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Outer Retinal Tubulation as a Predictor of the Enlargement Amount of Geographic Atrophy in Age-Related Macular Degeneration

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Purpose: To determine the prognostic value of outer retinal tubulation (ORT) in the enlargement amount of geographic atrophy (GA) in eyes with age-related macular degeneration (AMD).

Design: Cohort study.

Participants: One hundred eight fellow untreated eyes of 143 patients with GA resulting from AMD enrolled in the MAHALO study (clinicaltrials.gov identifier, NCT01229215) who completely satisfied the study term and had gradable spectral-domain optical coherence tomography (OCT) images obtained at both baseline and month 18 visits.

Methods: The MAHALO study enrolled 143 subjects into a phase 1b/2 multicenter, randomized, single-masked, sham-injection controlled clinical trial of the safety, tolerability, and evidence of activity of lampalizumab in patients with GA associated with AMD. Spectral-domain optical coherence tomography images were obtained at multiple time points in both eyes, although only the baseline and month 18 data of the fellow (nonstudy) eyes were considered in this exploratory analysis. The Cirrus HD-OCT review software was used for automatic segmentation and measurement of GA areas, with manual correction of segmentation errors by certified OCT graders. Baseline OCT images also were assessed for the presence of ORT. The enlargement amount of GA in eyes with ORT was compared with that of eyes without ORT.

Main Outcome Measures: Comparison of the enlargement amount of GA in eyes with and without ORT.

Results: Twenty-four of these 108 eyes demonstrated evidence of ORT. The amount of enlargement of GA in eyes with ORT was significantly slower than that of eyes without ORT (1.85±0.78 vs. 2.67±1.61; $P = 0.001$). This difference remained significant when considering subgroups with unifocal or multifocal GA lesions, because eyes with ORT in both subgroups had a slower enlargement amount of GA than eyes without ORT (2.91±1.70 vs. 2.08±0.88 [$P = 0.01$], in eyes with multifocal GA lesions; and 2.24±1.40 vs. 1.63±0.57 [$P = 0.02$], in eyes with unifocal GA lesions).

Conclusions: In eyes with ORT, GA lesions seem to enlarge at a significantly slower rate than those of eyes without ORT. The presence of ORT may need to be accounted for in longitudinal studies of GA. *Ophthalmology 2014;■:1–7 © 2014 by the American Academy of Ophthalmology.*

In 2009, Zweifel et al[1] first identified the novel optical coherence finding of outer retinal tubulation (ORT) in advanced diseases affecting the outer retina and retinal pigment epithelium (RPE). These ORT lesions were identified as branching tubular structures in the outer nuclear layer of the retina in en face optical coherence tomography (OCT) scans and appeared as round or ovoid hyporeflective spaces with hyperreflective borders on OCT B-scans. Zweifel et al speculated that ORTs formed as a result of a sublethal injury to the photoreceptors, probably through loss of the interdigitation of the outer segments with the RPE or through degeneration of the RPE itself. They theorized that either during or after the injury, there was a disruption of tight junctions with outward folding of the photoreceptor layer until opposite margins of the fold established contact to form a tubular structure. In 2011, Wolff et al[2] challenged this pathogenesis theory and hypothesized that ORT may correspond to giant cells.

Regardless of the precise pathogenic mechanisms, several additional reports have described the presence of ORTs in various outer retinal degenerative disorders and in both advanced neovascular and atrophic age-related macular degeneration (AMD).[3,4] Indeed, in a subgroup of the Geographic Atrophy Treatment Evaluation (GATE) study, Moussa et al[5] reported that ORT was extremely common in patients with geographic atrophy (GA), present in 65% of eyes in atrophic region and in 26% of eyes in junctional zone, which seemed to be associated with a significantly faster growth in the GA lesions.

The MAHALO study enrolled patients with geographic atrophy secondary to AMD into a phase 1b/2 randomized trial to evaluate the safety, tolerability, and efficacy of lampalizumab. Because the fellow eyes in the MAHALO study also were required to have evidence of atrophy and OCTs were obtained at multiple time points, the MAHALO study provided an opportunity to study the prevalence of

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ORTs and their influence on the progression of the atrophic lesions over time.

Methods

The MAHALO study (clinicaltrials.gov identifier, NCT01229215) enrolled 143 subjects into a phase 1b/2 multicenter, randomized, single-masked, sham-injection controlled clinical trial of the safety, tolerability, and evidence of activity of lampalizumab in patients with GA associated with AMD. To be included in the trial, patients had to have evidence of GA in both eyes resulting from AMD only and without evidence of past or present choroidal neovascularization. Spectral-domain (SD) OCT images were obtained at multiple time points in both eyes using the Cirrus HD-OCT (Carl Zeiss Meditec, Inc, Dublin, CA), with the 512 A-scans × 128 B-scans macular cube protocol over a 6×6-mm area centered on the foveal center. For this exploratory analysis, only the baseline and month 18 data of fellow (nonstudy) eyes were considered. The research protocol was approved by the Institutional Review Board of the University of Southern California, and the research adhered to the tenets set forth in the Declaration of Helsinki. Patients were not included in this analysis if the GA extended outside the OCT scan field at the baseline visit or if the signal strength was poor (less than 6) at any point during the study, thereby affecting the quality of the OCT data.

