Optical Coherence Tomography and Autofluorescence Findings in Areas with Geographic Atrophy Due to Age-Related Macular Degeneration

Steffen Schmitz-Valckenberg, Monika Fleckenstein, Arno P. Göbel, Thomas C. Hobman, and Frank G. Holz

PURPOSE. To analyze outer retinal changes within the atrophic lesion in patients with geographic atrophy (GA) secondary to age-related macular degeneration.

METHODS. Twenty-one simultaneously obtained fundus autofluorescence (FAF, excitation, 488 nm; emission, 500–700 nm) and spectral-domain optical coherence tomography (SD-OCT) scans (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) of 21 GA patients (mean age, 75.1 ± 7.4 years) were included and separately exported. Two readers independently graded the following parameters: width of the atrophic lesion on the FAF image at the site where the SD-OCT scan had been placed; and on the SD-OCT image, widths of the linear disruption of the outer nuclear layer, the external limiting membrane, and the inner and outer segments of the photoreceptor layer (IPRL) and width of the disruption of choroidal signal enhancement.

RESULTS. The mean width of the atrophic lesion by FAF imaging was 2.83 mm (95% confidence interval, 2.37–3.29). The linear disruption of choroidal hyperreflectivity showed the closest agreement with 2.83 mm (2.37–3.28), whereas the linear width of disrupted IPRL was larger (3.10 mm; 2.65–3.55). Overall, the width of the atrophic lesion correlated significantly with all five SD-OCT parameters ($P < 0.0001$, $r = 0.96–0.99$).

CONCLUSIONS. These findings demonstrate that the atrophic lesions identified with FAF represent irreversible underlying outer retinal damage. The observation that the width of the atrophic lesion identified with FAF, although significantly correlated but not identical with the width of disruption within the cellular layers of the retina, is consistent with the dynamic nature of the disease. (ClinicalTrials.gov numbers, NCT00599846, NCT00599846.) (Invest Ophthalmol Vis Sci. 2011;52:1–6) DOI: 10.1167/iovs.10-5619

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Age-related macular degeneration (AMD) is the leading cause of legal blindness in persons over the age of 65 years. Vision loss with this disease is attributable in large part to the advanced stages: exudative AMD and geographic atrophy (GA). Although antibody fragments directed against vascular endothelial growth factor are effective treatments for exudative AMD, GA remains a large, unmet medical need because of its high incidence and the absence of effective treatments. Klein et al.1,5 have reported that the 15-year cumulative incidence of GA in individuals 43 to 86 years old with early signs of AMD is 14%. In individuals older than 85 years, the incidence of GA is approximately four times that of exudative AMD.

Based on fundus images, GA is defined as any sharply demarcated area larger than 175 μm of apparent absence of retinal pigment epithelium (RPE), with visible choroidal vessels and no neovascular AMD. This definition is based on histopathologic studies that have characterized clinically visible areas of atrophy as areas with cell death in the RPE and outer neurosensory layers with occasionally visible choriocapillaris. These atrophic areas typically initially appear in the extrafoveal region and progress into the fovea only late in the course of the disease. Central vision becomes affected when atrophic lesions develop in the parafoveal region. Severe vision loss occurs when these areas of atrophy enlarge and expand into the foveola. When the center of the fovea is not fully involved, atrophic lesions impair visual performance by limiting the size of the functioning fovea so that only a portion of a word can fit in the seeing eye. When the center of the fovea becomes involved, patients experience severe vision loss.

Color fundus (CF) photographs have been used in most epidemiologic and natural history studies that have characterized the progression of GA. Although this technique can be used to detect the presence of GA, graders at reading centers have reported difficulty reproducibly measuring atrophic areas due to interobserver variability of fundus pigmentation, media opacities, and the presence of drusen and small satellites of atrophy.

