Peripheral Fundus Autofluorescence Is Increased in Age-Related Macular Degeneration

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Purpose. To evaluate peripheral fundus autofluorescence (FAF) in patients with AMD.

Methods. A consecutive series of 71 normal eyes, 71 eyes with neovascular AMD having received anti-VEGF treatment, and 43 eyes with untreated AMD were investigated. In all subjects, wide-field FAF imaging was performed, applying a wide-field scanning laser ophthalmoscope. FAF was quantified by image analysis software after defining peripheral and perifoveal central measurement zones with a grid scheme; age correction was performed by regression model.

Results. Fundus autofluorescence increased with age not only in the perifoveal retinal area, but also in the retinal periphery. For age-corrected measurements, peripheral FAF was significantly increased for both, treated and untreated AMD groups compared with normal subjects. No significant difference was observed in peripheral FAF between AMD eyes having received anti-VEGF treatment and those without treatment. Age-corrected normal FAF in the retinal center differed significantly from the anti-VEGF–treated group ($P < 0.01$), but not the untreated AMD group. Age-corrected peripheral FAF irregularity, defined as the standard deviation in the measurement field, was significantly increased in both AMD groups compared with normal subjects.

Conclusions. Detection of peripheral in addition to central FAF may provide additional information potentially helpful to detect and monitor the development of AMD. No differences in autofluorescence were observed in the retinal periphery between anti-VEGF–treated and untreated eyes. (*Invest Ophthalmol Vis Sci.* 2012;53:2193–2198) DOI:10.1167/iovs.11-8483

Age-related macular degeneration is the leading cause of blindness in industrialized countries and the third common leading cause of blindness worldwide. It is a chronic, complex, multifactorial disease with several risk factors for development and progression, including ethnicity, gender, smoking, arterial hypertension, genetics, diet, and sunlight exposure.[1–4] Documented early clinical symptoms prior to the onset of AMD are changes in color contrast sensitivity, central visual field deficits, macular recovery function, and spatiotemporal contrast sensitivity.[5–9]

Early stages of AMD are characterized by the formation of drusen and atrophy in the RPE.[10] With the progression of the disease, RPE degeneration with areas of geographic atrophy (GA) and consecutive photoreceptor degeneration in the macular region often occurs.[11] About 10% of AMD patients suffer from "wet," neovascular forms of AMD, due to choroidal neovascularization, that often lead to rapid loss of vision.[12,13] The remaining 90% of patients suffer from "dry" forms of the disease.

For "dry" AMD there are only limited treatment options available so far. In neovascular AMD, with the advent of intraocular applied anti-VEGF inhibitors (e.g., bevacizumab, ranibizumab) therapeutic options have greatly advanced.[14,15] Therefore, early detection and development of new diagnostic or prognostic markers for better evaluation of the disease stage or progression, together with these new therapeutic approaches, may help to improve visual outcome.[16]

FAF has proven to be a valuable tool for detection of RPE pathologies in several diseases.[17–20] It has been used for both detection and monitoring of diabetic maculopathy, hereditary retinal disorders, and GA secondary to AMD.[21–25]

In AMD patients, FAF of the posterior pole has been observed to be pathologic.[25–29] Lipofuscin accumulation in RPE cells results in an increased FAF signal. By contrast, RPE atrophy results in a loss of FAF in the affected area, where a clearly delineated dark patch can often be seen.[30]

FAF changes in early AMD have been described elaborately in the past 2 decades[24,30–34] as have findings in advanced stages of the disease.[22,25,30,35,36] FAF irregularities have also been observed in patients with neovascular AMD, indicating damage to the RPE more widespread than the area of hyperfluorescence observed in fluorescein angiography.[21,37]

Until now, all of the above-mentioned observations of FAF changes in patients with AMD have focused on the macula.[38] However, there is only very limited knowledge about peripheral FAF in eyes with AMD.

A recently developed novel ultra-wide-field scanning laser ophthalmoscope, the Optomap Panoramic 200Tx (Optos PLC, Dunfermline, Fife, Scotland, UK), allows non-mydriatic color, green, and red separation imaging, as well as FAF detection capability of up to 200° of the retina, often extending the equator. This imaging technique permits evaluation of FAF of the central and peripheral retina in one scan, without a need to dilate the pupil.

