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Outer Retinal Tubulation in the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT)

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Purpose: To determine the prevalence of, risk factors for, and visual acuity (VA) correlations with outer retinal tubulation (ORT) seen on spectral-domain optical coherence tomography (SD OCT) in eyes with neovascular age-related macular degeneration (AMD) after anti−vascular endothelial growth factor (VEGF) therapy.

Design: Prospective cohort study within a randomized clinical trial.

Participants: Patients with SD OCT images at weeks 56 and 104 in the Comparison of AMD Treatments Trials (CATT).

Methods: Participants in the CATT were assigned randomly to ranibizumab (0.5 mg) or bevacizumab (1.25 mg) treatment and to a monthly or pro re nata (PRN) injection-dosing regimen. A subset of eyes was imaged with SD OCT beginning at week 56. Cirrus 512×128 or Spectralis 20°×20° volume cube scan protocols were used to acquire SD OCT images. Two independent readers at the CATT OCT reading center graded scans, and a senior reader arbitrated discrepant grades. The prevalence of ORT, identified as tubular structures seen on at least 3 consecutive Cirrus B scans or 2 consecutive Spectralis B scans, was determined. The associations of patient-specific and ocular features at baseline and follow-up with ORT were evaluated by univariate and multivariate analyses.

Main Outcome Measures: Outer retinal tubulations.

Results: Seven of 69 eyes (10.1%) at 56 weeks and 64 of 368 eyes (17.4%) at week 104 had ORTs. Absence of diabetes, poor VA, blocked fluorescence, geographic atrophy, greater lesion size, and presence of subretinal hyperreflective material at baseline were associated independently with greater risk of ORT at 104 weeks ($P < 0.05$). Neither drug nor dosing regimen were associated significantly with ORT. The mean VA of eyes with ORT at week 104 (58.5 Early Treatment Diabetic Retinopathy Study letters) was worse than the mean VA of eyes without ORT (68.8 letters; $P < 0.0001$).

Conclusion: At 2 years after initiation of anti-VEGF therapy for neovascular AMD, ORTs are present in a substantial proportion of eyes. We identified baseline features that independently predict ORTs. It is important to identify ORTs because eyes with ORTs have worse VA outcomes than those without this finding. *Ophthalmology 2014;■:1−9 © 2014 by the American Academy of Ophthalmology.*

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Outer retinal tubulation (ORT) refers to tubular structures observed on optical coherence tomography (OCT) imaging within the outer retina. Photoreceptor rosettes with blue cone opsin immunoreactivity in eyes with retinitis pigmentosa are possible ORT histologic correlates.[1] Zweifel et al[2] were the first to describe these structures as ORTs, based on their OCT appearance. They described ORTs as branching tubular structures located in the retinal outer nuclear layer that occurred in eyes with a variety of advanced degenerative retinal disorders. On spectral-domain (SD) OCT B-scans, ORTs were seen as round hyporeflective spaces with hyperreflective borders. Since that report, ORTs have been observed in eyes with a variety of retinal diseases, including age-related macular degeneration (AMD), pseudoxanthoma elasticum, multifocal choroiditis, central serous chorioretinopathy, and other neovascular retinal disorders.[1−6]

The prevalence of ORTs in eyes with neovascular AMD and their association with ocular and nonocular characteristics has not been well described. We hypothesized that ORTs may be more common than previously thought in neovascular AMD and that the visual prognosis of eyes with ORTs may differ from those without ORTs. The purpose of this study was to determine the prevalence of ORT after anti−vascular endothelial growth factor (VEGF) therapy in subjects enrolled in the Comparison of AMD Treatments Trials (CATT) and to assess whether this prevalence depended on baseline nonocular and ocular features or on anti-VEGF drug and treatment regimen. A further aim was

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to evaluate the association of ORTs with other concurrent retinal morphologic findings and visual acuity (VA).

Methods

Subjects in this study were enrolled in the CATT. Written informed consent was obtained from all CATT study participants, and the protocol was approved by institutional review boards associated with each participating clinical center. The CATT study procedures have been published previously and can be found on clinical trials.gov (study identifier, NCT00593450).[7,8] Briefly, 1185 patients with neovascular AMD were enrolled in the CATT at 43 clinical centers in the United States. Patients were assigned randomly to 1 of 4 treatment groups: (1) ranibizumab monthly, (2) bevacizumab monthly, (3) ranibizumab pro re nata (PRN), or (4) bevacizumab PRN. At 52 weeks, patients originally assigned to monthly treatment were assigned randomly to continue monthly treatment or to PRN treatment of the same drug.

All patients underwent time-domain (TD) OCT with a Stratus system (Carl Zeiss Meditec, Dublin, CA) during year 1 of the study. Beginning in year 2 (defined as week 56), a subset of eyes were imaged with 1 of 2 SD OCT machines, a Cirrus HD-OCT unit (Carl Zeiss Meditec, Dublin, CA) or a Spectralis system (Heidelberg Engineering, Heidelberg, Germany). This subset of eyes was selected based on the availability of SD OCT machines at each participating clinical center; some eyes converted from TD OCT to SD OCT imaging at week 56, whereas others did not convert until later in the study period. A 512×128 macular cube and a 20°×20° 49-line high-speed macular cube were obtained with the Cirrus and Spectralis machines, respectively.

