Published in final edited form as:

Ophthalmology. 2013 May ; 120(5): 1038–1045. doi:10.1016/j.ophtha.2012.10.018.

Progression of intermediate age-related macular degeneration with proliferation and inner retinal migration of hyperreflective foci

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Abstract

Purpose—Drusen and migrating retinal pigment epithelium have been associated with hyperreflective foci (HF) detected by spectral domain optical coherence tomography (SDOCT). This study sought to quantify the change in intraretinal HF distribution and its correlation with age-related macular degeneration (AMD) disease progression.

Design—Prospective observational study from the multicenter Age-Related Eye Disease Study 2 (AREDS2) Ancillary SDOCT Study.

Participants—Patients (n = 299) with one enrolled eye with intermediate AMD and baseline SDOCT, followed by SDOCT imaging at 1-year and 2-year visits.

Methods—The number and location of HF were scored in SDOCT scans of all 299 eyes. The change in transverse (horizontal) and axial (vertical) distribution of HF in the macula were evaluated with pairwise signed rank tests. Two-year inner retinal HF migration was determined by the change in HF weighted axial distribution score (AxD) calculated for each eye. The correlation of HF with SDOCT features of AMD progression was evaluated with logistic regression analysis.

Main Outcome Measures—Mean change in number of HF, transverse and axial distribution of HF in the macula, and the AxD per eye.

Results—In 299 study eyes, the 2-year increase in the number of HF ($p < 0.001$) and AxD ($p < 0.001$) per eye represented longitudinal proliferation and shift to inner retinal layers, respectively.

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DISCLOSURES C. Presentation: Presented in part at the annual meeting of the American Academy of Ophthalmology, November 2012, Chicago, Illinois.

E. Financial Disclosure(s): The author(s) have made the following disclosure(s): Cynthia A. Toth: Genentech (research support), Biophtigen (research support), Physical Sciences Inc. (research support). Duke University has an equity and intellectual property interest in Biophtigen. Stephanie J. Chiu, Sina Farsiu, and Cynthia A. Toth have patents pending on image processing. The remaining authors do not have any financial disclosures.

Eyes with geographic atrophy (GA) at 2 years were correlated with presence of baseline HF ($p < 0.001$; odds ratio [OR], 4.72; 95% confidence interval [CI], 2.43–9.80), greater number of baseline HF ($p < 0.001$; OR, 1.61 per HF; 95% CI, 1.32–2.00) and greater baseline AxD ($p < 0.001$; OR, 1.58 per AxD point; 95% CI, 1.29–1.95).

Conclusions—Proliferation and inner retinal migration of SDOCT HF occurred during follow-up in eyes with intermediate AMD. These characteristics were associated with greater incidence of GA at year 2; therefore, SDOCT HF proliferation and migration may serve as biomarkers for AMD progression.

INTRODUCTION

In developed nations, age-related macular degeneration (AMD) is the most prevalent cause of central vision loss in the elderly population.¹ AMD is a progressive neuroretinal degenerative disease in which patients advance from early and intermediate stages characterized by changes in pigment and drusen deposits to more advanced pathology such as geographic atrophy (GA) and choroidal neovascularization (CNV). The exact pathogenic mechanisms of AMD are not completely understood, so precise clinical characterization of early lesions of AMD and their progression over time can provide insight into pathophysiology and guide research on treatments.^{2–5}

Optical coherence tomography (OCT) has become a valuable tool in characterizing the pathologic chorioretinal morphology that occurs in AMD. Spectral domain OCT (SDOCT) technology provides higher resolution and speed of image acquisition, compared with earlier time domain OCT imaging instruments. However, color fundus photographs (CFP) remain the gold standard for defining AMD-associated lesions, clinically staging AMD, and studying the epidemiology of this disease.^{6,7} Color photographs provide an *en face* view of the retina, but SDOCT allows cross-sectional visualization that permits image readers to characterize microstructural alterations in the different laminae of the retina.⁸

The appearance of changes in pigment in the retina on CFP is a characteristic defining feature of intermediate AMD. Large clinical trials have shown that the presence of macular hyperpigmentary changes in intermediate AMD increase the risk of progression to GA and CNV.^{2,9,10} The clinical significance of hyperpigmentary change has prompted further characterization of pathology associated with this feature of AMD. In a prospective pilot study of eyes with intermediate AMD, Jain et al. described how CFP hyperpigmentation corresponded to intraretinal pigmentary migration on SDOCT with obscuration of underlying large drusen that were not visible on CFP.¹¹ Ho et al. defined intraretinal hyperreflective foci (HF) as discrete, well-circumscribed lesions with greater reflectivity than the retinal pigment epithelium (RPE) band on SDOCT and associated with hyperpigmentation on CFP.¹² We have recently demonstrated the highly-specific spatial correlation of macular hyperpigmentation on CFP with SDOCT HF in the neurosensory retina and on the inner surface of the apex of drusen in a prospective study of intermediate AMD.¹³ On SDOCT imaging at baseline for all eyes enrolled in this prospective AMD study, intraretinal HF have been reported in the outer nuclear layer in areas with significant photoreceptor layer thinning, at the apex of drusen, and overlying areas of RPE atrophy.^{14,15} Therefore, it appears that HF are important both in the pathophysiology of AMD as well as the clinical course of the disease.

