Tracking Progression with Spectral-Domain Optical Coherence Tomography in Geographic Atrophy Caused by Age-Related Macular Degeneration

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PURPOSE. To investigate, with the use of spectral-domain optical coherence tomography (SD-OCT), microstructural alterations over time in eyes with progressive geographic atrophy (GA) due to age-related macular degeneration.

METHODS. Forty-six eyes of 26 patients (median age, 77.9 years [interquartile range (IQR), 71.8–81.0]) with GA without evidence of active or previous neovascular disease at baseline were examined by simultaneous confocal scanning laser ophthalmoscopy (cSLO) and SD-OCT. Serial examinations with alignment of follow-up to baseline scans were performed over a median period of 12.2 months (IQR, 10.2–15.3). Longitudinal SD-OCT variations were evaluated, including quantification of retinal thickness (RT) change and lateral spread of GA (LSGA) at a temporal, nasal, inferior, and superior GA border-section in each eye.

RESULTS. GA-enlargement was characterized by progressive loss of the outer hyperreflective SD-OCT bands and by thinning of the outer nuclear layer with subsequent approach of the outer plexiform layer toward Bruch’s membrane. In the perilesional zone, various dynamic changes were recorded, including migration of hyperreflective material and changes in drusen height. At the borders, there was a median RT change of −14.09 μm/y (IQR −26.21 to −7.48 μm/y). The median LSGA was 106.90 μm/y (IQR, 55.44–161.70 μm/y). Both parameters showed only moderate intraocular agreement (RT change: intraclass correlation coefficient [ICC], 0.54; 95% CI, 0.39–0.67; LSGA: ICC, 0.49; 95% CI, 0.34–0.64) and no statistical significant difference for one location (RT change, P = 0.125; LSGA, P = 0.516; likelihood ratio test).

CONCLUSIONS. Combined cSLO and SD-OCT imaging provides unprecedented insight into dynamic microstructural changes of GA enlargement that may help to better understand the pathogenesis of the disease. Quantitative progression data indicate local factors may exist that drive progression in junctional areas (ClinicalTrials.gov number, NCT00393692). (Invest Ophthalmol Vis Sci. 2010;51:3846–3852) DOI:10.1167/iovs.09-4533

Age-related macular degeneration (AMD) is a complex disease with both genetic and environmental factors. In aging societies, AMD has become the most common cause of legal blindness.1–9 AMD is a chronic, progressive disease with various phenotypic manifestations, different disease stages, and variable rates of progression. Early manifestations of AMD include focal hypopigmentation and hyperpigmentation and drusen.9 Geographic atrophy (GA) and choroidal neovascularization (CNV) characterize late stages of the disease. Although the overall incidence of neovascular AMD is still higher than that of GA, atrophic disease has recently been found to occur four times as often as neovascular AMD in persons 85 years of age and older, reflecting its important impact on public health in aging populations.4

The factors predisposing to the eventual development of atrophic AMD (i.e., GA) are still poorly understood. Natural history studies of GA revealed high-risk characteristics for disease progression, including the presence of soft drusen and hyperpigmentations as well as increased fundus autofluorescence (FAF) and specific abnormal patterns.9–12 In contrast to neovascular AMD, there is yet no therapy available for patients with GA. Current data on the natural course of GA are being used for the design of interventional trials.11,15–16 To identify potential targets for intervention and to complement available biomarkers for therapeutic interventions, better understanding of the pathogenesis and the natural history of late atrophic AMD appears mandatory.

Recent developments in retinal imaging technologies have allowed for more accurate phenotyping and further insights into disease processes. Abnormal FAF in atrophic AMD and the impact on disease progression have been studied using confocal scanning laser ophthalmoscopy (cSLO).10,12,17–21 With the advent of optical coherence tomography (OCT), cross-sectional analysis of the retina has become available in vivo. Recently, spectral-domain (SD)-OCT technology has been introduced with further improvements in imaging speed and resolution compared with previous time-domain OCT imaging.22–24 The simultaneous recording of cSLO and SD-OCT

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images in one instrument, with an exact topographic overlay during image acquisition, now allows for accurate orientation of cross-sectional SD-OCT scans at anatomic sites of interest and, furthermore, serial examinations at the same location over time.25–30 We and others20,29,31–34 have recently characterized morphologic alterations by SD-OCT imaging in eyes with GA. Here we longitudinally analyzed microstructural changes, including quantification of the change in retinal thickness (RT) and lateral spread of GA (LSGA).