Outer Retinal Tubulation Grading

Two independent readers at the CATT OCT reading center initially graded SD OCT scans for the presence and location of fluid, thickness of retinal layers at the foveal center point, elevation of the retinal pigment epithelium (RPE), and subretinal hyperreflective material (SHRM). Subretinal hyperreflective material was defined as hyperreflective material beneath the retina (or subretinal fluid, when present) and internal to the RPE, or, when the RPE was disrupted and not visible, it referred to the hyperreflective material beneath the retina (or subretinal fluid when present) and Bruch's membrane. This material may include choroidal neovascularization (CNV), blood, or scar tissue. Discrepant points were arbitrated by a third, independent senior reader. In the original CATT publications, the term *intraretinal fluid* (IRF) was used.[8,9] For these publications, readers identified IRF as a round or oval hyporeflective cystoid space, regardless of whether a hyperreflective outer border was present. Accordingly, eyes with IRF included those with intraretinal cystoid spaces alone, or together with ORTs. Furthermore, it is possible that round or elongated tubular structures near the outer retina boundary may have been classified as subretinal fluid. Therefore, in this report, to analyze ORTs at weeks 56 and 104, all SD OCT images from 52 eyes at week 56 and from 277 eyes at week 104 gradable for fluid status and with an initial finding of IRF or subretinal fluid were regraded by 2 independent readers (J.Y.L. and F.A.F.) in a masked fashion for presence and location of ORTs; by definition, eyes without any IRF or subretinal fluid would not have had ORTs. In cases of discrepant grades, a third independent senior reader arbitrated those parameters. In this report, to maintain consistency with previous publications, we retained the term *IRF*. However, we now differentiate IRF, defined as an intraretinal cystoid structure(s) without hyperreflective borders, from an ORT, which is defined as a round, ovoid, or tubular hyporeflective area with a hyperreflective border located in the outer retina. Furthermore, to capture the tubular nature of these structures, the hyporeflective structure with a hyperreflective border had to appear as a contiguous lesion on more than 1 consecutive scan. To be considered an ORT, 2 consecutive scans were required for Spectralis volume cubes. Because of the higher scan density on Cirrus volume cubes, and so that ORT prevalence rates would be comparable among OCT machine types, 3 consecutive scans were required for Cirrus scans.

If present, the location of the ORT was indicated (within the central 1-mm region or under the foveal center) and whether the ORT was located within an area of geographic atrophy (GA). Geographic atrophy on SD OCT was defined as an area of RPE cell layer loss, overlying retinal thinning, and associated penetration of the light signal into the choroid. On Cirrus images, the central 1-mm region was determined from the 512×128 volume cube and was defined as a 1000 μm × 1000 μm square that comprised 21 B-scans, 10 of which were above and 10 of which were below the most foveal-centered scan. On Spectralis images, the central 1-mm region was determined from the 49-line volume cube and was defined as a 1000 μm × 1000 μm square that comprised 11 B-scans, 5 of which were above and 5 of which were below the most foveal-centered scan. In both the Cirrus and Spectralis machines, the built-in software measurement tool was used to determine the boundaries of the 1-mm central region. In most cases, the foveal center was determined readily by the central foveal depression. In some cases, when the foveal depression was absent because of macular edema secondary to CNV, the foveal center was identified by the greatest outer nuclear complex thickness and by loss of the ganglion cell complex. To evaluate change in ORTs from week 52 to 104, we correlated the OCT B-scan location of the ORT with the corresponding location on the scanning laser ophthalmoscopic image (Spectralis) or OCT fundus image (Cirrus) to ensure that comparable locations were analyzed from one examination to the next.

Fluorescein Angiography and Fundus Photography Grading

Independent readers graded a variety of features on fundus photographs and fluorescein angiograms. Fibrotic and nonfibrotic scars were defined as follows. Fibrotic scars were defined as obvious white or yellow mounds of fibrous-appearing tissue that were well defined in shape and appeared solid on color stereo images. On fluorescein angiography, they were hyperfluorescent because of tissue staining or blocked fluorescence of the underlying choroid. Nonfibrotic scars were typically flat, small, well-circumscribed areas of pigmentation with varying degrees of central hypopigmentation on color fundus photographs. The peripheral pigmentary changes in these scars often followed the outline of previously active CNV. On early-phase fluorescein angiograms, the depigmented areas often were hyperfluorescent, and this hyperfluorescence persisted or increased in intensity on late-phase fluorescein angiograms. Hypofluorescence surrounding the hyperfluorescent center corresponded to the pigmented borders apparent in the color images. Blocked fluorescence was defined as hypofluorescence on fluorescein angiography that was contiguous with CNV and not related to hemorrhage or pigment on corresponding color fundus photographs.

Statistical Analysis

The comparisons of features between eyes with ORT versus those without ORT were performed using the Fisher exact test for comparing proportions and 2-group t tests for comparing means. To assess baseline predictors of ORT prevalence at week 104, we classified predictors, determined at study enrollment and measured

Table 1. Location of Outer Retinal Tubulation by Location of Fluid on Optical Coherence Tomography at Week 56 (N = 69)

Optical Coherence Tomography Fluid at Week 56	No.	Location of Outer Retinal Tubulation, No. (%)			
		Anywhere	*Central 1-mm Region*	*Under Foveal Center*	*In an Area of Geographic Atrophy*
Intraretinal only	19	6 (31.6)	0 (0)	0 (0)	2 (10.5)
Subretinal only	16	0 (0)	0 (0)	0 (0)	0 (0)
Both	17	1 (5.9)	1 (5.9)	0 (0)	0 (0)
Either intraretinal or subretinal	52	7 (13.5)	1 (1.9)	0 (0)	2 (3.8)
Total	69	7 (10.1)	1 (1.4)	0 (0)	2 (2.9)

on a continuous scale (e.g., VA, CNV area, OCT thickness), into categories for easier clinical interpretation based on either the normal range (for retinal thickness), quartiles of the distribution (for subretinal tissue complex thickness), or clinically relevant cut points (for baseline VA). We then performed univariate analysis for each of the baseline predictors, including demographic characteristics (e.g., age, sex, systemic diseases); ocular characteristics (e.g., intraocular pressure, history of glaucoma, CNV features); and OCT features (e.g., subretinal, retinal, and subretinal tissue complex thickness).