In previous reports characterizing the association between CFP hyperpigmentation and SDOCT HF, images were analyzed only from the baseline visit of eyes enrolled in a prospective multicenter SDOCT study for intermediate AMD.^{11,13–15} The purpose of this study is two-fold: first, to determine the change in quantity and distribution of SDOCT HF within the macula over a prospective 2-year follow-up period; second, to correlate SDOCT

HF with the development of morphologic features suggestive of advanced AMD. We hypothesize that tracking these lesions may serve as a predictive marker for estimating the odds of disease progression. Risk stratification based on SDOCT biomarkers may also become clinically useful for determining new points of future pharmacologic intervention.

METHODS

For the purpose of this study, OCT imaging was obtained from patients enrolled in a study auxiliary to the prospective randomized Age-Related Eye Disease Study 2 (AREDS2, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00345176) identifier NCT00345176) for AMD. The AREDS2 Ancillary SDOCT Study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00734487) identifier NCT00734487) recruited 345 participants from four AREDS2 clinical trial centers in the United States. Its purpose was to analyze changes seen with SDOCT imaging over time and determine whether these changes were associated with AMD progression and vision loss. The AREDS2 Ancillary SDOCT Study gained approval from the institutional review board of each of the four associated study centers, obtained written informed consent from each participant, and followed the tenets for human research set forth by the Declaration of Helsinki. Data management followed strict guidelines based on the Health Insurance Portability and Accountability Act.

Each participant in the AREDS2 Ancillary SDOCT Study contributed only one eye classified as category 3 AMD based on the presence of several medium drusen (diameter 63-124 μm) or at least one large druse (diameter $\geq 125 \mu\text{m}$) on color fundus photography graded by the Wisconsin Fundus Photograph Reading Center (University of Wisconsin, Madison, WI). For participants with bilateral category 3 AMD, the right eye was arbitrarily enrolled in the study. Three participants were excluded after inadequate SDOCT imaging due to insufficient media clarity. Another 28 participants were excluded due to bilateral category 4 AMD, based on central GA or CNV detected by color fundus photography.

The remaining 314 eyes that met the inclusion criteria had baseline OCT imaging performed by certified technicians with an investigational spectral domain ophthalmic imaging system (Bioptigen SDOIS; Bioptigen, Research Triangle Park, NC). At the same clinical center, OCT imaging was performed again at the year 1 and year 2 visits. The SDOCT imaging system consisted of an 840-nm wavelength superluminescent diode light source, a spectrometer capturing the interference signal, and Fourier signal transformation to resolve scan depth with an axial resolution of 4.5 μm per pixel. The AREDS2 Ancillary SDOCT Study imaging protocol required a raster scan that comprised 100 horizontal B-scan lines with 1000 A-scans per line, separated by an interval of 66 μm between lines, and spanning a 6.7-mm x 6.7-mm square surface area centered on the fovea. The unprocessed volume of B-scans from each visit was decoded and assigned a study identifier, then transferred to the Duke Advanced Research in SDOCT Imaging Laboratory (DARSI, Duke University, Durham, NC) for formal OCT grading by certified investigators.

For grading of OCT scans, images were displayed with the Duke OCT Retinal Analysis Program (DOCTRAP), which was developed with technical programming software (MATLAB; MathWorks, Natwick, MA) for retinal layer segmentation in SDOCT images, previously described in detail.^{16,17} The viewing program displayed SDOCT cross-sectional B-scans with lines delineating the circumference of three rings with 500- μm , 1-mm, and 6-mm diameters centered on the fovea. For the purpose of this study, two investigators (JGC, FAF) graded each eye and the principal investigator (CAT) served as arbiter of grading. Markers were placed over all focal hyperreflective lesions detected within the retina, adjacent to the RPE layer, or adjacent to the inner border of drusen. Markers were placed over lesions that were focal, well-circumscribed, hyperreflective (i.e. reflectivity greater

than the RPE layer), and clearly distinguishable from cross-sections of retinal blood vessels, as described in previous studies.^{12,13}