The aim of this study was to quantify central and peripheral FAF in patients with AMD with or without anti-VEGF treatment and to compare them with the FAF profiles in subjects without AMD.

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Methods

A consecutive series of 71 eyes, mean age 79 ± 6 years, with neovascular AMD having received anti-VEGF treatment and 43 eyes, mean age 77 ± 8 years, with untreated AMD were compared with 71 normal eyes, mean age 45 ± 18 years. After informed consent, mydriatic Optomap color and FAF images were obtained (Figs. 1a, 1b). Thereafter, a full retinal examination was performed by a retinal specialist. Basic operation of the Optomap Panoramic 200Tx is a scanning laser ophthalmoscope (SLO) with two laser wavelengths scanning: green (532 nm) and red (633 nm). The two images can be viewed separately or superimposed by specific software (Vantage V2, Optos PLC) to yield semirealistic color imaging.

FAF images were obtained using the same Optomap Panoramic 200Tx. An excitatory laser beam is produced at 532 nm wavelength, with the emitting light within the range from 540 nm to 800 nm detected by the machine. All research was conducted in accordance with institutional guidelines and board approval and conformed to the tenets of the World Medical Association Declaration of Helsinki.

Measurements

A standardized grid was used for assessments of the peripheral and central FAF (available at http://aaojournal.org). Images were not normalized, and the central field of the grid was centered on the macular region. The diameter of field "1" was adjusted to match one disc diameter.

Measurements of FAF were taken in the macular region (central four fields) and in the periphery in areas 34, 35, 38, and 39. Areas of altered pigmentation, scars, or blood vessels with altered FAF not due to AMD in all three study groups were excluded from measurements. Quantitative assessment of FAF was made using software Image J (available at http://rsb.info.nih.gov), see Figure 1b. Each pixel of all measured central and peripheral areas was measured for intensity on an intensity scale ranging from 0 to 255, followed by calculating the mean intensity and the standard deviation of intensity for all central and peripheral measured subfields.

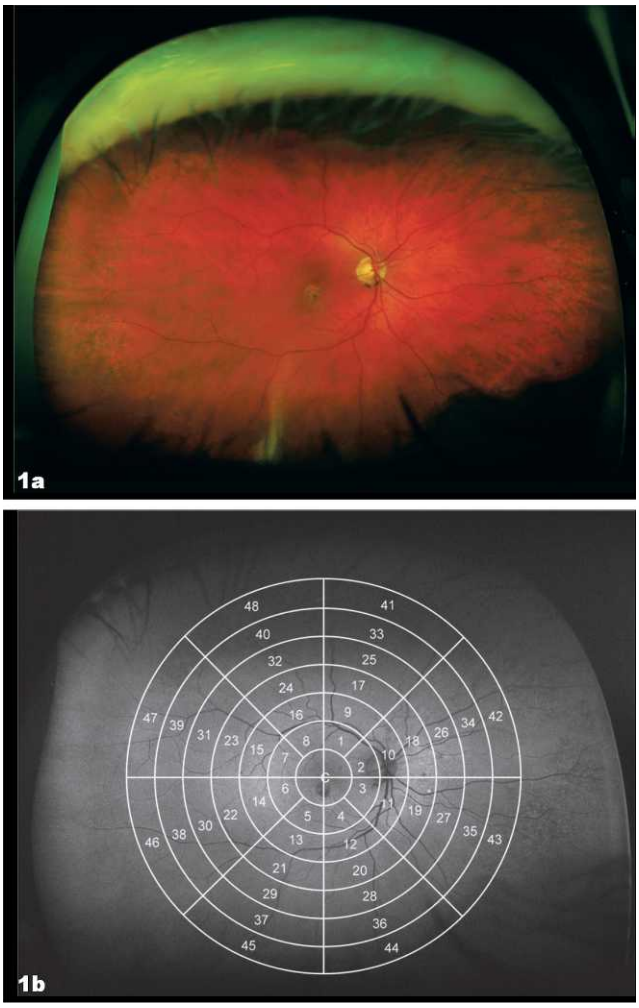

Figure 1. Pseudo-photographic color fundus scan. (**a**) AMD eye with anti-VEGF treatment imaged by Optomap Panoramic 200Tx. (**b**) Corresponding FAF scan with an overlaid grid of the same eye, showing peripheral irregularities.