The predictors with a P value less than 0.20 in the univariate analysis were included in a multivariate logistic regression so that the independent association of each baseline predictor with ORT could be assessed. The final multivariate model was created by applying a backward selection procedure that retained only those predictors with a P value less than 0.05, with the exception of drug and dosing regimen, which were included in the final multivariate model. Adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated from the final multivariate logistic regression model. All statistical analyses were performed using SAS software version 9.2 (SAS Inc., Cary, NC), and a 2-sided P value <0.05 was considered to be statistically significant.

Results

Frequency and Location of Outer Retinal Tubulations

CATT participants (n = 391) underwent SD OCT at weeks 56 and 104. Of these, the fluid status could be determined in 69 of 73 eyes (95%) at week 56 and in 368 of 384 eyes (96%) at week 104. In the remaining 4 eyes at week 56 and 16 eyes at week 104, the images were not of sufficient quality to determine definitively the presence or absence of IRF or subretinal fluid. Subsequent analyses were performed on these 69 and 368 eyes.

Of the 69 eyes with known fluid status at week 56, 7 (10.1%) had ORTs, and of 368 eyes with known fluid status at 104 weeks, 64 (17.4%) had ORTs. There were no differences in the prevalence of ORTs seen on Cirrus and those identified on Spectralis at week 56 (12.2% and 5.0% on Cirrus and Spectralis, respectively; P = 0.66) and at week 104 (16.6% and 18.5% on Cirrus and Spectralis, respectively; P = 0.68).

Outer retinal tubulations were located most commonly outside the central 1-mm region. At 56 weeks, only 1 of 7 eyes (14%) with ORTs was within the central 1-mm region and none were in the foveal center. At 104 weeks, 22 of 64 eyes (34%) with ORTs were within the central 1-mm region, and only 2 (3.1%) were under the foveal center. Outer retinal tubulations were found commonly in areas of GA, particularly by 104 weeks. At 56 weeks, 2 of 7 (28%) were within a GA area, and at 104 weeks, 34 of 64 (53%) were within a GA area (Tables 1 and 2).

In some eyes, ORTs appeared to change over time. Among the 7 eyes with ORTs at week 56, only 1 eye (14.3%) still had ORTs at week 104. Among 52 eyes that did not have ORTs at week 56, 8 (15.4%) had new ORTs at week 104. When serial OCT images from a given subject were reviewed, these structures disappeared in a variable manner. For example, in 2 subjects, there was disorganization of the retina associated with intraretinal cysts and subsequent disruption of the ORT. In 1 subject, the ORT appeared to shrink and then disappear (Fig 1). Of the 6 eyes in which ORTs were seen at week 56 but not at week 104, in 2 eyes GA was present in association with the ORTs at 56 weeks and was also visible at week 104. In 2 additional eyes, there was no GA at week 54, but GA was observed at week 104 in the area previously occupied by the ORTs. Of the 8 eyes in which new ORTs developed at week 104 that were not present at week 56, 6 eyes were associated with GA (Fig 2).

Baseline Features Associated with Outer Retinal Tubulations at Week 104

A variety of baseline nonocular and ocular features were evaluated for association with ORTs at week 104 (there were too few participants at week 56 for meaningful analysis). On univariate analysis, diabetes and baseline dietary supplement (e.g., β-carotene, vitamin C, vitamin E, and zinc as a combination) use were associated significantly with lower risk of ORT (Table 3, available at www.aaojournal.org). In addition, several baseline ocular characteristics were related significantly to ORT at week 104 (Table 4, available at

Table 2. Location of Outer Retinal Tubulation by Location of Fluid on Optical Coherence Tomography at Week 104 (N = 368)

Optical Coherence Tomography Fluid at Week 104	No.	Location of Outer Retinal Tubulation, No. (%)			
		Anywhere	*Central 1-mm Region*	*Under Foveal Center*	*In an Area of Geographic Atrophy*
Intraretinal only	131	39 (29.8)	15 (11.5)	2 (1.5)	27 (20.8)
Subretinal only	79	5 (6.3)	3 (3.8)	0 (0)	1 (1.3)
Both	67	20 (29.9)	4 (6.0)	0 (0)	6 (9.0)
Either intraretinal or subretinal	277	64 (23.1)	22 (7.9)	2 (0.7)	34 (12.3)
Total	368	64 (17.4)	22 (6.0)	2 (0.5)	34 (9.2)

