the application, we measured the distance between the ends of the images and determined 1.5 mm as the actual distance in pixels. (Fig 4A). Third, using “Segmented Line” mode of the application, we manually traced the border on the images and measured the border length (Fig 4B). To evaluate the extent of the jaggedness of the border, we drew a smooth line as a reference by drawing a spline curve through the middle points of each side on the polygonal line using “Spline Fit” mode of the application (Fig 4C) and measured the length of the spline curve. Finally, we defined the JR as the extent of the jaggedness of the border using the formula: $JR = \text{length of the border} / \text{length of the spline curve}$. Considering the numbers of anti-VEGF injections as the number of ME recurrences, we evaluated the correlation between the JR at the initial visit and the number of anti-VEGF injections during 1 year.

Factors Affecting ME Recurrences

To evaluate other factors affecting the number of anti-VEGF injections/year, we examined other OCT parameters, including the areas of the INL (Fig 5A), the number of cystoid spaces in the INL (Fig 5A, asterisks), and the areas of the outer retina (Fig 5B) at the initial visit. The area between the inner and outer borders of the INL was outlined manually using the “Polygon Selection” mode in the ImageJ software on the affected side of a vertical OCT scan (Fig 5A). The number of cystoid spaces seen in the INL also was counted within 1.5 mm from the fovea on the affected side in the OCT vertical scan (Fig 5A). The cystoid spaces also were seen in the OPL, but they were too large and vague at the borders to be counted. The area of the outer retina was outlined manually between the inner aspect of the OPL and inner border of the retinal pigment epithelium in the same manner (Fig 5B). These areas also were measured within the 1.5-mm vertical line from the fovea using ImageJ software. Two observers (H.S. and R.K.) independently evaluated four OCT parameters: JR, area of the INL, area of the outer retina, and number of cystoid spaces in the INL.

Statistical Analysis

A biostatistician (K.M.) performed the statistical analyses using SAS version 9.4 software (SAS Institute, Inc., Cary, NC). All values are expressed as the mean \pm standard deviation. The BCVA was measured using a Landolt chart, and the decimal VA was converted to the logarithm of the minimum angle of resolution units for the analyses. The ICCs were calculated to assess intergrader agreement on the JR, number of

INL cystoid spaces, area of the INL, and area of the outer retina in the OCT images. Considering the need for anti-VEGF injections with recurrent ME, we evaluated the correlation between the JR in the OCT images at the initial visit and the number of injections and factors affecting the ME recurrences. The Pearson correlation coefficient was used to investigate correlations between the parameters at the initial visit and the number of anti-VEGF injections/year and between the OCT parameters at the initial visit and the JR. Multiple regression analysis was performed to identify explanatory variables that contributed significantly to the number of anti-VEGF injections. Variables included in the multivariate models had a P value of 0.1 or less in univariate analysis. $P < 0.05$ was considered significant.

Results

Patient Characteristics

Sixty-five eyes of 65 patients with ME secondary to treatment-naïve BRVO were treated at Aichi Medical University Hospital during the current study. Twenty-eight eyes did not meet the study protocol: over 6 months from BRVO onset (3 eyes), hemicentral RVO (1 eye), epiretinal membrane (3 eyes), low OCT image quality due to dense hemorrhages (3 eyes) or progressed cataract at the initial visit (3 eyes), additional treatments during the study period such as phacoemulsification (3 eyes), laser photocoagulation (3 eyes), treatment with sub-Tenon triamcinolone acetonide injection (4 eyes), and follow-up periods less than 12 months (5 eyes). Thus, 37 eyes of 37 patients (mean age, 71.5 ± 8.1 years; range, 55-88) met the study criteria for analysis. The mean number of anti-VEGF injections/year was 4.1 ± 2.1 (range, 1-9). All 37 (100%) eyes had cystoid spaces in the OPL; 30 (81.1%) eyes had cystoid spaces in both the INL and OPL, and seven (18.9%) eyes only in the OPL. None had cystoid spaces only in the INL. Table 1 shows the clinical characteristics of the 37 patients at the initial visit. The intraclass correlation coefficients (ICCs) of the two observers were high for all measurements of interest (ICC, 0.95, 0.92, 0.92, and 0.97 for the JR, number of INL cystoid spaces, area of the INL, and area of the outer retina, respectively).

Baseline Factors Affecting the Number of Anti-VEGF Injections

We performed univariate and multivariate linear regression analyses using the baseline data to identify the factors directly affecting the number of anti-VEGF injections/year (Table 2). Correlated significantly with the number of anti-VEGF injections/year in the univariate analysis were only three of the parameters at the initial visit: the JR, the number of cystoid spaces in the INL, and the area of the INL (Pearson correlation coefficient, $r = 0.721$, $P < 0.0001$; $r = 0.348$, $P = 0.035$; and $r = 0.388$, $P = 0.018$, respectively). The correlations between the parameters at the initial visit and the number of anti-VEGF injections/year are shown in Fig 1. Among 37 eyes, nine (24.3%) eyes received two or fewer injections/year and their JRs were less than 1.05. Age, duration of symptoms until the initial treatment, VA, central subfield thickness (CST), and area of the outer retina were not correlated significantly with the number of anti-VEGF injections/year ($r = 0.0274$, $P = 0.87$; $r = 0.285$, $P = 0.087$; $r = -0.10$, $P = 0.55$; $r = 0.208$, $P = 0.22$; and $r = 0.207$, $P = 0.22$, respectively). The results of multivariate linear regression analysis showed that only the

JR was correlated significantly with the number of anti-VEGF injections/year ($P < 0.0001$; coefficient, 22.3) (Table 2).