Statistical Analysis

Data were collected and analyzed using SPSS Version 17.0 (SPSS Inc., Chicago, IL). A P value of < 0.05 was considered as statistically significant. Univariate analyses (ANOVA with post-hoc testing) were applied, adjusted for multiple testing by applying Bonferroni correction. To adjust for confounding potentially induced by age differences, we calculated a linear regression model for central and for peripheral changes of autofluorescence. These models served to correct autofluorescence values for age.

Results

Mean fundus autofluorescence of all included study eyes with and without AMD increased with age in the central and peripheral retinal areas (Fig. 2). Therefore, age correction was applied in all subsequent analyses.

Mean peripheral FAF intensity (intensity scale 0–255) of the four measured peripheral areas 34, 35, 38, and 39 in AMD eyes with and without anti-VEGF treatment were 80 ± 16 and 79 ± 16 (age-corrected). No statistically significant difference was observed in peripheral autofluorescence between the anti-VEGF–treated and untreated AMD group. The mean peripheral FAF intensity in normal eyes of the control group was 68 ± 21 (age-corrected) and significantly (all $P < 0.01$) lower compared with each group of eyes with AMD (Fig. 3).

The central perifoveal FAF intensity (intensity scale 0–255) of the central four fields in the macular region in AMD eyes with and without anti-VEGF treatment was 107 ± 24 and 102 ± 27 (age-corrected), respectively. No statistically significant difference was observed in central autofluorescence between the anti-VEGF–treated and untreated AMD group. The age-corrected control group showed a central FAF of 93 ± 25, which differs significantly from the anti-VEGF– treated AMD group ($P < 0.01$), but not from the untreated AMD group (Fig. 4).

Irregularity of peripheral FAF was defined as the FAF intensity SD within the measurement field itself. The SD of peripheral FAF in AMD eyes with and without anti-VEGF treatment was 5.39 ± 1.25 and 5.39 ± 1.14, respectively, compared with an SD of 4.06 ± 1.25 in normal eyes of the control group. The measured peripheral FAF irregularity of both AMD groups was significantly ($P < 0.001$) increased.

When comparing the perifoveal central/peripheral FAF standardized ratio, no significant differences exist between control group (1.26 ± 0.29) and each of the AMD groups (untreated 1.25 ± 0.07, anti-VEGF treated 1.26 ± 0.08), which suggests a similar alteration of central and peripheral retina.

Discussion

During the last years, tremendous research efforts aimed at the prevention and treatment of AMD have been driven. With the