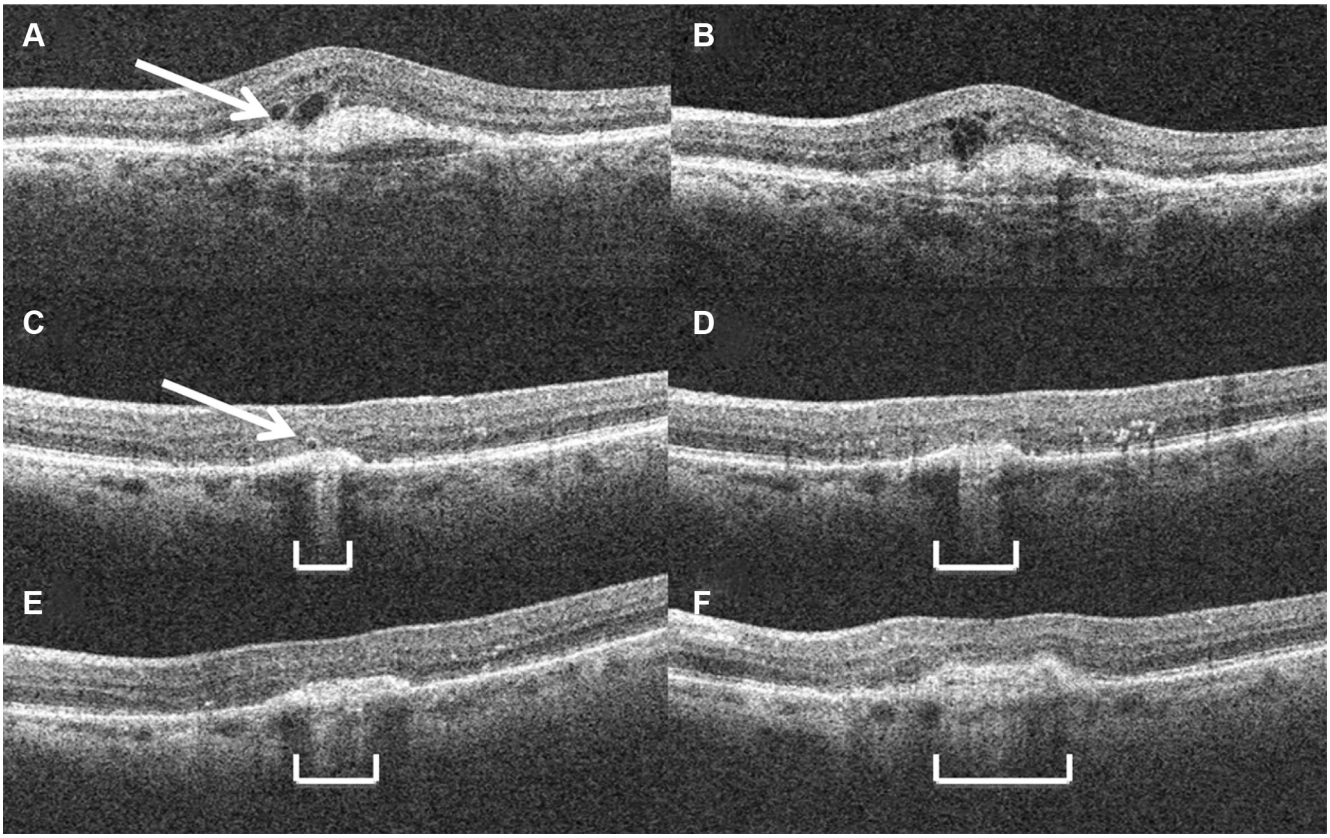

Figure 1. Representative images showing structural changes in outer retinal tabulations (ORTs) over time. **A**, Outer retinal tabulation (arrow) next to an intraretinal cystoid structure without a hyperreflective rim at week 56. **B**, Nine months later, the ORT and cystoid structure appear to have coalesced into a retinal area with multiple cystoid structures and disrupted layers. **C**, At 56 weeks, there was a small ORT overlying an area of retinal elevation (arrow). **D**, Three months later, in the same eye as in (**C**), the ORT has nearly disappeared, and by (**E**) 9 months and (**F**) 12 months, it is no longer apparent. The width of the atrophic area underlying this ORT, seen as the area with increased choroidal signal penetration, increased over time (brackets).

www.aaojournal.org). Lesions with blocked fluorescence, fibrotic or nonfibrotic scar, predominantly or minimally classic lesions, CNV without associated other lesion components (hemorrhage, blocked fluorescence, serous RPE detachment, and others), hemorrhage contiguous with a lesion, hemorrhages (>1 disc area), GA, large CNV lesions—all as determined by fluorescein angiography, color fundus photography, or both—and eyes with relatively worse VA all were associated significantly with increased risk of ORT at week 104 (Table 4, available at www.aaojournal.org). Of the eyes in which ORTs developed at week 104, 17% had GA at baseline, whereas of the eyes in which ORTs did not develop, only 5% had baseline GA. Furthermore, baseline OCT characteristics, which included increased sub-RPE tissue complex thickness, IRF, SHRM located anywhere in the macula, or within the center 1 mm subfield, were significantly associated with ORT (Table 5, available at www.aaojournal.org). Of note, the assigned anti-VEGF drug (bevacizumab or ranibizumab) and drug regimen (monthly or PRN) were not associated with ORTs at week 104.

To determine the factors that were associated independently with ORT, a multivariate analysis was conducted (Table 6). Diabetes was still associated with lower risk of ORTs (OR, 0.17; 95% CI, 0.05−0.56). Baseline ocular factors that were associated independently with increased risk of ORTs included lesions with blocked fluorescence (OR, 2.62; 95% CI, 1.12−6.13), GA (OR, 7.01; 95% CI, 2.27−21.7), large CNV lesions (OR, 4.62; 95% CI, 1.82−11.7 for >4 disc areas compared with ≤1 disc area), worse baseline VA (OR, 6.52; 95% CI, 1.92−22.1 for VA 20/200 or worse compared with 20/40 or better), and SHRM (OR, 2.50; 95% CI, 1.01−6.17).

Anatomic Features in Eyes with Outer Retinal Tubulations at Week 104

At week 104, several retinal anatomic features were compared between eyes with versus without ORTs at that point. Compared with eyes without ORT, eyes with ORT were more likely to have IRF, abnormally thin or thick retinas (<120 μm or >212 μm), larger CNV lesion size, fibrotic scar, and pathologic features in the foveal center (Table 7). They were less likely to have CNV; the proportion with ORTs among those with CNV was 10.9%, and among those without ORTs it was 18.1%. At week 104 there were no eyes with ORTs that did not also have associated fluid (intraretinal, subretinal, or sub-RPE). Conversely, among eyes without ORTs at week 104, 27.3% had fluid (intraretinal, subretinal, or sub-RPE). The incidence of de novo GA (those for whom GA was not evident at baseline but was present at 2 years) was 7.5% among those that had ORTs and was 15.5% among those who did not have associated ORTs. Of all eyes with GA at week 104, 10% had associated ORT and 90% did not.