Correlation between OCT Parameters at the Initial Visit and JR

OCT parameters including the number of cystoid spaces in the INL, area of the INL, CST, and area of the outer retina were evaluated to determine if they were relevant to the JR. The number of cystoid spaces in the INL and area of the INL were correlated significantly with the JR (Pearson correlation coefficient, $r = 0.55$, $P = 0.0005$, and $r = 0.39$, $P = 0.016$, respectively). The CST and area of the outer retina were not correlated significantly with the JR ($r = 0.031$, $P = 0.86$ and $r = 0.24$, $P = 0.16$, respectively).

Comparisons of the Locations of Intraretinal Cystoid Spaces Regarding the JR

The mean JR in eyes with cystoid spaces in both the INL and OPL was 1.11 ± 0.01 and that in only the OPL was 1.09 ± 0.03 . The JRs did not differ significantly ($P = 0.39$) between the locations of the cystoid spaces.

Comparisons between Clinical Characteristics at the Initial Visit and BCVA at the 12-Month Visit

The clinical characteristics including age, duration of symptoms until the initial treatment, best-corrected VA (BCVA), CST, and area in the outer retina at the initial visit were correlated significantly with the VA at 12 months in the univariate linear regression analysis ($P = 0.0027$, $P = 0.049$, $P = 0.0001$, $P = 0.0088$, and $P = 0.0094$, respectively); other clinical characteristics including the JR were not correlated with the VA at 12 months (Table 3). Multiple linear regression analysis showed that age, duration of symptoms until the initial treatment, and initial BCVA were correlated significantly with the VA at 12 months ($P = 0.0045$, $P = 0.02$, and $P = 0.0075$, respectively).

Case Reports

Case 1 was that of a 77-year-old woman who presented with visual loss due to ME secondary to BRVO. At the initial visit, ME was seen on the OCT images, which showed large cystoid spaces in the OPL and a small amount of subretinal fluid (Fig 2A). The border between the INL and OPL was relatively smooth (Fig 2B). The JR on the OCT image at the initial visit was 1.01. After one anti-VEGF injection, the ME did not recur during the 12-month study period.

Case 2 was that of a 62-year-old woman who presented with visual loss due to ME secondary to BRVO. At the initial visit, ME was seen on the OCT image, which showed small cystoid spaces in the INL and large cystoid spaces in the OPL (Fig 3A). The border between the INL and OPL was not smooth (Fig 3B). The JR on the OCT image at the initial visit was 1.10. The patient was treated with five anti-VEGF injections during the 12-month study period.

Discussion

We found that eyes with a smooth border between the INL and OPL on OCT images at the initial visit tended to receive fewer anti-VEGF injections and that only the JR was a predictor of the number of anti-VEGF injections based on multiple regression analysis. Previous studies²⁰⁻²³ have evaluated the potential biomarkers predictive of the visual prognosis of BRVO, but few studies have reported factors predictive of ME recurrences at the initial visit.²⁴ In the current study, we clarified that eyes that received two or fewer injections/year had lower JR values. Thus, the JR may be a novel biomarker that predicts fewer ME recurrences during anti-VEGF treatments for BRVO. Finding biomarkers that predict fewer ME recurrences is more important than finding factors predicting repeated injections or worse visual outcomes, because we can reduce the loading dose in the first few months for eyes with good signs while we have no choice but to repeat injections for eyes with bad signs.

We also found that baseline age, baseline BCVA, and duration of symptoms until the initial treatment were correlated significantly with the BCVA at 12 months by multivariate linear regression analysis, which is consistent with the results of previous studies²⁰⁻²³ regarding predictive factors for visual prognosis in BRVO. Meanwhile, the JR was not correlated significantly with the 12-month BCVA values, although higher JRs were associated with more anti-VEGF injections. A factor may not be correlated with BCVA but may be associated with the numbers of anti-VEGF injections. For example, in the RETAIN study, the final VA did not differ significantly between eyes with resolved ME and unresolved ME (74.9 vs. 76.5 letters, $P=0.9$), although eyes with resolved ME had significantly fewer ranibizumab injections over 4 years than eyes with unresolved ME (9.9 vs. 18.9, $P=0.002$).⁶ Hasegawa et al. also reported that the final VA did not differ significantly between eyes with resolved ME and eyes with recurrent ME (82 vs. 80 letters, $P=0.16$), but the number of injections differed significantly between those groups (2.1 vs. 5.1, $P<0.0001$).²⁵ Those and the current results may imply that the photoreceptors were damaged less because of proper management of the ME by repeating injections of anti-VEGF drugs. Further studies will determine whether the JR is associated with the VA prognosis.