During grading of SDOCT scans, the transverse (horizontal) location of each HF lesion was classified with respect to the center of the fovea: (1) within the central 500- μ m field, (2) between the 500- μ m and 1-mm rings, or (3) between the 1-mm and 6-mm rings. Retinal images *en face* were generated from the summed voxel projection (SVP) of the SDOCT scan, plotting the distribution of HF markers in the Page 6 of 18 transverse dimension (Fig 1). The axial (vertical) location of each lesion was classified with respect to the retinal layers: (1) adjacent to RPE or apex of drusen, (2) the inner segment band that approximates the location of the photoreceptor inner and outer segments, (3) the outer nuclear layer (ONL), (4) the outer plexiform layer (OPL), or (5) the inner retinal layers that include the inner nuclear, inner plexiform, ganglion cell, and nerve fiber layers. For each HF lesion, any morphologic pathology crossing the same axial plane of the same SDOCT B-scan was recorded as follows: (1) RPE elevation (such as drusen or RPE detachment), (2) RPE atrophy, (3) epiretinal membrane, (4) cystoid macular edema, and (5) subretinal fluid between the RPE and photoreceptor layers.

The transverse and axial distributions of HF were defined as the number of HF present in each concentric macular field and each retinal layer, respectively. The transverse density of HF was defined as the number of HF per mm² in each concentric macular field, and then reported as the mean $\pm$ standard deviation (SD) for all study eyes. The changes in transverse distribution, axial distribution, and associated pathology between baseline, year 1, and year 2 visits were compared for all eyes in pairwise analyses with the nonparametric Wilcoxon signed rank test.

In order to quantify inner retinal shift or migration of foci, an axial distribution score (AxD) was calculated for each eye at each study visit. The AxD is a novel weighted score defined as the sum of all SDOCT HF detected in one eye per visit, with each single HF multiplied by a correction factor (y) whose value was determined by its location in the RPE or drusen apex ($y = 1$), inner segment band ($y = 2$), ONL ($y = 3$), OPL ($y = 4$), or the inner retinal layers ($y = 5$). To obtain the final AxD value, the total sum of the products was divided by the total number of detected HF, which weighed the score against the confounding effect of different quantities of HF between two visits. The shift in axial distribution within the retinal layers was tested by comparing the AxD between baseline, year 1, and year 2 visits in a pairwise analysis with the nonparametric Wilcoxon signed rank test. An increase in AxD estimated a shift to the inner retinal layers, whereas a decrease in AxD estimated a distribution shift of foci towards the RPE.

In another arm of this study, baseline HF characteristics were correlated with the odds of progression to advanced AMD on SDOCT imaging. The presence or absence of the following features was recorded by the investigators exclusively based on SDOCT images: (1) non-central GA anywhere in the 6-mm diameter field, (2) central GA consistent with category 4 non-neovascular AMD, and (3) subretinal lesions likely caused by CNV and consistent with category 4 neovascular AMD. In order to exclude coincident parapapillary atrophy, we excluded isolated sections of retinal and RPE atrophy adjacent to the nasal rim of the 6-mm diameter field, which was adjacent to the temporal rim of the optic disc, from the analysis of non-central GA. The incidence of these disease progression features at the final year 2 visit was correlated with the following baseline characteristics: (1) presence or absence of HF at baseline (a binomial categorical variable), (2) the number of HF at baseline, and (3) the numerical AxD for each eye at baseline. Nominal logistic regression models were created to test the correlation of baseline characteristics with the appearance of advanced AMD pathology at year 2. The regression models were designed to control for the

confounding effect of advanced AMD pathology present on baseline SDOCT, which was not detected by baseline color fundus photography. All statistical analysis was performed with SAS analytics software (JMP 10; SAS Institute Inc, Cary, NC).

RESULTS

Total quantity and transverse distribution of hyperreflective foci

From the 314 eyes enrolled in the prospective multicenter AREDS Ancillary SDOCT Study, 299 eyes met the present study's inclusion criteria of protocol-compliant SDOCT imaging at the baseline visit, as well as imaging at year 1 or year 2 visits. There were 296 eyes with SDOCT imaging at both the baseline and year 1 visits. The rate of change in HF from the baseline to year 1 visit was determined by paired analysis of each of these 296 eyes. There were 284 eyes with SDOCT imaging at both the baseline and year 2 visits, and there were 281 eyes with imaging at both the year 1 and year 2 visits. The rate of change in HF from the year 1 to year 2 visit was determined by paired analysis of these 281 eyes with imaging available at both visits after baseline.

Table 1 shows that the total HF number increased from 367 HF among all eligible 299 eyes at baseline (1.33 ± 1.56 HF/eye), to 567 HF among all 296 eyes at year 1 (1.92 ± 1.82 HF/eye), and 846 HF among all 284 eyes at year 2 (2.98 ± 3.24 HF/eye). The increase in mean HF per eye from baseline to year 1, and from the year 1 to year 2, was significant ($p < 0.001$ for both). Within this cohort, there were zero HF detected in 124 eyes (41%) at baseline, then zero HF in 73 eyes (25%) at year 1, and 68 eyes (24%) at the year 2 visit.

