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Outer retinal tubulations response to anti-VEGF treatment

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ABSTRACT

Aim To review the longitudinal changes of outer retinal tubulations (ORTs) in wet age-related macular degeneration (AMD) and their response to anti-vascular endothelial growth factor (VEGF) therapy by spectral-domain optical coherence tomography (SD-OCT), and to correlate these observations with disease activity, presence or absence of fluid, and patients' demographics.

Methods Retrospective study of wet AMD eyes treated with anti-VEGF agents and showing ORTs on SD-OCT, and the patients' fellow eye with wet AMD but without ORTs.

Results Fifty-one wet AMD eyes from 31 patients diagnosed and treated for wet AMD were included in the review and analysis of data; 33 eyes showed ORTs at baseline, while 18 fellow eyes had no ORTs. During a median follow-up treatment period of 11 months, 23 eyes had stable ORTs and 10 eyes had ORT changes. Among the 10 eyes with ORTs changes, ORTs collapsed during anti-VEGF treatment in 5 eyes but then reappeared within 12 months after stopping treatment. In two eyes, ORTs increased in size during anti-VEGF treatment, while in two other eyes ORTs collapsed without any treatment. In a single eye, ORTs collapsed within 10 months of no treatment and did not reappear upon recurrence of fluid. Eyes with ORTs tended to have lower visual acuity than eyes with no ORTs due to greater disruption of the external limiting membrane in the fovea.

Conclusions ORTs documented by SD-OCT may exhibit multiple types of longitudinal changes, such as collapse, recurrence or enlargement, which could be associated with anti-VEGF treatment or spontaneous. Some ORTs may have a vascular component or may be vascular in nature, considering their response to anti-VEGF treatment, while other ORTs are likely composed only of degenerating photoreceptor cells and may collapse independently from anti-VEGF treatments.

INTRODUCTION

Outer retinal tubulations (ORTs) were first described by Zwiefel *et al*¹ in 2009 as branching tubular structures located in the outer nuclear layer of the retina and appeared as round or ovoid hyporeflective spaces with hyper-reflective borders on ocular coherence tomography (OCT) scans. ORTs-associated hyper-reflective borders appear to be a rearrangement of the photoreceptor cells and Muller cells in a reparative attempt or as a response to injury, mostly in wet age-related macular degeneration (AMD) eyes but also in other degenerative retinal diseases.¹ Wolff *et al*,² in 2012 using 'en face' OCT, differentiated between the main

subtypes of tubular formations in AMD; 'pseudo-dendritic' forms that develop next to fibrotic scars and 'perilesional' forms that develop at the edge of atrophic areas. The authors suggested that ORTs are stable structures and are not a sign of recurrent or active choroidal neovascularisation (CNV); hence, ORTs should not be used as a marker for disease activity. However, we have observed ORTs changes in some patients with wet AMD during treatment with anti-vascular endothelial growth factor (VEGF) agents. The aim of the present study was to review the behaviour of ORTs during anti-VEGF treatment and to correlate these observations with disease activity, presence or absence of fluid and demographics.

METHODS

This was a retrospective study of wet AMD patients with ORTs in at least one eye, imaged with SD-OCT scans, and treated with intravitreal anti-VEGF agents at the Jacobs Retina Center at Shiley Eye Institute, University of California San Diego, La Jolla, California, USA, between September 2009 and December 2013.

We included longitudinal scans of wet AMD eyes with ORTs, as well as the patients' fellow eye with wet AMD but without ORTs to serve as controls. The presence of ORTs was evaluated by trained physicians; ORTs were described as hyporeflective round or oval structures with hyper-reflective borders along the outer nuclear layer and mostly seen adjacent to a subretinal fibrovascular scar. We excluded eyes with CNV secondary to pathological myopia or hystoplasmosis, as well as eyes with non-exudative AMD. The collected baseline demographic data included patient age; sex; history of previous treatments for CNV, such as photodynamic therapy; number of previous anti-VEGF injections (pegaptanib, ranibizumab, bevacizumab, aflibercept); duration of treatment (in months); and type of fluid.