Other imaging modalities, such as FAF, may have improved precision and reproducibility for assessing atrophic lesion size. FAF imaging of atrophic lesions in GA is based on the normal autofluorescence properties of RPE cells. These cells continuously phagocytize shed photoreceptor cell outer segment discs; this process is an essential element of photoreceptor outer segment renewal and retinoid recycling. RPE cell degradation of these discs leads to an accumulation of lipofuscin granules that have autofluorescence properties. RPE autofluorescence can be visualized noninvasively, and the vivo visualization of this FAF serves as an assessment of the metabolic integrity of the RPE-photoreceptor complex and the loss of RPE autofluorescence indicates disruption of the...
RPE-photoreceptor complex possibly due to the death of photoreceptor cells and/or RPE cells.\textsuperscript{23} FAF has been used to follow GA progression in the Fundus Autofluorescence in Age-Related Macular Degeneration Study (FAM), a multicenter study conducted in Germany.\textsuperscript{24,25} Estimates of lesion growth based on FAF imaging from this study are in agreement with those in the natural history studies conducted by Sunness et al. based on FAF imaging from this study are in agreement with those in the natural history studies conducted by Sunness et al.\textsuperscript{11,12} in which CFs were used. However, there are no histopathology studies that have defined the cell loss that is localized in areas with severely reduced FAF. The objective of this study was to describe the morphologic changes that occur in these areas.

**METHODS**

**Image Acquisition**

High-resolution in vivo imaging was performed with a combined instrument (Spectralis HRA+OCT; Heidelberg Engineering) that allows for simultaneous recording of confocal scanning laser ophthalmoscopy (cSLO) and spectral-domain optical coherence tomography (SD-OCT).\textsuperscript{26} A minimum standardized imaging protocol was performed in all patients, which included acquisition of FAF images ($\lambda = 488$ nm; emission, 500–700 nm; field of view, $30^\circ \times 30^\circ$; image resolution, $768 \times 768$ pixels) and simultaneous SD-OCT scanning with a second, independent pair of scanning mirrors ($\lambda = 870$ nm; acquisition speed, 40,000 A-scans per second; scan depth, 1.8 mm; and digital depth resolution, $\pm 3.5$ mm/pixel).\textsuperscript{27} With the use of two independent pairs of scanning mirrors, eye movements were registered, and image acquisition was automatically corrected for generation of mean images from at least 30 single frames (the ART mode). For image analysis, cSLO and OCT scans were shown side-by-side with the same scaling. A line on the cSLO scan identified the placement of the SD-OCT scan. Thus, pixel-to-pixel correlation of cSLO and SD-OCT findings was possible. With the high-speed mode, as applied in this study, the transverse range of the B-scan encompassed $30^\circ$ and consisted of $768$ A-scans. In the high-speed mode, the vertical presentation of the OCT scan was magnified twice, as in other OCT instruments; therefore, morphologic alterations were disproportionately high in the vertical dimension.

**Inclusion and Exclusion Criteria**

The clinical research database of the Department of Ophthalmology, University of Bonn, was retrospectively screened for eligible patients. The study adhered to the tenets of the Declaration of Helsinki. All subjects had been enrolled in two natural history and imaging studies. Informed consent for additional imaging beyond routine clinical examination and use of retinal imaging data for additional research was obtained from each patient after explanation of the nature and possible consequences of participating in retinal imaging research. Study inclusion was limited to patients older than 50 years with GA secondary to AMD in at least one eye. Patients had to have drusen $\geq 50 \mu$m or GA as a manifestation of AMD in the fellow eye. Each patient had undergone a routine ophthalmic examination, including measurement of best corrected visual acuity with Snellen charts and stereo biomicroscopy with dilated pupils (1.0% tropicamide and 2.5% phenylephrine). One eye per patient was chosen as the study eye. If both eyes were eligible, the right eye was included. Exclusion criteria were a history of retinal surgery, including laser treatment; signs of diabetic retinopathy; a history of retinal vascular occlusion, and any signs or history of hereditary retinal dystrophy, as well as evidence of GA secondary to a hereditary retinal or macular dystrophy. Additional exclusion criteria were an accumulation of extracellular fluid, hemorrhage, exudates, or fibrosis. Fluorescein angiography was performed, and all images were screened by two graders; eyes with active or a history of neovascular AMD were excluded.