The HF quantity and density (HF/mm²) within the central 500 μ m of the fovea increased significantly from year 1 to year 2 only ($p = 0.001$), whereas the number and density of HF between the 500- μ m and 1000- μ m rings increased significantly only from baseline to year 1 ($p < 0.001$). The number and density of HF outside of the fovea, from the 1000- μ m to 6000- μ m rings, increased from baseline to year 1, as well as year 1 to year 2 ($p < 0.001$ for both). In a subanalysis of the central 500- μ m field, eyes without baseline HF had significantly greater increase in HF at year 1, whereas there was a significantly greater increment in HF detected from year 1 to year 2 in eyes with HF at baseline.

Axial distribution of hyperreflective foci among retinal layers

Table 2 shows the change in axial distribution of SDOCT HF from outer to inner retinal layers. From the baseline to year 1 visit, SDOCT HF increased significantly at the RPE and at the apex of drusen ($p < 0.001$), in the ONL ($p < 0.001$), and in the inner retinal layers ($p = 0.035$). The distribution of HF detected adjacent to the inner segment band did not increase significantly over time from baseline to year 2. From the year 1 to year 2 visits, the distribution of HF increased significantly in the ONL ($p < 0.001$), OPL ($p < 0.001$), and inner retinal layers ($p = 0.014$). The mean AxD per eye increased from 1.55 ± 1.47 at baseline to 1.85 ± 1.31 at year 1 ($p < 0.001$), and from 1.85 ± 1.31 at year 1 to 2.07 ± 1.39 at year 2 to ($p < 0.001$).

Comparison of yearly changes in distribution

The change in number and distribution of HF from baseline to year 1 was compared with the change from year 1 to year 2 in paired scans for each eligible study eye (Table 3). With regard to transverse distribution of HF, the change in distribution from year 1 to year 2 increased significantly over the change from baseline to year 1 in the total field ($p = 0.038$), 500- μ m to 1000- μ m field ($p = 0.007$), and outside 1000 μ m ($p < 0.001$). Not all eyes with intermediate AMD had an increase in HF over time. There was a decrease in number of HF from baseline to year 1 in 55 eyes (19%), and a decrease in number of HF from year 1 to

year 2 in 59 eyes (21%). There was no significant difference in the rate of increase from baseline to year 1 versus the increase from year 1 to year 2 in the AxD score. Although AxD increased significantly at each follow-up period in the pairwise analysis, not all individual eyes had an increase in AxD. There was a decrease in AxD from baseline to year 1 in 80 eyes (27%), and a decrease in AxD from year 1 to year 2 in 79 eyes (28%).

Vitreoretinal pathology associated with hyperreflective foci

The mean number of HF per eye associated with RPE elevation or drusen in the same axial plane increased significantly from baseline to year 1 ($p < 0.001$) and from year 1 to year 2 ($p = 0.001$). The mean number of HF associated with RPE atrophy significantly increased from year 1 to year 2 ($p = 0.003$), and mean number of HF associated with epiretinal membrane only significantly increased from the baseline to year 1 visit ($p = 0.031$) but remained almost equal from the year 1 to year 2 visit (Table 4). Among all study eyes, there was significant change in the frequency of pathology associated with progression of AMD. The total number of eyes with any GA detected in the total 6-mm diameter field increased significantly from baseline to year 1 ($p < 0.001$) and then from year 1 to year 2 ($p < 0.001$). The incidence of central GA and subretinal SDOCT lesions consistent with CNV also increased from each study period to the next, but the number of eyes was small in both groups. Nonetheless, the increase in eyes with CNV-like lesions was significant at the year 1 and year 2 visits ($p = 0.002$ for both, Table 4).

Predictors of age-related macular degeneration progression

Advanced AMD pathology observed at year 2 was correlated with baseline HF characteristics in 284 eligible eyes with SDOCT imaging at the baseline and year 2 visits (Table 5). Of 98 eyes that developed any GA in the total field by year 2, 76 eyes (78%) had HF detected at baseline, compared with only 45 eyes without baseline HF that had GA detected at year 2. Logistic regression determined that the presence of baseline HF was significantly associated the appearance of any GA at year 2, independent of the presence of baseline GA ($p < 0.001$; odds ratio [OR], 4.72; 95% confidence interval [CI], 2.43–9.80). Eyes that developed GA by year 2 possessed significantly more baseline HF per eye than eyes without any GA by year 2 ($p < 0.001$; OR, 1.61 per HF; 95% CI, 1.32–2.00). Moreover, eyes with any GA by year 2 had a higher baseline AxD score than eyes that did not develop GA ($p < 0.001$; OR, 1.58 per AxD point; 95% CI, 1.29–1.95). Baseline GA was not correlated with the presence of baseline HF ($p = 0.09$), number of baseline HF per eye ($p = 0.08$), or with baseline AxD per eye ($p = 0.18$). These baseline HF characteristics did not predict the development of central GA or likely CNV after 2 years of follow-up (Table 5).