For all patients, spectral-domain (SD)-OCT imaging was available for all visits and was carried out using the Spectralis (Heidelberg Engineering, Heidelberg, Germany). High-resolution single-line baseline and follow-up scans centred on the fovea were evaluated. The platform of the device simultaneously images the eye with two beams of light; one beam captures an image of the retina and maps over 1000 points to track eye movement. Using the mapped image as a reference, the second beam is directed to the desired location despite blinks or saccadic eye movements. The eye-tracking dual-beam technology (TruTrack Active Eye Tracking software, Heidelberg Engineering) mitigates eye

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motion artefact and ensures point-to-point correlations between the OCT scan and fundus images. The eye-tracking technology also permits precise scanning of the same location over consecutive visits and has been proven to provide repeatable macular thickness measurements.³ The co-localisation of structures on OCT scan and near-infrared fundus image were previously proved to be excellent, with an average maximum error of $15.35 \pm 6.29 \mu\text{m}$.⁴

For all patients and all visit scans, the observers evaluated the presence or changes of ORTs compared with baseline, integrity of external limiting membrane (ELM) in the foveal centre, subretinal scars' greatest linear diameter (GLD) and scar height at the highest point on the same follow-up single scans across the fovea. ORTs were described as 'collapsed' if from round or oval appearance at baseline they became flat with still identifiable hyper-reflective borders on follow-up scans; 'disappeared' if no identifiable hyper-reflective borders were noted on follow-up scans compared with baseline; or 'recurrent' if identifiable hyper-reflective borders reappeared on follow-up scans. A 'stable' ORT was defined as having no changes observed from baseline to the last follow-up scans. GLD and scar height were measured in microns with the calliper feature of the Spectralis device. Visual acuity by Early Treatment Diabetic Retinopathy Study (ETDRS) charts were recorded and converted to decimal notation for statistical analysis.

Descriptive statistical analyses were performed using the Fisher's exact test for categorical variables and the Wilcoxon tests for continuous variables. For all hypothesis tests, statistical significance was set at a level of $p < 0.05$. Statistical analyses were performed using SAS software V9.3 (SAS Institute, Cary, North Carolina, USA) and R V3.0.0 (<http://www.r-project.org/>).

RESULTS

Fifty-one eyes with wet AMD from 31 patients were included; in 33 eyes, there was evidence of ORTs at baseline, while 18 fellow eyes did not present any ORT. Baseline characteristics are summarised in table 1, dividing eyes by the presence or absence of ORTs. The median age of patients was 83 years (IQR 79–89). Fifty-five per cent of patients were females. The majority of eyes with ORTs at baseline (48.5%) had intraretinal fluid (IRF) only, followed by combined subretinal fluid (SRF) and IRF (42.4%), and by SRF only (9.1%). In eyes without ORTs at baseline, IRF only was present in 38.9% of the cases, combined IRF and SRF in 33.3%, and SRF only in 27.8%.

The median follow-up was 11 months. During follow-up, 23 eyes had stable ORTs and 10 eyes had dynamic changes in ORTs. Table 2 summarises the characteristics of eyes with ORTs that were either stable or had changes during the follow-up period. Although statistically not significant, patients with stable

Table 2 Characteristics of eyes with outer retinal tubulations

	Stable ORTs (n=23)	ORTs with changes (n=10)	p Value
Age, median (IQR)	85 (82–89)	80.5 (77–85)	0.107
Sex (female), n (%)	13 (56.5)	4 (40.0)	0.465
Type of fluid, n (%)			0.551
Intraretinal (IRF)	10 (43.5)	6 (60.0)	
Subretinal (SRF)	3 (13.0)	0 (0.0)	
Both (IRF and SRF)	10 (43.5)	4 (40.0)	

IRF, intraretinal fluid; ORTs, outer retinal tubulations; SRF, subretinal fluid.

ORTs tended to be older compared with patients that had changes in ORTs (85 vs 81 years old, $p = 0.107$). The majority of eyes with ORT changes during follow-up had IRF (60.0%) or combined IRF and SRF (40.0%) at baseline. Eyes with stable ORTs had (43.5%) both IRF and combined IRF and SRF at baseline ($p = 0.551$ compared with eyes with ORT changes).