We speculated about the formation of the jagged border. We evaluated the correlations between the OCT parameters at the initial visit and the JR and found that the number of cystoid spaces in the INL and the area of the INL were correlated significantly with the JR ($P=0.0005$ and $P=0.016$, respectively). This means that the jagged border becomes apparent with more cystoid spaces in the INL and larger area of the INL.

However, the locations of the cystoid spaces were not associated with the JR or the mean number of anti-VEGF injections/year. A reason for this discrepancy is that some eyes with cystoid spaces only in the OPL had jagged borders. A review of the data from those eyes with jagged borders without a cystoid space in the INL at the initial visit showed that five of the seven eyes had cystoid spaces in both the INL and OPL at the time of the ME recurrences (Table 4). Repeated anti-VEGF injections were administered (mean, 5.2; range, 3-8) in the eyes with cystoid spaces in the INL at the time of recurrence. In summary, a jagged border was observed not only in eyes with more cystoid spaces in the INL and a larger area of the INL but also in some eyes with no cystoid spaces in the INL at the initial visit but cysts that developed later.

Why did eyes with a smooth border require fewer anti-VEGF injections/year? As mentioned previously, a smooth border was seen in eyes with fewer cystoid spaces in the INL at the initial visit and time of recurrence. Retinal capillaries run in the inner retinal layers; the middle capillary plexus lies close to the top of the INL and the deep one the bottom.²⁶ Intraretinal cystoid spaces in the acute phase of BRVO indicate leakage from damaged capillaries. We speculated that fewer cystoid spaces in the INL might indicate less damage in these capillaries around the fovea or that fluid absorption exceeds leakage from the capillaries. However, in eyes with ME with a smooth border, the cystoid spaces were primarily in the OPL. In the acute phase in BRVO, extensive leakage from the relatively thick retinal vein at the vascular arcade travels preferentially through the OPL and causes ME mainly within Henle's layer. A previous study reported that even in cases with extramacular BRVO, leakage from the capillaries travelled through the OPL and caused ME.²⁷ This implied that ME in the OPL does not necessarily indicate damage to the capillaries around the fovea. A smooth border on the OCT images, therefore, might suggest that the fluid in the OPL originated from a lesion apart from the fovea and the damage in the deep capillary plexus around the fovea was less. However, the exact pathogenesis of the fewer ME recurrences in eyes with a lower JR remains unclear. Further studies are needed to clarify that the eyes with a smooth border had fewer ME recurrences in BRVO.

The current study had several limitations. First, this was a retrospective study with its inherent sampling bias and relatively small sample size. Second, we manually traced the border between the INL and OPL and traced the spline curve through the middle points of the border on the OCT images using ImageJ software. However, this study is highly reproducible and the first to show that the jagged border on the OCT image could be a useful biomarker of ME recurrences in eyes with BRVO.

In conclusion, lower JRs, which indicate a smooth border, on the OCT images at the initial visit may predict fewer ME recurrences secondary to BRVO. This new finding also may shed some light on the pathogenesis of BRVO. Considering that the jagged border, not the exact values of the JR, on the OCT images could be identified easily even by non-retinal specialists and, thus, the jagged border could be a useful biomarker in routine clinical practice. We also suggest that a loading dose of anti-VEGF drugs may not be needed in cases with a smooth border.

Declarations

Author Contributions

Conception and Design of the study (H.S., M.K.); conduct of the study (H.S., M.K.); data collection (H.S., R.K., A.F.); management, analysis, and interpretation of the data (H.S., K.T., K.M., M.K.); preparation of the manuscript (H.S.); review of the manuscript (M.K.); approval of the manuscript (M.K.). All authors accepted the final version of the manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

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Tables

Table 1. Patient characteristics and optical coherence tomography findings at the initial visit. Fluorescein angiography was not performed in 1 eye. The data are expressed as the mean \pm standard deviation. INL = inner nuclear layer; logMAR = logarithm of the minimum angle of resolution; No. = number; OPL = outer plexiform layer.

Baseline characteristic	
No. eyes	37
Age (years)	71.5 ± 8.1
Gender (male/female)	11/26
Eye (right/left)	17/20
Hypertension, No. (%)	26 (70.3)
Diabetes mellitus, No. (%)	2 (5.4)
Duration of symptoms before initial treatment (weeks)	7.8 ± 7.0
LogMAR visual acuity	0.53 ± 0.4
Central subfield thickness (µm)	508.4 ± 154.8
Location of intraretinal cystoid space (INL/OPL/both), No.	0/7/30
Subretinal fluid, No. (%)	14 (37.8)
Subtype (macular/major), No.	10/27
Perfusion status (perfused/ischemic), No.	15/21
Jagged ratio	1.1 ± 0.07
No. INL cystoid spaces	3.6 ± 2.3
Area of INL (mm ²)	0.29 ± 0.09
Area of outer retina (mm ²)	1.20 ± 0.37

Table 2. Baseline factors affecting the number of anti-vascular endothelial growth factor injections/year. INL = inner nuclear layer; logMAR = logarithm of the minimum angle of resolution; CI = confidence interval; No. = number.