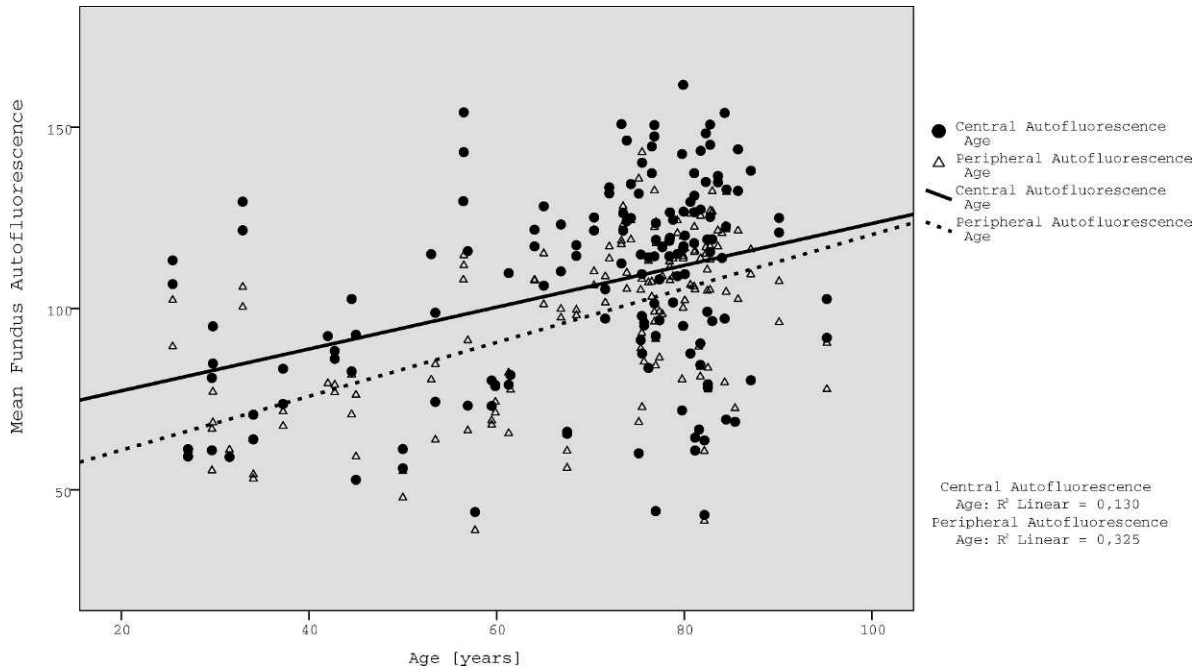

Figure 2. Perifoveal central and peripheral FAF intensities relationship with age. Peripheral more than central autofluorescence increased significantly with age (linear regression $R^2 = 0.325$).

advent of intraocular anti-VEGF treatment, therapeutic options especially for patients with neovascular AMD have greatly advanced.[14,15] However, AMD is still the leading cause of severe, irreversible vision loss in individuals over the age of 60 in the Western world,[14,39] and the number of individuals with AMD is supposed to almost double during the next 2 decades in Europe and the United States.[14,39] More precise diagnostics in combination with new and available medication may lead to better treatment of patients with AMD.

Therefore, FAF imaging has gained a growing interest in recent years, as alterations of the tissue fluorescence may be an early indicator for aging and disease on a molecular level. FAF in patients with AMD is a widely and thoroughly examined issue.[25,26,28,29] So far, the focus of pathologic alterations in FAF signals in AMD eyes has always been on the central area, mainly within vascular arcades in the macular region.[38,40] This is because the observed pathology in AMD, together with clinical symptoms, is seen in and can be related to the macular region. Another important reason may be the lack of appropriate imaging tools to reliably capture peripheral FAF during a routine imaging procedure.

The Optomap Panoramic 200Tx SLO is a recently developed prototype of a non-mydriatic wide-field fundus imaging system for detection of FAF. The Optomap imaging system allows for imaging FAF of up to 200° of the retina with one scan.[41–43] The Panoramic 200Tx uses a green laser (532 nm)

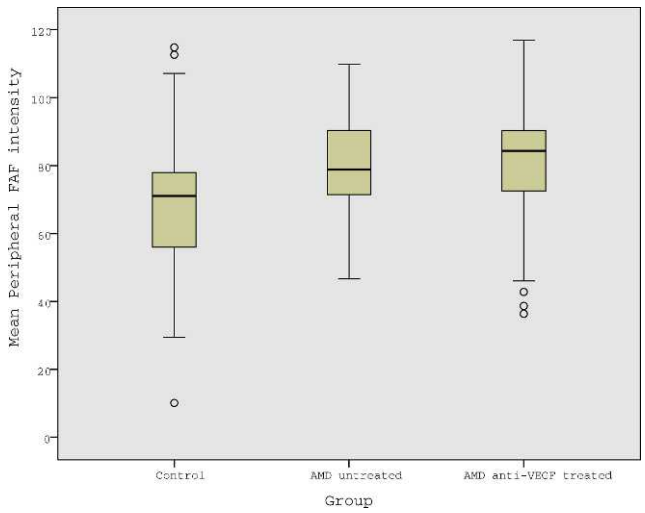

Figure 3. Age-corrected mean peripheral FAF intensities measured in AMD eyes with and without anti-VEGF therapy and normal eyes (control). The control group shows significantly less peripheral autofluorescence than each of the two AMD groups ($P < 0.01$).