Among the 10 eyes with ORT changes during the treatment period, 5 eyes from five patients had collapsed ORTs during treatment with bevacizumab (figure 1). In two eyes from two patients in this group, the ORTs reappeared within 12 months of stopped anti-VEGF treatment, with no recurrence of fluid on OCT or leakage on fluorescein angiogram (figure 2). In two eyes from two patients, the ORTs increased in size during treatment with bevacizumab (figure 3). ORTs that collapsed without

Figure 1 (A) An outer retinal tubulation (ORT) is present in the outer nuclear layer nasal to the fovea (white thin arrow) adjacent to cystic intraretinal fluid (white solid arrowheads). (B) Two months after start of bevacizumab treatment, the ORT collapsed or disappeared, along with the resolution of the intraretinal fluid.

Table 1 Characteristics of eyes with and without outer retinal tubulations

	Eyes with ORTs (n=33)	Eyes without ORTs (n=18)	All eyes (n=51)	p Value
Age, median (IQR)	83 (79–89)	84 (77–89)	83 (79–89)	0.685
Sex (female), n (%)	17 (51.5)	12 (66.7)	29 (56.9)	0.380
Type of fluid, n (%)				0.259
IRF	16 (48.5)	7 (38.9)	23 (45.1)	
SRF	3 (9.1)	5 (27.8)	8 (15.7)	
Both (IRF and SRF)	14 (42.4)	6 (33.3)	20 (39.2)	

IRF, intraretinal fluid; ORTs, outer retinal tubulations; SRF, subretinal fluid.

Figure 2 (A) An outer retinal tubulation (ORT) is noted in the outer nuclear layer inferior to the fovea (black open arrow) adjacent to cystic intraretinal fluid (white open arrow). (B) Two months after starting bevacizumab treatment, the ORT collapsed or disappeared. (C) The ORT reappeared 13 months after last treatment without any evidence of intraretinal or subretinal fluid.

treatment were noted in two eyes from two patients, but these patients had prior treatment with photodynamic therapy plus pegaptanib with and without bevacizumab (figure 4). In a single eye, the ORT collapsed within 10 months of no treatment and did not reappear upon recurrence of fluid (figure 5).

Table 3 summarises the visual acuity, subfoveal scar characteristics and ELM integrity in the foveal centre in eyes with and without ORTs. In eyes with ORTs, the mean visual acuity was $0.13 (\pm 0.13)$ decimals while eyes without ORTs had a mean of

Figure 3 (A) Two outer retinal tubulations (ORTs) are noted in the outer nuclear layer temporal to the fovea (white thin arrows) adjacent to cystic intraretinal fluid (white solid arrowheads), with nasal subretinal fluid (white open arrowhead). (B) After 31 months of bevacizumab treatment, one ORT increased in size while the other ORT was stable compared with baseline.

Figure 4 (A) A small outer retinal tubulation (ORT) is present in the outer nuclear layer temporal to the fovea (white thin arrow) without any evidence of intraretinal fluid or subretinal fluid. (B) Seven months after starting bevacizumab treatment, the ORT was collapsed or disappeared. (C) Thirteen months after last treatment, the ORT reappeared and slightly increased in size, but still without evidence of fluid. Further retinal thinning and distortion of the foveal contour was also noted.

$0.25 (\pm 0.20)$ decimals ($p=0.071$). Although this is not statistically significant and could be due to the small sample size, this trend suggests that eyes with ORTs may have a lower visual acuity than eyes with no ORTs. Eyes with ORTs had larger scar measured by its GLD compared with eyes without ORTs, with a mean length of $2871.79 (\pm 1587.13) \mu\text{m}$ and of $1637.89 (\pm 1830.37) \mu\text{m}$, respectively ($p=0.027$). Eyes with ORTs

Figure 5 (A) Outer retinal tubulations (ORTs) are present in the outer nuclear layer nasal and temporal to the fovea (white open arrows) above a large disciform scar, with small cystic intraretinal fluid (white thin arrow) temporal to the fovea. (B) After 5 months without any treatment due to very low visual acuity, the ORTs collapsed (white open arrow) and disappeared with shallow elevation of the photoreceptor layer (white thin arrow) in the peripapillary area due to mild appearance of subretinal fluid.