Effect of Outer Retinal Tubulations and Fluid on Visual Acuity at Week 104

Overall, eyes with ORTs at week 104, when compared with those without ORTs, had worse VA at week 104 (mean VA, 59 vs. 69

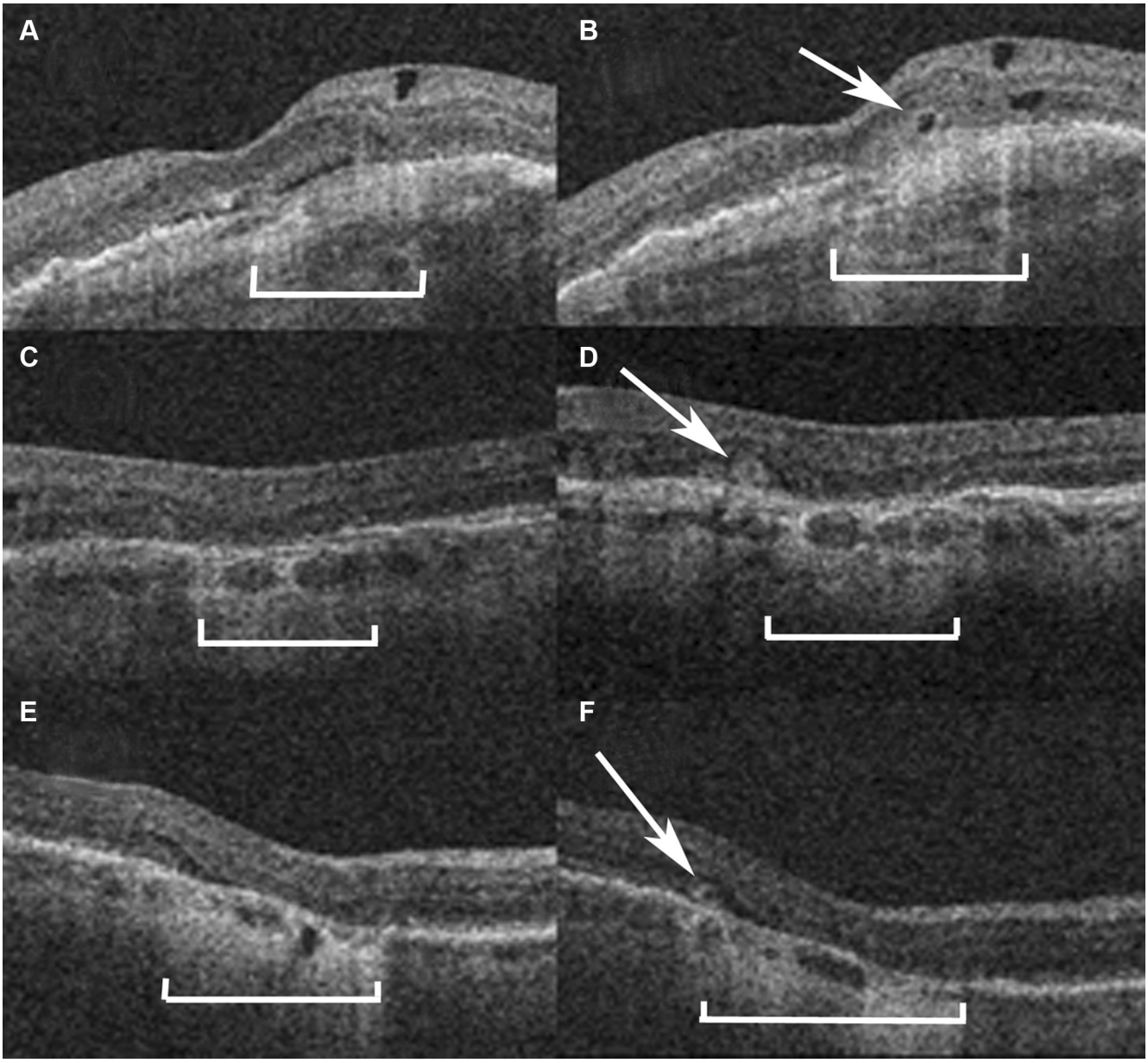

Figure 2. Representative optical coherence tomography images obtained at (**A**, **C**, **E**) week 56 and (**B**, **D**, **F**) week 104 in 3 eyes without outer retinal tabulations (ORTs) at week 56 but with ORTs (arrows) at week 104. The ORTs are seen adjacent to areas of geographic atrophy, seen as photoreceptor layer thinning above an area of increased light penetration into the choroid (brackets).

letters; $P < 0.0001$), a higher proportion with VA 20/200 or worse (20% vs. 5%; $P < 0.0001$), and a smaller percentage with VA 20/40 or better (45% vs. 66%; $P = 0.004$; Table 7). Furthermore, baseline VA was worse by 6 letters in eyes with ORTs and IRF at week 104 than in eyes with IRF but no ORTs at week 104, which was worse by 3 letters than in eyes without IRF. The 59 eyes with both IRF and ORTs had worse VA at week 104 (mean VA, 57.3 letters) compared with eyes with IRF but no ORTs (mean VA, 63.3 letters; $P < 0.0001$; Table 8). All eyes with ORTs at week 104 had associated IRF, subretinal fluid, or both that were distinct from the ORTs. Furthermore, week 104 VA in eyes with both IRF and ORTs was 6 letters worse than eyes with IRF and no ORTs, which was approximately 10 letters worse than in eyes without IRF (Table 8).

It is conceivable that the prevalence of ORTs among subjects who underwent SD OCT differed from those who underwent TD OCT only. To confirm that the population evaluated in the present study was similar to the CATT study population as a whole, we did an additional analysis to compare the key baseline predictive factors and the 2-year outcomes in the eyes that had SD OCT and those in which only TD OCT was performed. There were no significant differences in any of these features between the groups either at baseline or at 2 years (Table 9, available at www.aaojournal.org).