DISCUSSION

The purpose of this study was to examine SDOCT scans of eyes enrolled in the AREDS2 Ancillary SDOCT Study, in order to determine the longitudinal change in SDOCT HF seen in intermediate AMD and the concurrent appearance of AMD-associated pathology. The results of the present study showed that the total number of HF increased significantly over 2 years of follow-up. A weighted AxD score developed for the purpose of this study also increased significantly over 2 years, suggesting that over time the HF distribution in the inner retinal layers increases in AMD and that HF may migrate from outer to inner retinal layers. Eyes with features of GA detected on SDOCT at 2 years were significantly correlated with the presence of HF, greater HF number, and greater AxD score at baseline.

After corroborating the association of SDOCT HF with drusen and RPE atrophy at baseline in non-advanced AMD, this study has also shown an increase in the mean number of HF associated with drusen and RPE atrophy from baseline to year 2. The mechanism behind

why there is a correlation of HF with drusen and RPE atrophy may be explained by a combination of findings in previous studies. Schuman et al. found that SDOCT intraretinal HF were associated with photoreceptor thinning and underlying drusen, suggesting these features were a precursor to geographic atrophy.¹⁵ Subsequently, Leuschen et al. showed that HF were associated with the drusen apex, and that these HF were significantly associated with RPE atrophy at the baseline visit of eyes enrolled in the AREDS2 Ancillary SDOCT Study.¹⁴ The present longitudinal study suggests that these associations become more significant over time, with more HF per eye associated with drusen and RPE atrophy at each subsequent time point.

Basic research to correlate SDOCT HF with an underlying histopathology has revealed several likely etiologies for these characteristic lesions. Ma et al. demonstrated that activated intraretinal microglia migrated to the subretinal space and RPE layer below the retina, and these microglia altered the structure and function of RPE cells with respect to their ability to nourish the photoreceptors and choriocapillaris.¹⁸ Microglial cell migration has also been described in human retinitis pigmentosa, in addition to AMD. Gupta et al. concluded that microglial migration observed in these diseases occurred secondary to photoreceptor cell layer death.¹⁹ In AMD, photoreceptor layer death likely results from RPE cell hyperplasia, fibroblast transformation, or atrophy, secondary to oxidative damage²⁰ and inflammation.²¹ Some predisposed individuals are believed to be especially susceptible by alternative complement pathway dysregulation.^{22,23} When RPE cells are healthy, they secrete immunosuppressants into the retina that suppress microglial activation;²⁴ however, under chronic injury or demise the RPE no longer performs this function and microglia migrate into the subretinal space.

Although there is evidence for activated glial cell migration to outer retinal layers, the longitudinal distribution changes in this study warrant an explanation for HF shift to inner retinal layers in AMD. In intermediate AMD, SDOCT HF may represent activated RPE cells migrating into the inner nuclear and inner plexiform layers.^{12,25} Laboratory studies have demonstrated the potential for RPE cell migration in response to factors associated with AMD pathogenesis. The combination of oxidative damage, complement activation, and other factors inducing RPE cell injury activate macrophages that release cytokines and other inflammatory mediators including tumor necrosis factor- α and transforming growth factor- β . Evidence suggests that these factors induce RPE cell migration.^{26,27} Moreover, dysfunction of the RPE is known to cause atrophy of the underlying choriocapillaris.²⁸ With choriocapillaris atrophy, secretion of vascular endothelial growth factor becomes upregulated, and this factor also induces reactive RPE cell proliferation and migration.²⁹⁻³¹ However, intraretinal RPE cell migration in early or intermediate AMD eyes has not yet been well described with definitive immunostaining techniques.