Table 3 Correlation of visual acuity, scar greatest linear diameter, scar height and ELM integrity in the foveal centre in patients with and without outer retinal tubulations

	Eyes with ORTs (n=33)	Eyes without ORTs (n=18)	p Value
Visual acuity (decimals±SD)	0.13 (±0.13)	0.25 (±0.20)	0.071
Scar GLD (µm±SD)	2871.79 (±1587.13)	1637.89 (±1830.37)	0.027
Scar height (µm ±SD)	158 (±145.83)	83.94 (±96.68)	0.055
ELM in the foveal centre			0.017
Intact (%)	2 (6.1)	6 (33.3)	
Disrupted (%)	31 (93.9)	12 (66.7)	

ELM, external limiting membrane; GLD, greatest linear diameter, ORTs, outer retinal tubulations.

tended to have a higher scar height than eyes without ORTs, with a mean height of 158 (±145.83) µm and of 83.94 (±96.68) µm, respectively (p=0.055). The ELM was found disrupted in the foveal centre in around 94% (30 of 33) of the eyes with ORTs and in 67% (12 of 18) of the eyes without ORTs (p=0.017).

DISCUSSION

The purpose of this study was to review the behaviour of ORTs in eyes with wet AMD and to correlate ORT changes with disease activity, presence or absence of fluid, and patient demographic characteristics. The median age of our patients was 83 years old, similar to the age of the patients included in a previous manuscript by Zweifel *et al.*,¹ which first described ORTs (median age 80 years old). In that report, the authors looked into all patients with ORTs regardless of disease type and found that many eyes had wet AMD undergoing anti-VEGF treatment, as well as CNV secondary to causes other than AMD.¹ Our study demonstrated dynamic changes in 10 wet AMD eyes with ORTs as documented by SD-OCT. Changes ranged from collapse, recurrence or enlargement that could be associated with anti-VEGF treatment or spontaneous. Such findings, clearly demonstrating that ORTs may not be stable as thought, have never been reported before. Some ORTs may have a vascular component or may be vascular in nature, considering their response to anti-VEGF treatment, while other ORTs are likely composed only of degenerating photoreceptor cells and may collapse independently from anti-VEGF treatments.

In the CNV classification proposed by Gass,⁵ type 1 (occult) CNV is located between the retinal pigment epithelium (RPE) and Bruch's membrane, while type 2 (classic) CNV is located above the RPE and proliferating in the subretinal space. A study by Faria-Correia *et al.*⁶ published in 2012 found that patients with ORTs had mostly type 2 (classic) CNV, supporting the hypothesis that CNV lesions located above the RPE are more prone to induce ORT formation. In our study, we went further and investigated the association between types of fluid and ORT formation, and found that the majority of eyes with ORTs (48.5%) had IRF, followed by combined SRF and IRF (42.4%), and by SRF only (9.1%). These proportions were not statistically different compared with eyes without ORTs, wherein 38.9% of eyes showed IRF, 33.3% showed combined IRF and SRF, and 27.8% showed SRF only. Our data show that ORTs will most likely have IRF (48.5%) or combined IRF and SRF (42.4%) than SRF alone (9.1%). This is in agreement with the recent data from the Comparison of Age-related Macular

Degeneration Treatments Trials (CATT) study in which eyes with ORTs were more likely to have IRF (92.2%), or SRF and sub-RPE fluid (39.1%).⁷

This study confirms results from previous investigators^{1 2 7} that ORTs are associated with worse visual acuity at baseline and worse prognosis due to their association with disciform scars. The trend is clear that eyes with ORTs have lower visual acuity than eyes with no ORTs, and this is supported by the integrity of the subfoveal ELM where we found that 94% of eyes with ORTs had disrupted ELM. The correlation of the ELM integrity to visual acuity has been previously described.⁸⁻¹⁰ We note that contradictory data about final visual acuity in ORT eyes have been reported. Faria-Correia *et al.*⁶ reported visual improvement during follow-up in the majority of their patients (mean improvement of 3.17 ETDRS letters), while Zweifel *et al.*¹ noted that temporary collapse of ORTs structures was not associated with visual improvement.