Discussion

In this study, we found that the prevalence of ORTs at weeks 56 and 104 was substantial in eyes with neovascular

Table 6. Multivariate Analysis of the Baseline Predictors of Outer Retinal Tubulation in Any Area at Week 104 (N = 347)

Baseline Features	No.*	Outer Retinal Tubulation, No. (%)	Univariate Analysis		Multivariate Analysis†	
			Odds Ratio (95% Confidence Interval)	P Value‡	*Odds Ratio (95% Confidence Interval)*	P Value§
Diabetes						
No	283	58 (20.5)	1.00	0.012	1.00	**0.004**
Yes	64	4 (6.25)	0.26 (0.09–0.74)		0.17 (0.05–0.56)	
Blocked fluorescence						
No	300	49 (16.3)	1.00	0.063	1.00	**0.03**
Yes	47	13 (27.7)	1.96 (0.96–3.98)		2.62 (1.12–6.13)	
Geographic atrophy in study eye						
None/questionable	325	52 (16.0)	1.00	0.001	1.00	**0.0007**
Present	22	10 (45.5)	4.38 (1.80–10.7)		7.01 (2.27–21.7)	
Baseline total area of CNV lesion (DA)						
≤1	117	12 (10.3)	1.00	0.029	1.00	**0.01**
>1–≤2	79	14 (17.7)	1.89 (0.82–4.33)		1.97 (0.75–5.15)	
>2–≤4	82	17 (20.7)	2.29 (1.03–5.10)		2.75 (1.09–6.95)	
>4	69	19 (27.5)	3.33 (1.50–7.38)		4.62 (1.82–11.7)	
Baseline VA in study eye						
20/25–40	133	13 (9.77)	1.00	0.0002	1.00	**0.003**
20/50–80	126	20 (15.9)	1.74 (0.83–3.67)		1.38 (0.61–3.10)	
20/100–160	67	20 (29.9)	3.93 (1.81–8.53)		3.22 (1.36–7.63)	
20/200–320	21	9 (42.9)	6.92 (2.46–19.5)		6.52 (1.92–22.1)	
Subretinal hyperreflective material						
No	84	7 (8.33)	1.00	0.012	1.00	**0.047**
Yes	263	55 (20.9)	2.91 (1.27–6.66)		2.50 (1.01–6.17)	
Drug						
Lucentis	175	25 (14.3)	1.00	0.08	1.00	0.08
Avastin	172	37 (21.5)	1.64 (0.94–2.87)		1.75 (0.94–3.27)	
Regimen						
Monthly	74	10 (13.5)	1.00	0.43	1.00	0.40
Switched	94	20 (21.3)	1.73 (0.75–3.96)		1.90 (0.74–4.89)	
PRN	179	32 (17.9)	1.39 (0.65–3.00)		1.36 (0.58–3.18)	

CNV = choroidal neovascularization; DA = disc area; PRN = pro re nata; VA = visual acuity.
Boldface indicates statistical significance in multivariate analysis.
*Three hundred forty-seven patients were included in the final multivariate model; 21 patients were excluded because of missing data in any of the baseline predictors in the final model.
†The initial multivariate model includes diabetes, dietary supplement use, blocked fluorescence lesion, fibrotic or nonfibrotic scar, lesion type, hemorrhage contiguous with lesion (yes or no), geographic atrophy, baseline total area of choroidal neovascularization lesion (DA), baseline VA in study eye, subretinal tissue complex thickness in the foveal center, intraretinal fluid, subretinal hyperreflective material, and drug and regimen.
‡*P* values are from univariate logistic regression.
§*P* values are from multivariate logistic regression.

AMD that had been treated with anti-VEGF therapy. Baseline features identified at subject enrollment that were associated independently and positively with ORT prevalence included worse baseline VA, presence of GA, larger CNV lesion area, blocked fluorescence on fluorescein angiography, and SHRM on OCT, whereas baseline diabetes was associated negatively with ORTs. Furthermore, after treatment for 2 years, eyes with ORTs were more likely to have abnormally thin or thick retinas, a fibrotic scar, and a large CNV lesion complex and were less likely to have a CNV-only lesion than treated eyes without ORTs. Eyes with IRF had worse VA than those without any intraretinal cystoid structures, and VA was even worse in eyes that had both IRF and ORTs.

Outer retinal tubulations were identified by SD OCT in the subgroup of CATT participants in whom it was performed. All eyes underwent Stratus TD OCT in the first year of the CATT. Initially, we attempted to identify ORTs on these TD OCT images. However, we found that it was not possible to identify them reproducibly with this methodology. Spectral-domain OCT allowed us to identify the ORTs as tubular structures on contiguous SD B-scans. For this study, the requirements for visibility of ORT on multiple SD OCT scans was conservative, and it is likely that ORTs thus are more common than predicted here. It is likely that we could not identify these structures reliably on TD OCT scans because of inadequate scan density, particularly because ORTs typically were seen distal to the foveal center and the radial TD OCT B-scans are separated widely outside the foveal area. In addition, even on SD OCT imaging, ORTs may not always have prominent hyperreflective ORT rim, and decreased TD OCT image resolution compared with SD image resolution more often precluded clear differentiation of ORTs from the surrounding reflective tissue.