Patients and Methods

In this prospective study, patients were examined by simultaneous cSLO and SD-OCT imaging. By June 2009, 26 patients were examined at least twice. Data of these patients were included in the analysis. The study followed the tenets of the Declaration of Helsinki, and informed consent was obtained from each patient. Patients older than 50 years of age, with advanced atrophic AMD in at least one eye (unifocal or multifocal GA), were enrolled. If both eyes met the inclusion criteria, both eyes were included. Eyes were excluded from the study if there was any additional sign of active or previous neovascular disease manifestation at baseline, a history of retinal surgery (including laser treatment) or retinal vascular occlusion, signs of diabetic retinopathy, or any evidence suggestive of hereditary retinal dystrophy. Each patient underwent a routine ophthalmologic examination, including a determination of best-corrected visual acuity using ETDRS charts.

Image Acquisition

Before retinal imaging, pupils were dilated with 1.0% tropicamide and 2.5% phenylephrine. Imaging was carried out with a combined instrument (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), which allowed for simultaneous recording of cSLO and SD-OCT with two independent scanning mirrors, as described previously.30 cSLO images were obtained according to a standardized operation protocol, which includes acquisition of near-infrared reflectance (λ = 815 nm) and FAF (excitation at λ = 488 nm, emission 500–700 nm) images.11 The size of the field of view encompassed 30° × 30°, with an image resolution of 768 × 768 pixels. Simultaneous SD-OCT imaging was carried out with an illumination wavelength of 870 nm, an acquisition speed of 40,000 A-scans, and a scan depth of 1.8 mm. The digital depth resolution was approximately 3.5 μm/pixel. Live B-scans can be acquired and observed simultaneously in real time with the cSLO image. For automated alignment, the software uses an algorithm to detect eye movements between different images.30 A set of hundreds of landmarks is extracted automatically for every image. A combinatorial algorithm is used to match the landmarks between the different images. The automated alignment is used for real-time compensation of eye movements, averaging multiple images, and allows for exact alignment of follow-up scans with baseline scans. Therefore, it is possible to track morphologic changes over time at the same retinal location (Fig. 1).

At baseline, one horizontal and one vertical SD-OCT scan were placed through the foveal center, and multiple scans were places through the horizontal and vertical diameters of GA, including the border and the perilesional zone. At least five baseline scans per eye were used as references for follow-up scans. Thereby, scans with initially relatively high background noise or poorly definable retinal structures (mostly attributed to media opacities) were excluded. The number of consecutive scans further depended on the patient’s cooperation and fixation.

Image Analysis

Before evaluation of retinal changes over time, the alignment of the follow-up SD-OCT scans with the baseline images was verified by comparing the position of blood vessels.

Individual hands below the hyperreflective band of the outer nuclear layer were identified based on recent descriptions35, a thin hyperreflective band, presumably corresponding to the external limiting membrane (ELM); a slightly thicker hyperreflective band, presumably corresponding to the interface of the inner and outer segments of the photoreceptor layer (IPL); a thin—only occasionally visible—hyperreflective band, presumably corresponding to the outer segment-RPE interdigitation; and a broad hyperreflective band, thought to correspond to the RPE/Bruch’s membrane (BM) complex.

Measurement of Geographic Atrophy Progression

Fundus Autofluorescence Images. The total GA size was measured in the processed FAF images (baseline and latest follow-up image, respectively) by automated imaging analysis software that uses region-growing techniques to segment the areas of GA.56,57 The measurement strategy was described in detail previously.16 A linear mixed-effects model was used to quantify overall GA growth. The two-level random effects model separates eye-specific and patient-specific effects and helps to address related observations. The mixed model methodology allows analysis of variance components and fixed effects simultaneously. A detailed description of the longitudinal modeling process is given by Dreyhaupt et al.36

SD-OCT Images. For quantitative analyses, four GA borders per eye were analyzed at baseline and at latest follow-up. These were the nasal and temporal borders of a horizontal scan and the inferior and superior borders of a vertical scan, respectively. In eyes with multifocal GA, the borders of the largest atrophic spot were analyzed.

Change in RT (Fig. 2) was measured using the OCT analysis window – thickness profile of the commercially available analysis software of the HRA+OCT (Eye Explorer; Heidelberg Engineering). Measurements were taken at the site of the GA border at baseline. The exact position of the border was defined as the point with an abrupt beginning of choroidal signal enhancement on the SD-OCT scan—that is, change of the normal hyporeflective to an abnormal hyperreflective signal at
the level of the choroid below BM. This hyperreflective area has been shown to spatially correlate with the severe reduction of the FAF signal over atrophy in the corresponding cSLO image (Schmitz-Valckenberg S, et al., manuscript submitted). To determine RT change over time, the following standardized operation procedures were performed.