**Image Processing**

For each study eye, one combined cSLO FAF and SD-OCT image was selected for further analysis and exported as a bitmap to image-analysis software (Photoshop 7.0; Adobe Systems Inc., Mountain View, CA). Selected images were those in which the SD-OCT scans were placed through a well-demarcated atrophic area equal to or larger than $1.25 \text{mm}^2$ (0.5 disc area; DA). To acquire separate cSLO and corresponding SD-OCT scans, each image file was cropped in half; two separate files were saved, and individual brightness and contrast settings were optimized for each file. The optimization process consisted of improving image brightness by stretching the pixel histogram using the entire range of available pixel values (0–255). Image contrast was manually adjusted so that the visualization of the details was subjectively maximally enhanced. Finally, the graders were presented with a single image and were unable to pair the cSLO and SD-OCT images for each patient.

The individual bands below the outer nuclear layer (ONL) in the SD-OCT images were identified according to the definitions described by Schmidt-Erfurth et al.\textsuperscript{28} These authors defined the hyperreflective bands in SD-OCT images, shown in Figure 1, by comparing images from intact normal retina with those of disease-damaged retina.

**Image Grading**

Using the mouse-driven arrow on the computer screen (Image 1.32); developed by Wayne Rashband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html), two graders independently measured (Fig. 2) the linear dimensions of the disrupted retinal layers by placing a line between the proximal and distal points of the following disruption of the structures.

1. **FAF loss**: The linear dimension was defined by the sharp step in the recorded signal (i.e., the abrupt transition from severely reduced FAF intensities to the normal background or increased intensity) and was quantified on the FAF images exactly at the site of the line identifying the placement of the SD-OCT scan.
2. **ONL loss**: the points at each site where the bands of the photoreceptor cell nuclei were completely lost on the SD-OCT images.
3. **ELM (external limiting membrane) loss**: the points at each site where the ELM (band 1 in Fig. 1) were completely lost on the SD-OCT images.
4. **IPRL (interface of the inner and outer segments of the photoreceptor layer) loss**: the complete loss of IPRL (band 2 in Fig. 1) on the SD-OCT scans; small focal alterations of this band away from edges of atrophy were not included in this parameter; IPRL loss was recorded only if band 2 was no longer visible.
5. **RPE attenuation**: the point on the SD-OCT scan where the

**Figure 1.** SD-OCT scan through the fovea of a normal retina of a 31-year-old man. Different hyperreflective bands were defined that appeared to correlate with the anatomic layers of the retina: RNFL, retina nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; ONL, outer nuclear layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; C, choriocapillaris and choroid.
upper third (toward the vitreous) of band 4 was lost. The lower third which presumably reflects Bruch’s membrane was not considered.

6. **Choroidal signal enhancement**: the points at which there is an abrupt transition from a hyporeflective to hyperreflective area in the choriocapillaris below Bruch’s membrane on the SD-OCT scan at each site of atrophy.

Graders were instructed to exclude any measurement in case of insufficient image quality. Linear dimensions were always quantified at the most nasal and most temporal margins of atrophy in cases of multiple patches of GA. Pixel values were calculated in micrometers by using the scale factor given by the instrument (Spectralis HRA+OCT; Heidelberg Engineering). This factor is based on a Gullstrand eye, assuming standard corneal radii and taking into account the individual spherical refraction as adjusted by the operator during acquisition. Correlations between the width of disruption of the retinal cell layers identified in the SD-OCT images (i.e., ONL, ELM, IPRL, and RPE), choroidal signal enhancement, and the width of the atrophic lesion, identified with FAF and transected by the OCT scan, were analyzed by the Pearson correlation test. All tests were two-tailed (SAS software, ver. 9.2; SAS Institute, Cary, NC). Bland-Altman analysis was used to assess interobserver variability. Finally, a third masked reader quantified atrophic areas in square millimeters on the FAF images by customized image analysis software, as previously described.

### RESULTS

A total of 21 eyes of 21 patients with a mean age of 75.1 ± 7.4 years (range, 53 – 87) were enrolled in the study. There were 13 women and 8 men. Bilateral GA was presented in 17 patients. The remaining four study patients had unilateral GA with signs of exudative AMD in the fellow eye. For the study eyes, the mean of the total area of atrophy visualized with FAF imaging was 8.54 ± 5.52 mm² (range, 1.25–22.38).