Several *in vivo* OCT studies have now proposed that HF in intermediate AMD represent alterations of RPE cells, which may relocate spatially to interior layers of the retina. In addition to reports from the AREDS2 Ancillary SDOCT Study, Fleckenstein et al., Pieroni et al., and Ho et al. proposed evidence that hyperreflective lesions in the retina represented migration of the RPE.^{12,25,32} Fleckenstein et al. reported that hyperreflective clumps throughout the retinal layers, plaques of the outer retinal bands, and increased or decreased band reflectivity were all present in eyes with advanced non-neovascular AMD.³² While these eyes had progressed beyond intermediate AMD, the correlation between HF and microstructural changes in the RPE layers has been established in a reproducible manner. Pieroni et al. found that 47 of 52 eyes with a clinical diagnosis of non-exudative AMD displayed drusen or RPE changes on ultra high resolution OCT. Upon closer examination of RPE changes, 15% of eyes had RPE hyperpigmentary changes on CFP, which all showed “clumping of hyperreflective material” adjacent to the RPE on OCT, and 4 of these eyes had

HF in the inner retinal layers suggestive of RPE migration.²⁵ In this report from the AREDS2 Ancillary SDOCT Study, the increase in AxD score for intermediate AMD eyes at each subsequent annual visit supports our hypothesis that SDOCT HF are markers for migration of RPE cells in response to chemotactic stimuli. These factors are either released primarily from the neurosensory retina or secondarily from RPE injury during the course of AMD progression.

During SDOCT grading for the present study, we sought to exclude other intraretinal hyperreflectivities that we believed did not correspond to transformed and/or migrating RPE cells on SDOCT. In neovascular AMD, CNV produces subretinal fluid and fibrovascular membranes, which may generate a myriad of punctate hyperreflective specks in the subretinal space. The process of CNV introduces abnormal friable blood vessels that leak into subretinal and intraretinal potential spaces, and the hyperreflectivities likely represent lipid exudates or inflammatory crystalline deposits due to vascular leakage. Subretinal punctate hyperreflectivities observed in the few eyes with likely CNV lesions were not marked as SDOCT HF; however, any focal intraretinal lesions meeting criteria for HF were marked as such.

There were limitations to this study with regards to AMD progression endpoints and histopathological correlation. First, the AREDS2 Ancillary SDOCT Study was prospective in design, and the subjects in this study have been followed for 2 years with SDOCT imaging thus far. This duration of time may not have been sufficient to obtain a cohort of patients that was large enough to achieve statistically significant conversion from intermediate to advanced AMD. More years of follow-up will produce more patients developing central GA or CNV in this cohort, permitting more robust conclusions about SDOCT biomarkers that predict progression. However, longer follow-up periods will also be subject to higher study drop-out rates due to the advanced age of these patients. Second, while SDOCT imaging and color fundus photography was available for subjects enrolled in AREDS2, no histological samples of chorioretinal tissue was collected. Without histological correlation of the lesions producing SDOCT HF with immunohistochemistry markers for reactive RPE or microglial cells, we cannot definitively conclude that these SDOCT lesions are RPE cells migrating to inner retinal layers or, alternatively, activated microglial cells. We speculate that postmortem specimens donated from subjects previously enrolled in these clinical trials could elucidate the definitive cell type, or multiple cellular etiologies, of intraretinal HF in eyes with intermediate AMD.

In this study of SDOCT HF detected in eyes with intermediate AMD, we showed that HF proliferate significantly over time and the distribution of HF shifts gradually but significantly from the RPE and outer retina to the inner retinal layers during follow-up. The presence of HF at baseline and greater distribution in the inner retinal layers were predictive of development of areas of GA in the macula. The duration of follow-up may not have been sufficient to find a significant correlation between inner retinal migration and progression to central GA or CNV. This study introduced a novel AxD score, which was determined for each eye at a specific time point based on the quantity and axial distribution of SDOCT HF. The AxD increased with greater accumulation of HF in the inner retinal layers, and it was weighted to account for the effect of the number of HF on the AxD value. Future directions of investigation include refinement of this weighted HF score to provide physicians with a reproducible calculation to risk stratify patients with intermediate AMD. Software algorithms for coregistration of sequential SDOCT scans may be able to track the appearance or movement of HF in the same eye over multiple clinical visits without manual grading. Further investigation is required to determine whether this biomarker may serve to develop new endpoints for clinical imaging trials and more accurate disease monitoring in clinical practice.

Acknowledgments

D. Financial Support: The AREDS2 Ancillary SDOCT Study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00734487) identifier: NCT00734487) is supported in part by Biopogen (equipment), Genentech (IST-4400S grant), and Alcon Laboratories (unrestricted startup grant). The laboratory of Sina Farsiu is supported in part by the American Health Assistance Foundation (research support) and the NIH (P30 EY-005722 grant). The laboratories of Drs. Toth and Farsiu are both supported by Research to Prevent Blindness (unrestricted grant).

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Figure 1.

Magnified spectral domain optical coherence tomography (SDOCT) images (*top*) correspond to the fovea of SDOCT-derived retinal maps (*bottom*) with rings indicating the 500- μ m (*blue*), 1-mm (*pink*), and 6-mm (*white*) fields in a single study eye at the (A) baseline, (B) year 1, and (C) year 2 visits. The foveal SDOCT images (*top*) show the appearance at year 1 and subsequent inner retinal migration at year 2 of a hyperreflective focal lesion (*arrowheads*) in the central 500- μ m field, associated with growth of underlying drusen. The corresponding retinal maps (*bottom*) show proliferation of total hyperreflective foci (*asterisks*) over time.