First, automated segmentation for retinal thickness measurements performed by the software was verified in the baseline and in the follow-up image, respectively, and, if required, was manually corrected. Retinal thickness was defined as the distance between the first signal from the vitreoretinal interface (corresponding to the internal limiting membrane) and the signal from the posterior boundary of the outermost high-reflective retinal band that presumably correlated with BM.

Second, for baseline scans, the green vertical line was positioned at the GA border, and the automatically displayed retinal thickness at this location was documented.

Third, by using the show next/previous image button, the latest follow-up scan in the progression series was opened. At the same time, because of the alignment of follow-up scans to the baseline scan, the position of the green vertical line was not changed. Thus, it still marked the site of the GA border at baseline. It was now located, because of the growth of atrophy, within the atrophic area of the displayed follow-up scan. Both the actual RT at the time of the follow-up imaging and the change compared with baseline at this location was then given by the software and documented for statistical analysis.

For measurement of LSGA at the border over time (Fig. 2), the lateral growth of the choroidal signal enhancement over atrophy between baseline and the latest follow-up scan was determined. This was achieved by measuring the length between the GA border at baseline and the latest follow-up using the distance tool of the software (Eye Explorer; Heidelberg Engineering) and by placing the green vertical line exactly at the GA border at high magnification.

Measurements were taken by two independent readers. Interrater reliability was assessed using ICC. Further analyses were based on the average values of the two readers. All statistical calculations were carried out with the free software package R 2.10.1 for Windows.

RESULTS

Patient Characteristics

In 46 eyes of 26 patients (19 women, 7 men; median age at baseline, 77.9 years [IQR, 71.8–81.0; minimum, 61.1; maximum, 87.6]) with GA due to AMD, serial simultaneous cSLO and SD-OCT imaging was performed over a median follow-up period of 12.2 months (IQR, 10.2–15.3; minimum, 4.5; maximum, 23.9). Median number of examinations was 2.25 (IQR, 2–3; minimum, 2; maximum, 4). Median visual acuity at baseline was 0.6 logMAR (IQR, 0.3–1.1; minimum, 1.6; maximum, 0). Bilateral GA was present in 20 patients, while six patients had unilateral GA at baseline; four of those patients had active or previous neovascular disease, and two patients had a macular hole in the fellow eye. In one patient with bilateral GA, subfoveal occult CNV developed in the left eye during the review period.

Overall, 337 single scans were followed over time, of which 18 had to be excluded because of high background noise or poorly definable retinal structures in the follow-up scans. Therefore, a total of 319 serial scans (median number of scan series per eye, 6 [IQR, 5–10; minimum, 5; maximum, 16]) were evaluated to describe qualitative microstructural changes over time.

Progression of Preexisting GA

Preexisting GA areas showed enlargement on FAF images in all eyes. The population-averaged median progression rate measured based on cSLO images was 1.46 mm²/y (IQR, 0.89–1.86; minimum, 0.28; maximum, 5.22; overall mean progression rate, 1.64 mm²/y; 95% CI, 1.250–2.043). Expansion of GA patches was accompanied by advancing loss of bands 1 to 3 (band 3 was only occasionally visible), the inner part of band 4, and thinning of the outer nuclear layer (ONL; Fig. 3).

In most eyes, beyond the border, bands 1 to 4 were already severely altered and the ONL was thinned. Only in two eyes (one patient), the outer retinal bands appeared unaffected through their abrupt ending directly at the margin of GA.

Within the atrophic lesion, the irregular structure of the remaining part of band 4 became more homogeneous over time, and a thin line in extension of the outer part of band 4 remained throughout the atrophy. The ONL progressively thinned, and, subsequently, the outer plexiform layer (OPL) approached the remaining part of band 4 (Fig. 3).

For estimation of interrater reliability, all measurements of RT change and LSGA, respectively, were used (at 180 border sections, four GA borders in each eye; one study eye [i.e., four borders] was excluded from quantitative analyses because of CNV development). Both RT change and LSGA showed high agreement, with an ICC >0.9.