The linear FAF dimension of GA was determinable in all 21 FAF images (Fig. 3). Both graders were unable to measure the linear dimension of ELM and ONL loss in two eyes because of insufficient image quality. They were able to quantify IPRL loss, RPE attenuation, and the transition to choroidal signal enhancement in those two patients. Table 1 summarizes the results for the six different parameters. For both graders, the largest linear dimensions were measured for IPRL loss, followed by choroidal hyperreflectivity, FAF loss, RPE attenuation, ELM loss, and ONL loss. The interobserver agreement between both graders was similar for the different dimensions, ranging between 0.02 and 0.05 mm, except for a markedly larger variability of 0.1 mm for the linear dimension of the IPRL loss. Although higher, the interobserver agreement for the latter was still low relative to the absolute values; both graders encountered difficulties in precisely identifying the exact point of complete IPRL loss at the edge of FAF loss. Instead of an abrupt ending point, there was a transition zone between the clear presence of and clear loss of IPRL. The graders also experienced difficulty measuring the linear dimension of RPE attenuation.

**Figure 2.** Illustrations of the six linear dimensions that were separately and independently assessed by two graders. The six linear dimensions include the width of the fundus autofluorescence atrophic lesion at the site transected by the SD-OCT scan, the width of disruption of the ONL, the ELM, the IPRL, and the width of choroidal hyperreflectivity. The disruption of each parameter was defined at both linear ends of atrophy and a line was placed horizontally, to determine the respective linear dimension. The images in the figure were cropped for illustration purposes and do not show the entire 30° field of the original scans.

**Figure 3.** SD-OCT at the margin of GA with illustration of disruption of individual retinal layers at high magnification. **Right inset:** Normal-appearing retinal bands outside the area of atrophy: red, choroidal hyporeflectivity; green, RPE/Bruch’s membrane complex; blue, IPRL; yellow, ELM; purple, ONL. The bands end at the site of the disruption.
although the interobserver variability was similar compared with that for the five remaining parameters. Rather than an abrupt thinning of band 4, the graders were challenged by differentiating the loss of the upper part of band 4 and the accumulation of abnormal hyperreflective material within band 4. Indeed, even in the very center of the FAF loss, small focal areas with a normal-appearing band 4 were observed.

When values from the two graders were averaged, the mean width of the atrophic lesion transected by the SD-OCT scan was 2.83 mm. This measurement was not different from the 2.83 mm of linear disruption of choroidal hyporeflectivity, was smaller than the 3.06 mm linear width of disrupted inner and outer photoreceptor segments, but was larger than the 2.68 mm linear width of RPE cell loss and the 2.62 mm width of ELM loss. The greatest difference was between the width of the atrophic lesion and the ONL loss, 2.83 and 2.18 mm, respectively. With the Pearson correlation test, the width of the atrophic lesion that was transected by the OCT scan correlated significantly ($P < 0.0001$) with the width of the linear disruption of the ONL (Fig. 4, top left), the ELM (Fig. 4, top middle), and the IPRL (Fig. 4, bottom left); the attenuation of the RPE (Fig. 4, bottom middle), and the width of the disruption of choroidal hyporeflectivity (Fig. 4, right). The $r$ values for these correlations ranged between 0.96 and 0.99.

**DISCUSSION**

The systematic analysis of a representative cohort of GA patients in this study demonstrates that the severe reduction in the FAF signal correlates spatially to the abrupt transition from a hypo- to hyperreflective area in the choriocapillaris below Bruch’s membrane on the SD-OCT scan. This transition from a hypo- to hyperreflective area is secondary to the notable neural and RPE cell loss localized between the IPRL and Bruch’s membrane. These results underscore the assumption that areas with severe reduced FAF correspond to severe outer retinal damage. Furthermore, they confirm that semiautomated detection and quantification of atrophic areas by FAF imaging may be a suitable method for monitoring disease progression in GA over time.