Optical Coherence Tomography Grading

All OCT volume scans of the fellow (nonstudy) eye at baseline and month 18 were reviewed by 2 independent, masked, certified OCT graders (A.H., M.G.N.) at the Doheny Image Reading Center. For this assessment, all 128 B-scans for each case, as well as en face images at the level of the outer nuclear layer, were reviewed carefully for the presence of ORT. En face images were viewed using the advanced visualization function of the Cirrus HD-OCT review software (version 6.0.2). Figure 1 shows a single OCT B-scan and an en face image of an eye with ORT. Criteria for ORT identification and detection were based on the definition by Zweifel et al.[1] For this analysis, graders only needed to determine whether ORT was present; the severity or extent of ORT was not quantified. The results from the 2 graders were compared, and for discrepant cases, the 2 graders met in open adjudication to try to arrive at a consensus result. If the 2 graders could not resolve their disagreement, a final determination regarding the presence of ORT was made by the reading center director (S.R.S.).

Grading of GA areas at the reading center also was performed using the Food and Drug Administration–cleared Advanced RPE Analysis Tool, which is available in Cirrus HD-OCT review software (version 6.0.2 being used in this analysis). The Advanced RPE Analysis Tool segments and identifies GA based on sub-RPE illumination. Previous studies have shown excellent agreement between GA lesion area as determined by OCT or by fundus autofluorescence.[6] Nonetheless, the segmentation result of the RPE analysis was reviewed by trained, certified OCT graders to assess for the presence of segmentation errors. The Advanced RPE Analysis Tool does permit graders to adjust the segmentation boundaries manually to the correct location before calculation of the GA area. Initial correction of all segmentation errors was performed by 1 grader (A.H.). To be considered as an atrophic lesion, the diameter of the lesion should be more than the diameter of a retinal vein (125 μm). Figure 2 shows an example of an automatically generated GA segmentation map with evidence of segmentation error and the same image after manual correction by the grader. To evaluate the reproducibility of this method, a second grader (M.G.N.) regraded 30% of the cases (both the baseline and month 18 visit for each case) in a masked fashion.

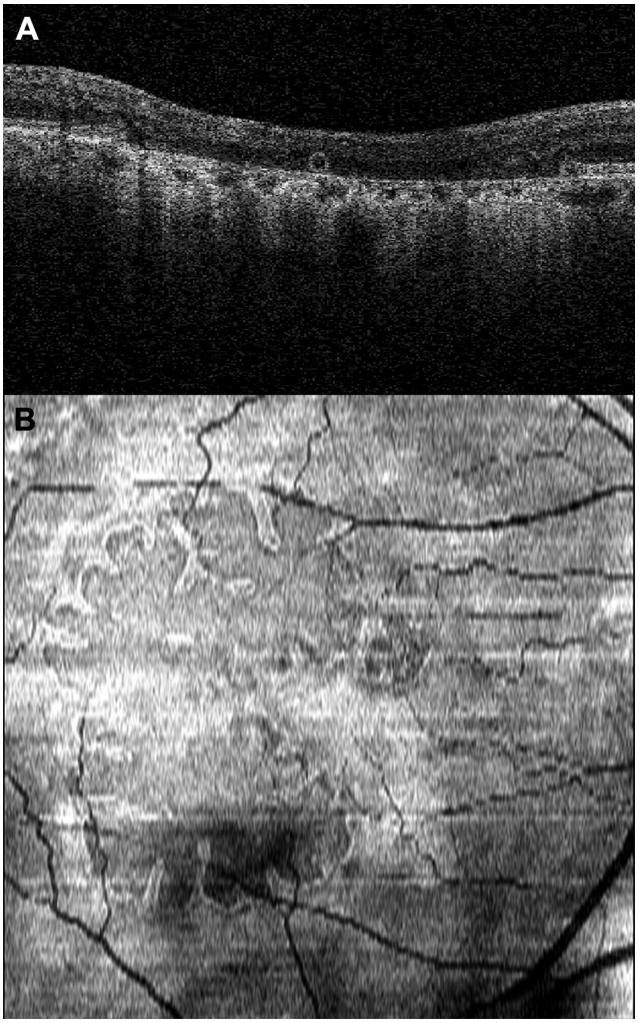

Figure 1. A, Optical coherence tomography (OCT) B-scan from an eye with geographic atrophy. An area of outer retinal tubulation (ORT) is identified by a hyperreflective ring-like structure in the outer nuclear layer, with a hyporeflective center. **B**, Corresponding en face OCT slab image at the level of the outer nuclear layer. The perilesional pattern of the ORTs can be appreciated easily.

The study by Yehoshua et al[7] showed that when GA areas were analyzed after applying a square root transformation (SQRT), the enlargement amount did not show any dependence on the baseline lesion size; thus, for all GA lesions, SQRT was calculated and comparisons were made based on this parameter also. The enlargement amount of GA at 18 months for all eyes was calculated and the amount was compared between eyes with ORT and eyes without ORT.

Statistical Methods

To compare the enlargement amount of GA and the enlargement amount of SQRT of GA in eyes with ORT at baseline versus eyes without ORT, a Student *t* test was performed. Significance was set at a $P<0.05$ for the test. Intraclass correlation coefficients, Bland-Altman plots, and interrater agreement (κ value) were used to