Median retinal thickness at baseline at lesion borders was 278.25 μm (IQR, 251.38–302.50; minimum, 197; maximum, 377 μm). Within the review period, there was a median RT change of −14.09 μm/y (IQR, −26.18 to −7.39; minimum, +18.79; maximum, −54.96). Herein, at 17 borders, an increase in RT was detected. This observation was made at sites with the development or growth of sub-RPE deposits in the neighborhood of the GA border (n = 12) or in the event of epiretinal membrane development (n = 5).

In all but seven borders, GA spread over time in the lateral direction could be quantified by SD-OCT within the follow-up time (median LSGA, 106.90 μm/y [IQR, 55.44–161.70; minimum, 0; maximum, 838.50]). Follow-up times of the seven borders (seven eyes of seven patients) at which no lateral spread could be detected ranged from 4.46 to 20.39 months (median, 10 months; IQR, 8.1–11.97).
ent at any specific location measured (RT change, 631.46). Between eyes, progression was not statistically differ-
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LSGA,
zone of atrophy over time. Serial examination revealed marked changes in the perilesional

FIGURE 3. Progression of preexisting GA visualized by serial SD-OCT imaging (14 months’ follow-up). There is marked enlargement of the atrophic area over time with progressive loss of the RPE layer (inner part of band 4), IPRL (band 2), and ELM (band 1), respectively, and thinning of ONL at the border of atrophy. Within the atrophic lesion, the remaining part of band 4 becomes more homogeneous, and the ONL progressively thins; subsequently, the OPL approaches the remaining part of band 4 (assumed BM).

Within the same eye, the progression of RT and LSGA was variable at different GA border locations (RT change: ICC, 0.54; 95% CI, 0.39–0.67; LSGA: ICC, 0.49; 95% CI, 0.34–0.64). The median difference between minimal and maximal LSGA for different border locations within the same eye was 121.95 μm/y (QQR, 76.99–196.74; minimum, 18.72; maximum, 651.46). Between eyes, progression was not statistically different at any specific location measured (RT change, P = 0.125; LSGA, P = 0.516; likelihood-ratio test).

Perilesional Zone
Serial examination revealed marked changes in the perilesional zone of atrophy over time.

In 44 eyes, dome-shaped elevations of the outer retinal bands could be detected that correlated funduscopically with soft drusen (Figs. 4A, 4B). Over time, these elevations underwent variable changes; increase in height could be observed (Fig. 4A), as could fading (Figs. 4A, 4B). Within one eye such changes could occur concurrently and adjacent to each other (Fig. 4A). Occasionally, decreases in drusen height preceded the development of GA (observed in five eyes during the follow-up period; Fig. 5A).

In all eyes, a thickened band 4, spikes of band 4, and hyperreflective clumps in more inner retinal layers in extension of those spikes were present in the perilesional zone (Figs. 4C, 4D). Over time, changes in these alterations included an increase in vertical extension resulting in the penetration of band 2 and band 1 (Figs. 4C, 4D), migration into more inner retinal layers (Fig. 4D), and fading of hyperreflective material that could result in the evolution of new atrophic lesions (observed in two eyes during the follow-up period; Fig. 5B).

DISCUSSION
While previous studies using high-resolution imaging in GA were limited to cross-sectional analysis,26,28,31–34 this is, to the best of our knowledge, the first study to analyze the progression of GA secondary to AMD by high-resolution SD-OCT imaging. The identification of microstructural changes over time, including the advancing loss of the RPE and photoreceptor bands at the GA border or the subsequent apposition of the OPL with BM within the atrophic lesion, underscores the relevant information obtained by this imaging method. Previous nondetectable dynamic changes can be now monitored in the same person over time. Of note, these observations are made within a relatively short period (i.e., a few months). Furthermore, the in vivo findings may be correlated with observations from histopathologic examinations.39–42 For example, it would be conceivable that the in vivo findings disclosed by SD-OCT imaging in the perilesional zone may correspond to previous postmortem descriptions of enlargement and fading of soft drusen or to migration of clusters of dead RPE cells and melanin-containing macrophages.

In addition, this study demonstrates the advantages of simultaneous cSLO and SD-OCT imaging. This imaging technology allows accurate serial imaging of the same retinal location at different time points with the use of two independent mirrors and eye tracking software. Therefore, in addition to the description of relative changes, we were able to quantifiably determine the spread of GA and the change in retinal thickness. This was achieved by evaluating changes in the SD-OCT signal exactly at, and in relation to, the location of the GA border as defined at the baseline visit.