Linear regression	Univariate		Multivariate	
	Coefficient (95% CI)	<i>P</i> Value	Coefficient (95% CI)	<i>P</i> Value
Parameters at initial visit				
Age	0.007 (-0.08-0.09)	0.87		
Sex, female	-0.014 (-1.54-1.5)	0.99		
Eye, left	-0.068 (-1.47-1.3)	0.92		
Hypertension	-1.18 (-2.65-0.29)	0.11		
Diabetes mellitus	1.5 (-1.54-4.54)	0.32		
Duration of symptoms before initial treatment	0.084 (-0.013-0.18)	0.087	-0.022 (-0.11-0.06)	0.61
LogMAR visual acuity	-0.53 (-2.28-1.23)	0.55		
Central subfield thickness	0.0028 (-0.0017-0.0072)	0.22		
Subretinal fluid	0.44 (-0.99-1.87)	0.53		
Subtype (major)	1.48 (-0.004-2.97)	0.051	-0.17 (-1.9-1.6)	0.85
Perfused	-1.2 (-2.55-0.15)	0.079	-0.46 (-1.9-1.0)	0.52
Jagged ratio	22.1 (14.8-29.4)	<0.0001	22.3 (12-32.3)	<0.0001
No. INL cystoid spaces	0.25 (0.018-0.48)	0.035	-0.16 (-0.5-0.2)	0.31
Area of the INL	8.81 (1.63-16.0)	0.018	6.1 (-2.9-15.1)	0.17
Area of outer retina	1.17 (-0.73-3.1)	0.22		

Table 3. Baseline factors affecting best-corrected visual acuity at the 12-Month visit. INL = inner nuclear layer; logMAR = logarithm of the minimum angle of resolution; CI = confidence interval; No. = number.

Linear regression	Univariate		Multivariate	
	Coefficient (95% CI)	<i>P</i> Value	Coefficient (95% CI)	<i>P</i> Value
Parameters at initial visit				
Age	0.016 (0.006-0.026)	0.0027	0.014 (0.0047-0.02)	0.0045
Sex, female	-0.013 (-0.21-0.19)	0.9		
Eye, left	-0.094 (-0.27-0.084)	0.29		
Hypertension	-0.10 (-0.30-0.093)	0.29		
Diabetes mellitus	-0.19 (-0.58-0.21)	0.34		
Duration of symptoms before initial treatment	0.011 (-0.0018-0.023)	0.049	0.012 (0.0019-0.021)	0.02
LogMAR visual acuity	0.39 (0.20-0.57)	0.0001	0.30 (0.086-0.51)	0.0075
Central subfield thickness	0.00073 (0.0002-0.0013)	0.0088	-0.00026 (-0.00097-0.00045)	0.46
Subretinal fluid	0.14 (-0.037-0.32)	0.11		
Subtype (major)	0.12 (-0.082-0.32)	0.24		
Perfused	-0.16 (-0.34-0.013)	0.068	-0.11 (-0.3-0.075)	0.23
Jagged ratio	1.1 (-0.26-2.4)	0.11		
No. INL cystoid spaces	0.012 (-0.02-0.044)	0.45		
Area of the INL	0.031 (-0.98-1.0)	0.95		
Area of outer retina	0.31 (0.080-0.54)	0.0094	0.078 (-0.22-0.37)	0.59

Table 4. Characteristics of seven cases with cystoid spaces only in the outer plexiform layer at the initial visit. INL = inner nuclear layer; VEGF = vascular endothelial growth factor; No. = number.

Case	Jagged ratio	Duration before initial recurrence (weeks)	No. cystoid spaces in INL at initial recurrence	No. anti-VEGF injections/year
1	1.037704	16	0	2
2	1.013793	8	0	2
3	1.051238	12	4	3
4	1.159429	8	7	6
5	1.10028	4	6	8
6	1.138357	16	5	5
7	1.114708	8	6	4