Previously published studies have reported that ORTs are stable and do not represent signs of CNV activity.^{1 6} However, we report in this study several changes in ORTs with closed configuration, as described by Schaal *et al.*,¹¹ that may show response to anti-VEGF treatment. In particular, five eyes had collapsed ORTs during treatment with bevacizumab; this could suggest that ORTs collapsing during anti-VEGF treatment and ORTs collapsing after successful anti-VEGF treatment may be vascular-related structures, with the latter being more resistant. The reappearance of ORTs noted in two eyes but without recurrence of fluid could also suggest their vascular nature or that ORTs may have vascular-related structures. On the other side, in two patients who had prior treatment with photodynamic therapy plus pegaptanib with and without bevacizumab, ORTs collapsed several months after treatment; this would suggest that ORTs may be composed of degenerating photoreceptor cells as described by Schaal *et al.*¹¹ Lastly, the persistence of ORTs in eyes with a dry retina after anti-VEGF treatment would suggest a non-vascular entity. Although our data are too small to make a definite conclusion, we demonstrated that not all ORTs are stable but instead can significantly change over time. This finding is also in agreement with previous results of the CATT study;⁷ although seven eyes showed ORTs at week 56, only one eye had persistent ORTs at week 104. This could suggest that some ORTs may be vascular in nature and may respond to anti-VEGF treatment, while other ORTs could alternatively be coincidental in nature.

Schaal *et al.* have described two ORT histological configurations; 'open form', which has horizontally elongated cross sections with curving ELM at the ends and non-photoreceptor cells on the outer aspect, and 'closed form', which are circular or oval with ELM on the outer border and photoreceptors completely encircling the lumen.¹¹ Zweifel *et al.* hypothesised that in some cases ORTs communicating with the subretinal space (referring to the 'open form' described by Schaal *et al.*¹¹) may transiently collapse with anti-VEGF treatment by eliminating fluid from the ORTs.¹ In our study, however, we documented only oval or round ORTs, which refer to the 'closed form' described by Schaal *et al.*¹¹ We found that in 10 eyes there were dynamic changes over time in ORTs with closed configuration; in particular, collapsed ORTs during treatment, recurrent ORTs during no treatment and enlarged or collapsed ORTs without treatment. Considering such dynamic changes, we believe that there must be a direct or indirect response to anti-VEGF treatment. In addition, because some ORTs are not stable, they may not only be composed of photoreceptor cells but at some point they may present vascular-related structures.

Limitations of this study are the retrospective nature and the relatively small amount of wet AMD eyes that showed ORTs that could be evaluated longitudinally. The retrospective OCT imaging review allowed analysis of only a single scan line across the fovea; therefore, ORTs in the remaining macula may have been missed. However, thanks to the use of an accurate and reliable progression scan software as described in the 'Methods' section, we were able to follow precisely the behaviour of ORTs and reported new evidence about these peculiar structures.

In conclusion, this study demonstrated dynamic changes in 10 wet AMD eyes with ORTs as documented by SD-OCT that could be associated with anti-VEGF treatment or spontaneous. Such findings, clearly demonstrating that ORTs may not be stable as thought, have never been reported before. Some ORTs may have a vascular component or may be vascular in nature, considering their response to anti-VEGF treatment, while other ORTs are likely composed only of degenerating photoreceptor cells and may collapse independently from anti-VEGF treatments. This study also confirmed that ORTs are associated with worse visual acuity and worse visual prognosis; in particular, eyes with ORTs have lower visual acuity than eyes with no ORTs due to greater disruption of the subfoveal photoreceptors.

Collaborators Dirk-Uwe Bartsch.

Contributors ME is the lead author. CAA, FM, NC and GB are coauthors. NM is statistician. NF is coauthor and consultant. WRF is chairman and corresponding author.

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Competing interests GB is a full-time employee at Genentech Inc.

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