The larger linear dimension of the IPRL loss compared to the width of severe reduction of the FAF signal suggests that damage to the photoreceptor inner and outer segments precedes complete RPE cell loss. The difference in the linear

<table>
<thead>
<tr>
<th>Grader 1</th>
<th>Grader 2</th>
<th>Average of Grader 1 and Grader 2</th>
<th>Interobserver Difference</th>
</tr>
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<tbody>
<tr>
<td>FAF 2.85 (2.38 to 3.31)</td>
<td>2.81 (2.35 to 3.26)</td>
<td>2.83 (2.37 to 3.29)</td>
<td>0.04 (0.02 to 0.07)</td>
</tr>
<tr>
<td>IPRL loss 3.06 (2.60 to 3.51)</td>
<td>3.14 (2.69 to 3.59)</td>
<td>3.10 (2.65 to 3.55)</td>
<td>0.09 (~0.15 to ~0.02)</td>
</tr>
<tr>
<td>RPE attenuation 2.69 (2.22 to 3.16)</td>
<td>2.66 (2.17 to 3.14)</td>
<td>2.67 (2.20 to 3.15)</td>
<td>0.04 (~0.01 to 0.08)</td>
</tr>
<tr>
<td>ELM loss 2.61 (2.11 to 3.10)</td>
<td>2.61 (2.11 to 3.11)</td>
<td>2.61 (2.11 to 3.11)</td>
<td>0.00 (~0.04 to 0.03)</td>
</tr>
<tr>
<td>ONL loss 2.21 (1.68 to 2.75)</td>
<td>2.19 (1.66 to 2.71)</td>
<td>2.20 (1.67 to 2.72)</td>
<td>0.02 (~0.03 to 0.07)</td>
</tr>
<tr>
<td>Choroidal signal enhancement 2.84 (2.38 to 3.29)</td>
<td>2.82 (2.36 to 3.27)</td>
<td>2.83 (2.37 to 3.28)</td>
<td>0.02 (~0.01 to 0.05)</td>
</tr>
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</table>

Tabulation of the mean width of the FAF atrophic lesions bisected by the SD-OCT scan and the mean linear dimensions of the disruption of the ONL, ELM, and the IPRL and the width of the disruption of choroidal hyporeflectivity (choroidal signal enhancement) identified in the SD-OCT images. The tabulation includes measurements from both readers, along with an estimate of interobserver variability.
dimension between the IPLR and the ONL and ELM may suggest that the photoreceptor cell damage characteristic of GA affects the inner and outer segments before the photoreceptor cell body.

Compared with previous evaluations of SD-OCT changes at the margin of atrophy, in all subjects we consistently used SLO and SD-OCT images that were recorded simultaneously and with real-time eye tracking technology. The simultaneous recording of SLO and SD-OCT images allows for the calculation of mean images with increased signal-to-noise ratio, better visualization of details, and more accurate orientation of both modalities to each other permitting a better comparison of measurements.

Consistent with other studies, the results reported herein confirm that SD-OCT imaging provides greater insights into the retinal alterations of atrophy lesions in patients with GA than does en face imaging such as FAF or CF. Unlike these other SD-OCT studies that described the retinal damage at the margins of the atrophic lesions, the present study characterized the extent of retinal damage that underlies the atrophic lesions identified with FAF imaging.

Compared with previous available imaging methods, combined cSLO+OCT imaging allowed for more detailed in vivo visualization of retinal microstructures. However, the resolution was still inferior compared with postmortem histologic analyses (e.g., individual cellular elements are not distinguishable). The identification of an exact point of loss for a specific OCT band can be challenging. However, given the good agreement between both independent readers and the severe SD-OCT band can be challenging. However, given the good agreement between both independent readers and the severe SD-OCT changes seen at the GA border, it is assumed the morphologic parameters used in this study are meaningful and reliable and would reflect irreversible damage to retinal tissue along with the atrophic process.

In summary, this study showed that the markedly reduced FAF signal over atrophic lesions in patients with GA is defined by the transition of the choriocapillaris from a hypo- to hyper-reflective area. The results also show that in FA-imaged atrophic lesion changes in photoreceptor inner and outer segments occur before the loss of photoreceptor nuclei and before RPE cell loss. Together, these results indicate that the areas with severely reduced FAF correlate with retinal dysfunction. Based on these findings and the advantages of a noninvasive, semi-automated, and rapid approach for lesion boundary visualization of details, and more accurate orientation of both modalities to each other permitting a better comparison of measurements.20

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References