Figures

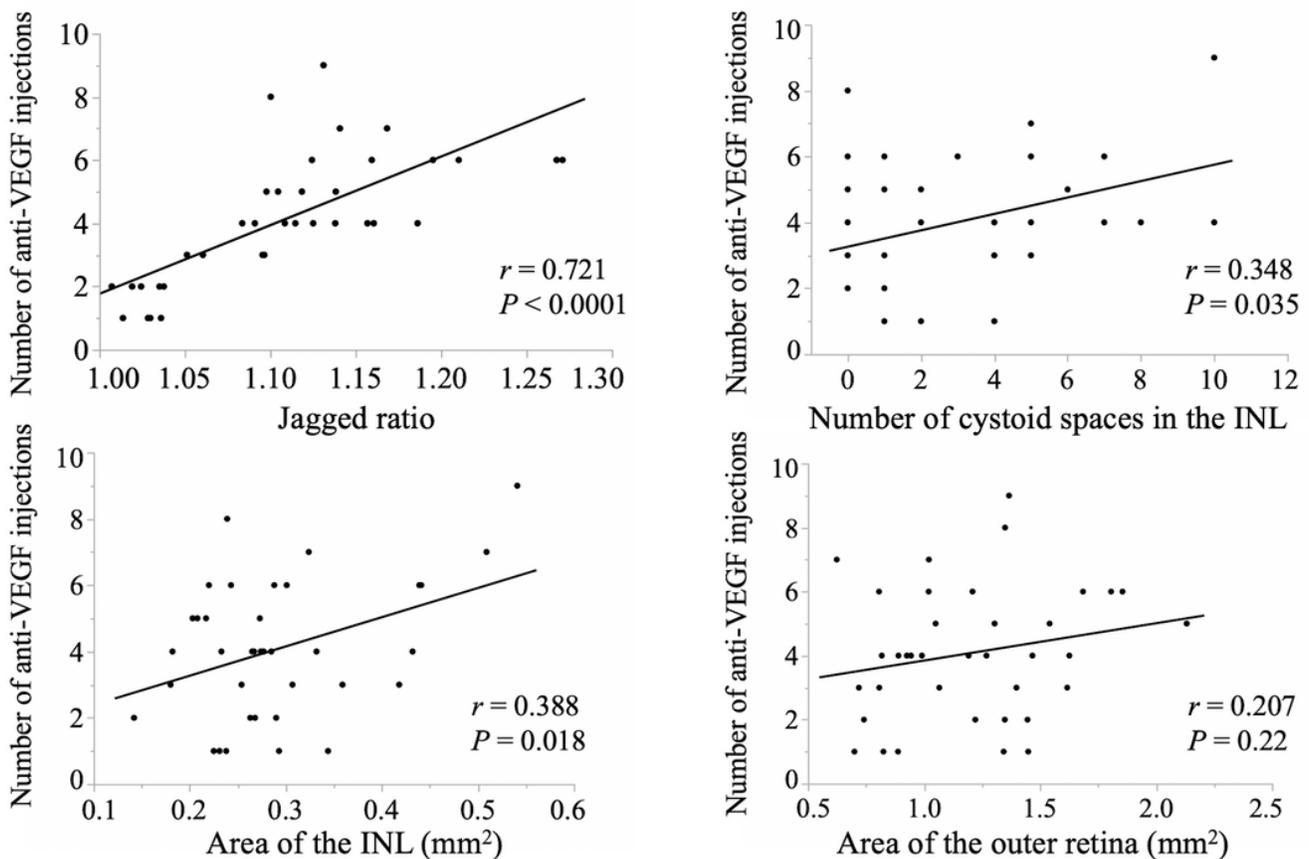


Figure 1

The scatterplots show the correlations between the parameters at the initial visit and the number of anti-vascular endothelial growth factor (VEGF) injections/year. The jagged ratio, number of cystoid spaces in the inner nuclear layer (INL), and area of the INL were correlated significantly with the number of anti-

VEGF injections/year (Pearson correlation coefficient, $r = 0.721$, $P < 0.0001$; $r = 0.348$, $P = 0.035$; and $r = 0.388$, $P = 0.018$, respectively).