In our previous 1-year and 2-year CATT outcomes data, we used the term *intraretinal fluid* to describe hyporeflective

Table 7. Comparison of Week 104 Outcomes between Patients with and without Outer Retinal Tubulation at Week 104 (N = 368)

Week 104 Outcomes	With Outer Retinal Tubulation (n = 64)	Without Outer Retinal Tubulation (n = 304)	*P* Value*
VA categories, no. (%)			<0.0001
20/200 or worse	13 (20.3)	15 (4.9)	
20/50–20/160	22 (34.4)	90 (29.6)	
20/12–20/40	29 (45.3)	199 (65.5)	
Mean VA in letters (SE)	58.5 (3.11)	68.8 (0.97)	<0.0001
Mean VA change from baseline in letters (SE)	3.69 (2.81)	6.15 (0.94)	0.31
Increase of ≥15 letters from baseline (%)	20 (31.3)	87 (28.6)	0.65
Mean no. of injections in year 1 in PRN groups (SE)†	12.8 (1.03)	13.7 (0.51)	0.43
Retinal thickness, no. (%)			0.03
<120 μm	23 (35.9)	77 (25.4)	
120–212 μm	30 (46.1)	194 (64.0)	
>212 μm	11 (17.2)	32 (10.6)	
Mean change of total foveal thickness from baseline μm, (SE)	−168 (25.2)	−162 (11.0)	0.83
Intraretinal fluid, no. (%)	59 (92.2)	139 (45.7)	<0.0001
Subretinal fluid, no. (%)	25 (39.1)	121 (39.8)	1.00
Sub-RPE fluid, no. (%)	25 (39.1)	109 (36.1)	0.67
Leakage on angiography, no. (%)	18 (28.6)	83 (27.9)	0.88
Mean lesion size at 2 yrs, disc areas (SE)	4.47 (0.45)	3.10 (0.18)	0.002
Mean change in lesion size from baseline, disc areas (SE)	0.70 (0.29)	0.68 (0.15)	0.95
Fibrotic scar, anywhere, no. (%)	31 (48.4)	68 (22.4)	<0.0001
Nonfibrotic scar, anywhere, no. (%)	12 (18.8)	52 (17.1)	0.72
Geographic atrophy, anywhere, no. (%)	11 (17.2)	58 (19.1)	0.86
Pathology in fovea center, no. (%)			0.03
No pathology	9 (14.1)	70 (23.0)	
Fluid only	0 (0)	13 (4.3)	
Choroidal neovascularization	7 (10.9)	55 (18.1)	
Serous pigment epithelial detachment	0 (0)	2 (0.7)	
Scar	27 (42.2)	59 (19.4)	
Geographic atrophy	3 (4.7)	11 (3.6)	
Nongeographic atrophy	14 (21.9)	58 (19.1)	
Hemorrhage	0 (0)	3 (1.0)	
Retinal pigment epithelium tear	0 (0)	5 (1.6)	
Blocked fluorescence	0 (0)	9 (3.0)	
Other	3 (4.7)	10 (3.3)	
Unknown	1 (1.6)	9 (3.0)	

PRN = pro re nata; RPE = retinal pigment epithelium; SE = spherical equivalent; VA = visual acuity.
*From Fisher exact test for comparison of proportions and 2-group *t* test for comparison of means.
†Only patients in PRN group were included in the analysis.

intraretinal cystoid structures but did not distinguish those intraretinal cystic structures without a hyperreflective border from those with such a border. In this report, we defined ORTs as those intraretinal cystic structures with a hyperreflective border. The hyperreflective border likely corresponds to intact photoreceptor inner segment ellipsoids that surround the photoreceptor outer segment rosettes. However, histologically, these rosettes also may be seen without intact surrounding ellipsoids (Schaal KB. Outer Retinal Tubulation (ORT) in AMD: OCT Findings Correlate with Histology. 11th International SPECTRALIS Symposium, OCT 18, 2013; New York). To avoid confusion and maintain consistent terminology, we retained the term *intraretinal fluid* in this report, recognizing that some of these structures may represent outer tubulations because it is not possible to distinguish hyporeflective cystoid structures that originate from fluid leakage from those that represent outer tubulations without a hyperreflective border on OCT. Furthermore, because it was not possible by OCT to identify outer retinal tubulations without a hyperreflective border in eyes with neovascular AMD treated with anti-VEGF therapy, the prevalence of ORTs is likely even higher than that reported here.

It was notable that diabetic patients had lower risk of developing ORTs. The reason for the negative association between diabetes and ORTs in eyes with neovascular AMD treated with anti-VEGF agents is unclear, and further studies are needed to explain this relationship.

The relationship between ORTs and neovascular AMD disease activity and the need for anti-VEGF therapy is unknown. We have initiated a follow-up CATT study that will extend our observations to 5 years after initiation of anti-VEGF therapy to treatment-naïve patients. We then will be able to correlate ORTs at week 104 with subsequent need for treatment.

Eyes with ORTs had significantly worse VA at baseline and week 104 than eyes without this finding. These results are consistent with those described in a recent study in which ORTs were associated significantly with worse VA before and after intravitreal anti-VEGF treatment.[10] Previously, we showed that IRF, which included eyes with ORTs,

Table 8. Visual Acuity at Baseline and at Week 104 between Eyes with and without Outer Retinal Tubulation at Week 104 (N = 368)

Fluid and Outer Retinal Tubulation Status at Week 104	No.	Visual Acuity				
		Baseline, Mean (Standard Error)	*Week 104, Mean (Standard Error)*	*Change at Week 104, Mean (Standard Error)*	*≥20/40 at Week 104, No. (%)*	*≤20/200 at Week 104, No. (%)*
ORT and IRF						
No IRF	170	63.9 (1.0)	73.5 (1.4)	9.55 (1.33)	127 (74.7)	1 (0.6)
IRF with ORT	59	54.8 (1.6)	57.3 (2.33)	2.42 (2.25)	25 (42.4)	13 (22.0)
IRF without ORT	139	60.8 (1.1)	63.3 (1.52)	2.44 (1.47)	76 (54.7)	14 (10.1)
P value*		<0.0001	<0.0001	0.0005	<0.0001	<0.0001
ORT and SRF						
No SRF	221	59.4 (0.8)	64.3 (1.25)	4.88 (1.19)	125 (56.6)	23 (10.4)
SRF with ORT	25	60.2 (2.5)	64.7 (3.72)	4.44 (3.53)	14 (56.0)	2 (8.0)
SRF without ORT	121	65.2 (1.2)	72.5 (1.69)	7.34 (1.60)	89 (73.6)	3 (2.5)
P value*		0.0003	0.0004	0.44	0.005	0.02
ORT and IRF or SRF						
No IRF or SRF	91	61.4 (1.3)	71.0 (1.9)	9.64 (1.84)	60 (65.9)	1 (1.1)
IRF or SRF with ORT	64	54.8 (1.6)	58.5 (2.3)	3.69 (2.19)	29 (45.3)	13 (20.3)
IRF or SRF without ORT	213	63.2 (0.87)	67.9 (1.27)	4.66 (1.20)	139 (65.3)	14 (6.6)
P value*		<0.0001	0.0001	0.047	0.014	<0.0001
ORT						
No	304	62.6 (0.73)	68.8 (1.06)	6.15 (1.01)	199 (65.5)	15 (4.9)
Yes	64	54.8 (1.58)	58.5 (2.32)	3.69 (2.20)	29 (45.3)	13 (20.3)
P value*		<0.0001	<0.0001	0.31	0.004	<0.0001