Pairwise longitudinal comparison of the mean transverse distribution of SDOCT HF per study eye from each subject from baseline to year two. P values determined by the Wilcoxon signed rank test.

Table 1

Group	Baseline (Y0)		Year 1 (Y1)		Y0 vs Y1		Year 2 (Y2)		Y1 vs Y2	
	N	mean ± SD	N	mean ± SD	P value	N	mean ± SD	P value	N	P value
Total eyes	299		296			284				
Transverse distribution (HF)										
Total field	398	1.33 ± 1.56	567	1.92 ± 1.82	<0.001	846	2.98 ± 3.24	<0.001		
Central 500 µm	42	0.14 ± 0.43	52	0.18 ± 0.42	0.182	84	0.30 ± 0.74	0.001		
500 – 1000 µm	70	0.23 ± 0.57	123	0.42 ± 0.69	<0.001	114	0.40 ± 0.85	0.565		
Outside 1000 µm	286	0.96 ± 1.23	392	1.32 ± 1.50	<0.001	646	2.27 ± 2.59	<0.001		
Transverse density (HF/mm²)										
Total field		0.05 ± 0.06		0.07 ± 0.06	<0.001		0.11 ± 0.11	<0.001		
Central 500 µm		0.72 ± 2.21		0.90 ± 2.16	0.182		1.51 ± 3.75	0.001		
500 – 1000 µm		0.40 ± 0.96		0.71 ± 1.18	<0.001		0.68 ± 1.44	0.565		
Outside 1000 µm		0.03 ± 0.04		0.05 ± 0.05	<0.001		0.08 ± 0.09	<0.001		
Subanalysis of central 500 µm										
Eyes without baseline HF	124									
Transverse distribution (HF)	0	0	7	0.06 ± 0.23	0.016	11	0.09 ± 0.34	0.395		
Transverse density (HF/mm ²)		0		0.29 ± 1.18	0.016		0.46 ± 1.72	0.395		
Eyes with baseline HF	175									
Transverse distribution (HF)	42	0.24 ± 0.55	45	0.26 ± 0.50	0.673	73	0.45 ± 0.87	0.001		
Transverse density (HF/mm ²)		1.22 ± 2.79		1.32 ± 2.55	0.673		2.28 ± 4.45	0.001		

SDOCT = spectral domain optical coherence tomography; HF = hyperreflective foci; SD = standard deviation.

Table 2

Pairwise longitudinal comparison of the mean axial distribution of SDOCT HF per study eye from each subject from baseline to year two. P values determined by the Wilcoxon signed rank test.

Group	Baseline (Y0)		Year 1 (Y1)		Y0 vs Y1		Year 2 (Y2)		Y1 vs Y2	
	N	mean ± SD	N	mean ± SD	P value	N	mean ± SD	P value	N	P value
Total eyes	299		296			284				
Axial distribution (HF)										
RPE / drusen apex	58	0.19 ± 0.51	104	0.35 ± 0.57	<0.001	100	0.35 ± 0.79		100	0.561
Inner segment band	99	0.33 ± 0.71	118	0.40 ± 0.63	0.149	97	0.34 ± 0.65		97	0.289
Outer nuclear layer	178	0.60 ± 1.04	248	0.84 ± 1.27	<0.001	428	1.51 ± 2.07		428	<0.001
Outer plexiform layer	54	0.18 ± 0.45	74	0.25 ± 0.61	0.055	164	0.58 ± 1.25		164	<0.001
Inner retinal layers	9	0.03 ± 0.19	23	0.08 ± 0.33	0.035	49	0.17 ± 0.49		49	0.014
Axial distribution score (AxD) *		1.55 ± 1.47		1.85 ± 1.31	<0.001		2.07 ± 1.39			<0.001

SDOCT = spectral domain optical coherence tomography; HF = hyperreflective foci; SD = standard deviation; RPE = retinal pigment epithelium; AMD = age-related macular degeneration; GA = intraretinal and subretinal atrophy consistent with geographic atrophy; CNV = subretinal lesion consistent with choroidal neovascularization.

* AxD = weighted score for each eye, defined as the sum of all HF after multiplying each single HF by a correction factor (y) based on location at the RPE (y = 1), inner segment band (y = 2), outer nuclear (y = 3), outer plexiform (y = 4), or inner retinal layers (y = 5), divided by the total number of HF.

Table 3

Pairwise longitudinal comparison of the mean change (Δ) in distribution of SDOCT HF per study eye from baseline to year one (296 eyes) versus year one to year two (281 eyes). P values determined by the Wilcoxon signed rank test.