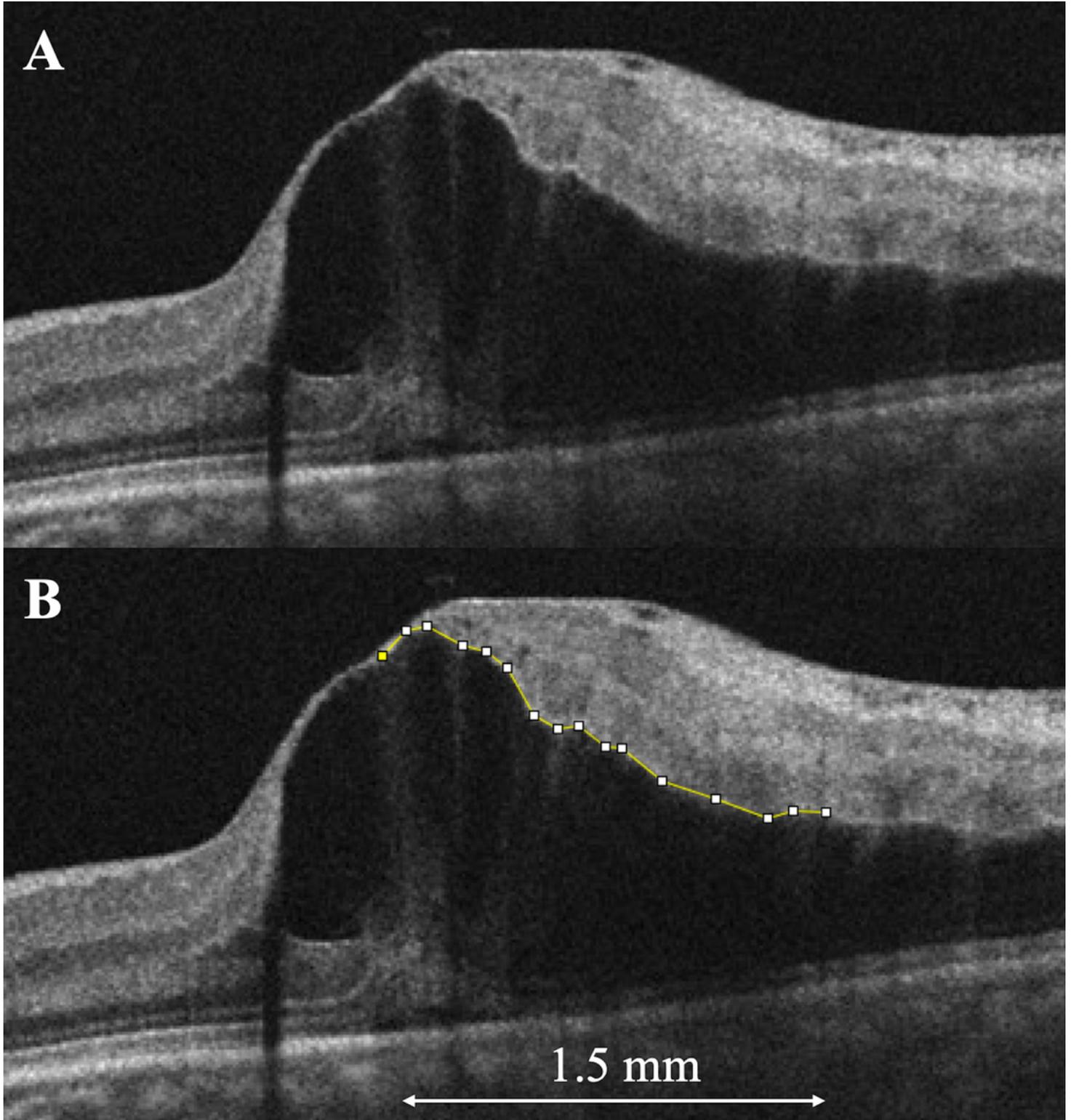


Figure 2

A spectral-domain optical coherence tomography (OCT) image of an eye with branch retinal vein occlusion of a 77-year-old woman who required only one anti-vascular endothelial growth factor injection/year. A, An OCT image that ran vertically through the fovea at the initial visit shows macular

edema, with large cystoid spaces in the outer plexiform layer (OPL). B, The border between the inner nuclear layer and OPL is relatively smooth (segmented line). In this case, the jagged ratio is 1.01.