Quantification of GA progression has been accomplished using fundus photography and cSLO imaging.11,14,15,45-47 Previous studies have reported an overall mean enlargement of the en face visible area of GA between 1.3 and 2.6 mm²/y. Interestingly, a high interindividual variability of progression rates was found in these studies. These observations would be in accordance with a high-variability of the lateral spread of GA within the study population as shown here. The high degree of variability of the lateral spread of GA within eyes and the overall nonstatistical significant difference for nasal, temporal, inferior, or superior location would indicate that local rather than general disease-related markers influence the enlargement of atrophic patches. This would confirm previous studies that failed to show a statistically significant effect on GA progression over time for population-specific risk factors, including age, smoking, and even the genetic risk loci CFH, C3, and ARMS2, although the importance of all these factors in terms of AMD susceptibility is not questioned.13,14,47–50 Furthermore, this assumption would be in accordance with FAF imaging.
findings, indicating that the area size of baseline atrophy and abnormal FAF patterns in the perilesional zone at baseline have an impact on GA enlargement over time. These data and considerations imply that additional visible focal changes seen by simultaneous high-resolution SD-OCT and cSLO imaging directly at or near the individual GA border influence the spread of atrophy over the fundus. Because of the limited number of eyes, we did not compare the lateral spread of GA by SD-OCT scans with GA area progression rates and phenotypic patterns as determined by FAF imaging. Of note, the FAF pattern classification is based on the overall phenotypic FAF alterations seen on a 30° × 30° central fundus image. By contrast, the current analysis is based on four single GA borders of two SD-OCT scans. Furthermore, lateral spread of GA using SD-OCT reflects the progression of atrophy in one dimension whereas GA area progression using FAF images is based on two dimensions. The obvious advantage of SD-OCT imaging is its visualization of anterior-posterior retinal changes at high resolution. It would be preferable using more SD-OCT scans of one eye for further evaluation. However, the accurate evaluation of the two parameters quantified in this study requires high-quality images consisting of a mean image out of several single scans. Acquisition of these scans, particularly real-time aligned follow-up images, is time consuming with current SD-OCT technology. In addition, difficulties in performing retinal imaging in the elderly population, such as GA patients, would limit the practical implementation of multiple SD-OCT scans in longitudinal multicenter trials.

Based on theoretical considerations and experimental data derived from animal studies, it might be assumed that a reduction in retinal thickness, as measured by SD-OCT, may correspond to increasing loss of the outer nuclear layer and thus correlate with a decrease in retinal function. Indeed, we could confirm in this study that a reduction in retinal thickness is a hallmark of disease progression at the GA border that was both quantifiable and confined to loss of photoreceptor cell morphology. Although segmentation for measurements was manually corrected in case of misalignment by the analysis software, we observed an increase in retinal thickness in 17 eyes that was related to confounders such as epiretinal membrane formation or marked collateral changes such as development of RPE elevations or sub-RPE deposits. These observations indicate that tracking of GA progression by changes in retinal thickness measurements alone, even when accurately segmented, should be interpreted with caution.

Various limitations of this study must be considered. First, alterations of reflectivity are yet not readily correlated with anatomic changes. Interpretation becomes even more difficult in the presence of complex pathologic findings. Moreover, optical phenomena may evoke artifacts that simulate pathologic findings. For example, it has been shown that elevations in the RPE layer may result in optical disruption, causing disappearance of the IPRL. Therefore, SD-OCT changes (e.g., penetration of retinal layers) may not reflect disintegration of the retinal tissue and should be interpreted with caution. Second, because of the small size of microstructural alterations, even minor changes of the scan location in follow-up examinations would result in misinterpretation of changes over time.

Finally, the review period and the number of analyzed eyes and SD-OCT scans were limited. Patients with bilateral and
unilateral GA were included, and follow-up times between patients were variable. In this regard, expansion of the study is aimed at the development of appropriate progression models that separate “eye-specific” and “patient-specific” effects and help to handle related observations.

In conclusion, this longitudinal study provides insight into dynamic microstructural retinal changes in progressive GA by high-resolution in vivo imaging. The simultaneous application of SD-OCT and cSLO imaging in one instrument also allows for quantitative tracking of GA progression at specific retinal locations. This approach may be helpful for monitoring the natural course of the disease and for elucidating pathogenetic mechanisms. In particular, the identification of structural risk factors reflecting disease activity and future lateral spread of GA may be important for prognosis and visual function. Finally, the effects of new therapeutic agents aimed at slowing down GA progression may be evaluated at high resolution and in a three-dimensional fashion.

References