	Δ Baseline to Year 1	Δ Year 1 to Year 2	
Group	mean ± SD	mean ± SD	P value
Transverse distribution (HF)			
Total field	+0.57 ± 1.47	+1.08 ± 2.50	0.038
Central 500 μm	+0.03 ± 0.43	+0.12 ± 0.66	0.221
500 – 1000 μm	+0.18 ± 0.71	–0.01 ± 0.86	0.007
Outside 1000 μm	+0.36 ± 1.29	+0.95 ± 2.08	<0.001
Transverse density (HF/mm ²)			
Total field	+0.02 ± 0.05	+0.04 ± 0.09	0.038
Central 500 μm	+0.17 ± 2.18	+0.62 ± 3.25	0.221
500 – 1000 μm	+0.30 ± 1.21	–0.01 ± 1.46	0.007
Outside 1000 μm	+0.01 ± 0.05	+0.03 ± 0.08	<0.001
Subanalysis of central 500 μm			
Eyes without baseline HF			
Transverse distribution (HF)	+0.06 ± 0.23	+0.03 ± 0.42	0.464
Transverse density (HF/mm ²)	+0.29 ± 1.18	+0.17 ± 2.15	0.464
Eyes with baseline foci			
Transverse distribution (HF)	+0.02 ± 0.52	+0.19 ± 0.72	0.062
Transverse density (HF/mm ²)	+0.09 ± 2.65	+0.94 ± 3.69	0.062
Axial distribution score (AxD)	+0.30 ± 1.57	+0.23 ± 1.36	0.664

SDOCT = spectral domain optical coherence tomography; HF = hyperreflective foci; SD = standard deviation.

Table 4

Pairwise longitudinal comparison of pathology associated with SDOCT HF per study eye from baseline to year two. P values determined by the Wilcoxon signed rank test or McNemar chi squared test.

Group	Baseline (Y0)		Year 1 (Y1)		Y0 vs Y1		Year 2 (Y2)		Y1 vs Y2	
	N	mean ± SD	N	mean ± SD	P value	N	mean ± SD	N	mean ± SD	P value
Total eyes	299		296					284		
Associated pathology in the same axial plane (HF)										
RPE elevation / drusen	293	0.98 ± 1.33	458	1.55 ± 1.65	<0.001	571	2.01 ± 2.63			0.001
RPE atrophy	17	0.06 ± 0.28	36	0.12 ± 0.49	0.062	74	0.26 ± 0.89			0.003
Epiretinal membrane	8	0.03 ± 0.23	22	0.07 ± 0.47	0.031	17	0.06 ± 0.46			0.832
Cystoid macular edema	10	0.03 ± 0.47	13	0.04 ± 0.25	0.062	28	0.10 ± 0.60			0.147
Subretinal fluid	9	0.03 ± 0.25	21	0.07 ± 0.39	0.144	40	0.14 ± 0.76			0.131
Advanced AMD pathology in the total field (eyes)										
Any GA	41		75		<0.001	98				<0.001
Central GA	7		11		0.045	13				0.083
Likely CNV	13		23		0.002	33				0.002

SDOCT = spectral domain optical coherence tomography; HF = hyperreflective foci; SD = standard deviation; RPE = retinal pigment epithelium; GA = geographic atrophy; CNV = subretinal lesion consistent with choroidal neovascularization.

Table 5

Correlation of advanced pathology detected with SDOCT at year two to the mean baseline SDOCT HF characteristics per study eye (284 eyes). P values determined by logistic regression analysis.

Group	Any GA in the total field at Year 2			Central GA at Year 2			Likely CNV at Year 2		
	absent n = 186	present n = 98	P value	absent n = 271	present n = 13	P value	absent n = 251	present n = 33	P value
Eyes with baseline HF, n (%)	87 (47)	76 (78)	<0.001	153 (56)	10 (77)	0.437	139 (55)	24 (73)	0.320
Baseline HF, mean $\pm$ SD	0.96 $\pm$ 1.31	1.90 $\pm$ 1.64	<0.001	1.26 $\pm$ 1.50	1.76 $\pm$ 1.42	0.135	1.20 $\pm$ 1.42	1.88 $\pm$ 1.93	0.485
Baseline AxD, mean $\pm$ SD	1.21 $\pm$ 1.46	2.07 $\pm$ 1.30	<0.001	1.49 $\pm$ 1.47	1.99 $\pm$ 1.29	0.461	1.47 $\pm$ 1.47	1.85 $\pm$ 1.38	0.571

SDOCT = spectral domain optical coherence tomography; HF = hyperreflective foci; SD = standard deviation; AxD = axial distribution score; GA = intraretinal and subretinal atrophy consistent with geographic atrophy; CNV = subretinal lesion consistent with choroidal neovascularization.