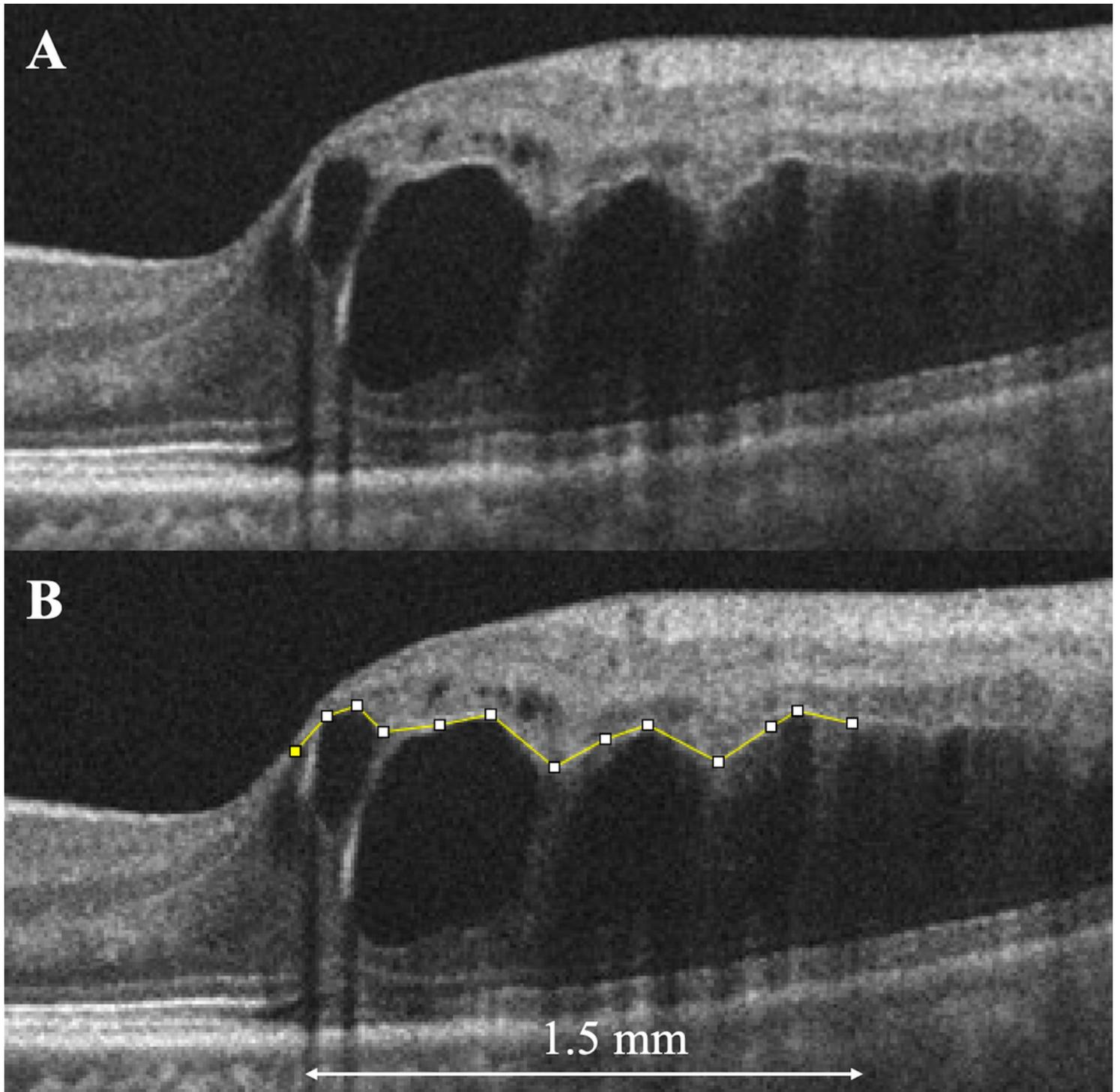


Figure 3

An optical coherence tomography (OCT) image of an eye with branch retinal vein occlusion of a 62-year-old woman who required five anti-vascular endothelial growth factor injections/year. A, An OCT image that ran vertically through the fovea at the initial visit shows macular edema, with small cystoid spaces in the inner nuclear layer (INL) and large cystoid spaces in the outer plexiform layer (OPL). B, The border between the INL and OPL is not smooth (segmented line). In this case, the jagged ratio is 1.10.