IRF = intraretinal fluid; ORT = outer retinal tubulation; SRF = subretinal fluid.
*From Fisher exact test for comparison of proportions and 2-group *t* test for comparison of means.

was an independent predictor of poor VA at all points evaluated through 1 year.[11] In this report, we have extended and further clarified this observation. At 2 years, eyes with IRF without ORTs had significantly worse VA than those without any IRF, and eyes with both IRF and ORTs had significantly worse VA than eyes with IRF without ORTs. The reason for the association of ORTs with worse VA is unclear, but presumably eyes with ORTs have a greater degree of photoreceptor degeneration than eyes with IRF alone.

Baseline SHRM and GA predicted ORT at 2 years. Furthermore, in 2 eyes, GA was observed at week 104 as a footprint of ORTs that were seen at week 56 but had disappeared by week 104. There is preliminary evidence that combination treatments such as anti−platelet-derived growth factor and anti-VEGF causes resolution of subretinal hyperreflective tissue, an OCT correlate of subretinal CNV tissue complex, more effectively than does anti-VEGF therapy alone and may also result in better VA outcomes.[12] It will be of interest in future studies to determine whether these types of combination therapies, or future treatments to limit GA progression, more effectively reduce ORTs and, if so, whether the eyes with ORT resolution have better VA than those without ORT resolution.

The current study investigated associations of ORT with various systemic and anatomic factors but was not designed to determine whether anti-VEGF therapy can cause resolution of ORTs, as it does with IRF from leaking CNV, nor whether resolution influences VA. Zweifel et al[2] suggested that the tubular arrangements seen in ORT may be a response to degenerating photoreceptors and may represent a common final pathway in a variety of retinal degenerative conditions, rather than a specific response to leaking CNV. Although this hypothesis may be correct for many conditions, data from the present report indicate that, once formed, ORTs are not static in neovascular AMD treated with anti-VEGF therapy. The dynamic character of ORTs is supported by the comparison of ORTs at weeks 56 and 104 among 59 eyes with fluid status known at both weeks 56 and 104. Among 7 eyes with ORT at week 56, only 1 eye had ORT at week 104. The ORT disappearance that we observed in some eyes after anti-VEGF therapy indicates that in some cases, ORTs may respond to anti-VEGF treatment. Alternatively, ORT disappearance may be coincidental and unrelated to anti-VEGF therapy.

The above data suggest that, once formed, ORTs can resolve. To state definitively that by OCT, on a single OCT B-scan, ORTs disappeared from one point to the next, it would be necessary to ensure that the OCT B-scan images were registered precisely on consecutive visits. However, most commonly, ORTs are tubular, branching structures that would be seen on more than 1 consecutive OCT-B scan. We could not be certain that single B-scan OCT images were always registered precisely from one visit to the next. Therefore, to consider a structure as an ORT, we required the circular or ovoid structure with a hyperreflective border to be seen on 2 (Spectralis) or 3 (Cirrus) consecutive scans. More importantly, when we evaluated change in ORT over time, we correlated the location on OCT with the scanning laser ophthalmoscopic fundus image (Spectralis) or OCT fundus image (Cirrus) that accompanied it to ensure that the same location was evaluated from one point to the next. Accordingly, we believe that it is very unlikely that ORT appearance or disappearance could be attributed solely to registration errors.

Our results apply specifically to ORT prevalence 1 and 2 years after anti-VEGF therapy in treatment-naïve patients

and cannot be extrapolated to untreated eyes or to eyes treated for less than 1 year after anti-VEGF therapy initiation. It is possible that ORTs were present during the first year but could not be identified by the imaging methods used. To determine precisely the time to ORT onset and whether they can be present in treatment-naïve patients, it would be necessary to obtain SD OCTs at earlier time points than evaluated in the current study. The increased ORT prevalence at week 104 compared with that at week 56 suggests that in treatment-naïve eyes, ORTs may be relatively uncommon and supports the hypothesis that ORTs develop from degenerating photoreceptors and are a late stage of treated neovascular AMD. Further studies that evaluate ORTs at the time of anti-VEGF therapy initiation, and during the first year of treatment, will be necessary to characterize more definitively the longitudinal development and changes in appearance of ORTs, regardless of whether they respond to therapy.

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **CATT** = Comparison of Age-Related Macular Degeneration Treatments Trials; **CI** = confidence interval; **CNV** = choroidal neovascularization; **GA** = geographic atrophy; **IRF** = intraretinal fluid; **OCT** = optical coherence tomography; **OR** = odds ratio; **ORT** = outer retinal tubulation; **PRN** = pro re nata; **RPE** = retinal pigment epithelium; **SD** = spectral domain; **SHRM** = subretinal hyperreflective material; **TD** = time domain; **VA** = visual acuity; **VEGF** = vascular endothelial growth factor.

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