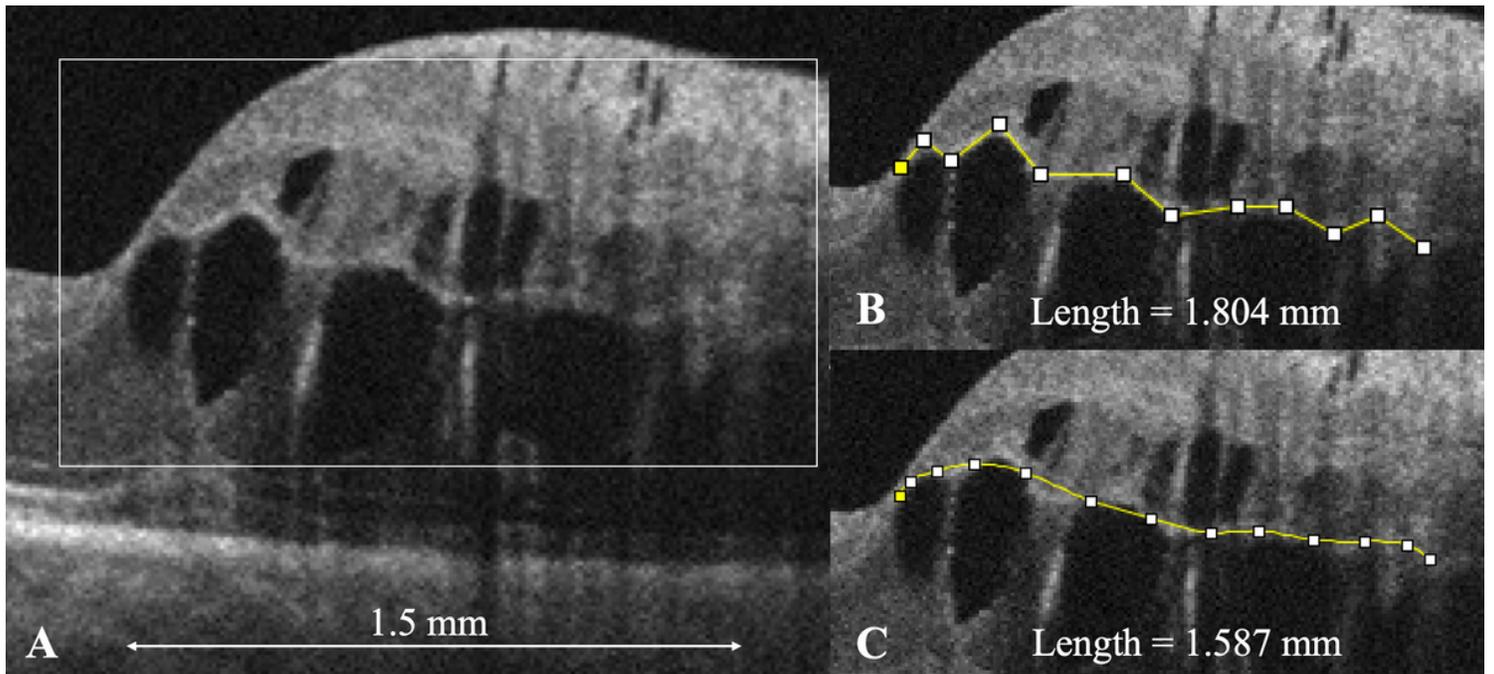


Figure 4

The jaggedness of the border between the inner nuclear layer (INL) and the outer plexiform layer (OPL) in eyes with macular edema (ME) secondary to branch retinal vein occlusion. A, At the initial visit, an optical coherence tomography (OCT) vertical scan through the fovea shows ME. B, C, Magnified images of the box in A. B, We manually traced the border (segmented line) between the INL and OPL on the images within 1.5 mm from the fovea using ImageJ software. The length of the border in this case was 1.804 mm. C, We also manually drew a spline curve through the middle points of the border using the “Spline Fit” mode in the software and measured its length (1.587 mm). The jagged ratio (JR) then was calculated as the length of the border/length of the spline curve; the JR is 1.14 in this case.

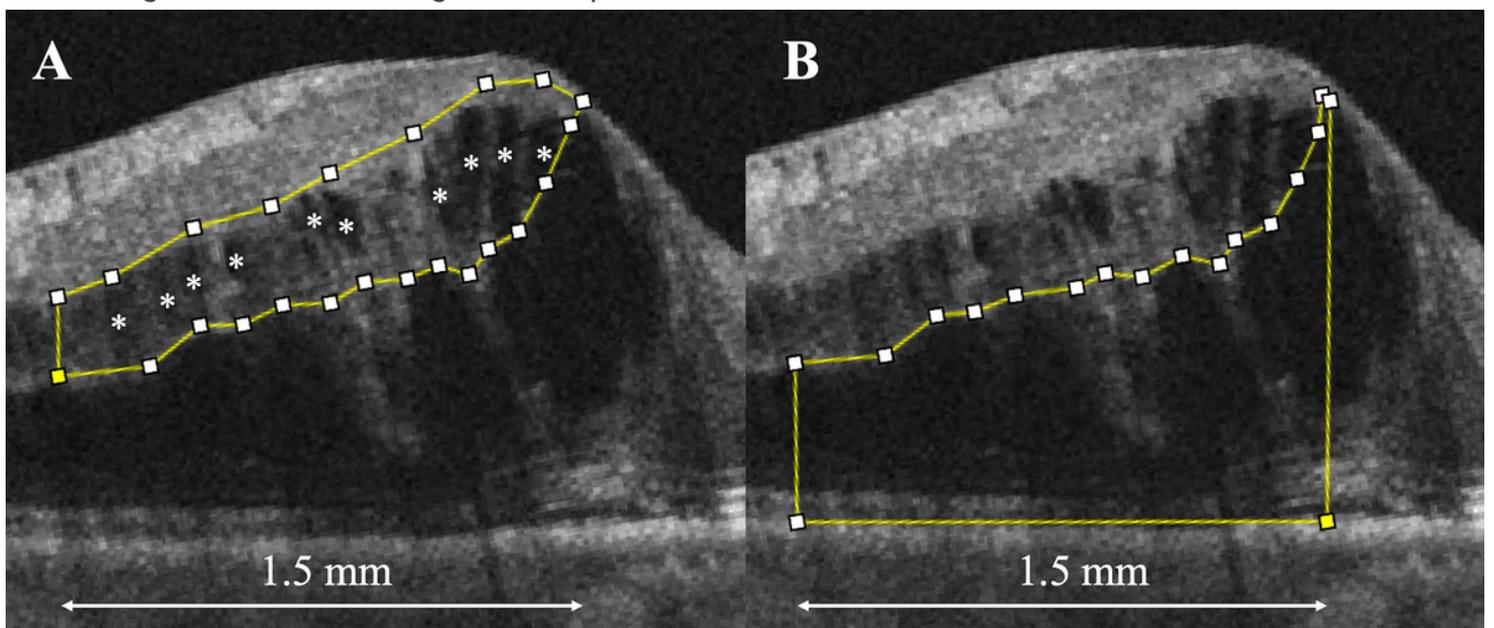


Figure 5

Assessment of areas in the inner nuclear layer (INL), the number of INL cystoid spaces, and areas in the outer retina associated with branch retinal vein occlusion. A, The area of the INL (between the inner and outer borders of the INL) was manually outlined within the affected 1.5-mm vertical optical coherence tomography (OCT) line from the fovea. The area in the INL is 0.494 mm² in this case. The number of INL cystoid spaces (asterisks) also was counted on this image. B, The area in the outer retina (between the inner aspect of the outer plexiform layer and the inner border of the retinal pigment epithelium) was manually outlined in the same way and is 1.007 mm² in this case. These OCT parameters were measured within the affected 1.5-mm vertical line from the fovea (double-headed arrow).