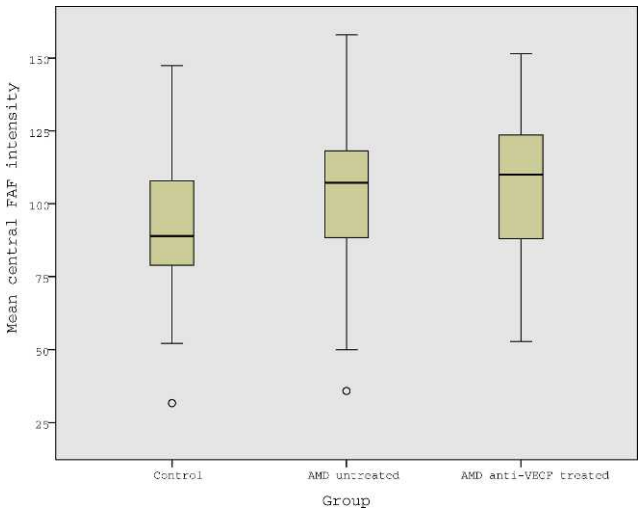

Figure 4. Age-corrected mean central FAF intensities measured in AMD eyes with and without anti-VEGF therapy and normal eyes (control). The two AMD groups do not differ significantly. The control group differs significantly from the anti-VEGF–treated group ($P < 0.01$), but not the untreated AMD group.

for excitation and a broad-band detector within the yellow-orange-red range for detection of FAF. This system potentially results in a more specific detection of lipofuscin-associated FAF, as side-signals from collagen autofluorescence may be less than when using a lower excitation wavelength.[44–46] These unique properties of the Optomap Panoramic 200Tx system allow better detection of FAF related to peripheral RPE alterations and may provide additional information regarding RPE-related retinal dysfunction of the peripheral retina.

In our study, wide-field FAF imaging revealed a significantly increased central and peripheral FAF signal as well as an increased peripheral FAF irregularity in patients with AMD with or without anti-VEGF treatment compared with normal eyes in the control group. Central FAF profiles differed only between the AMD group treated with anti-VEGF and the control group. The obtained FAF intensity pointed to slightly increasing signals in AMD eyes with anti-VEGF treatment compared with AMD eyes without treatment but did not significantly vary between these two groups in the periphery. In contrast, in central FAF, a difference in the anti-VEGF treatment group was observed versus the control group.

The FAF observed in healthy eyes is a cumulative signal of a variety of fluophores, each with its assumed own excitation and emission spectra including lipofuscin, which itself is a mixture of different compounds and is considered to be the strongest fluophore of the ocular fundus.[47–54] A2E, the product of hydrolysis of vitamin A aldehyde and phosphatidylethanolamine, is supposed to be mainly responsible for the autofluorescence properties of lipofuscin, which accumulates intracellularly during aging and plays an important role in the pathogenesis of AMD.[55–57]

FAF properties vary according to age, media opacities, and excitation wavelengths.[58] Optomap Panoramic 200Tx uses a 532-nm laser for excitation compared with the 488-nm excitation wavelength used, for example, by HRA (Heidelberg Retina Angiograph-Optical Coherence Tomography, Heidelberg Engineering, Heidelberg, Germany)[24,29] for FAF imaging. By exciting with a higher wavelength, Optomap Panoramic 200Tx is able to detect autofluorescence signals that can be attributed to a higher proportion of lipofuscin autofluorescence than if excited with 488 nm, and therefore may be more sensitive in detecting pathologic changes due to lipofuscin accumulation in the RPE layer in AMD.[44–46]

Our results revealed a higher peripheral FAF in AMD patients with or without anti-VEGF treatment. This altered FAF may indicate an accumulation of lipofuscin-like fluophores in the peripheral fundus. A potential explanation is an accumulation of lipofuscin in the peripheral RPE due to degradation in aging.

The observed pathologic changes in AMD are mainly concentrated on the macular region. However, it is reasonable to assume that lipofuscin accumulation of RPE cells occurs, at least to a certain degree, in the peripheral retina as well. An observed significantly higher peripheral FAF irregularity, defined as a higher intensity standard deviation in the measurement fields in AMD eyes, supports our theory of possible lipofuscin accumulation in RPE cells not only in the macular region but also in the peripheral fundus. Our additional observation of a non-significant increase in peripheral FAF in eyes that have received anti-VEGF treatment compared with AMD patients with no anti-VEGF treatment may be explained by a more advanced disease with longer duration, resulting in higher levels of RPE lipofuscin pigment.

A limitation of our study is that we are not able to exclude other fluophores with autofluorescence properties similar to those of lipofuscin. Melanolipofuscin, for example, which is commonly found in the inner retina, is difficult to distinguish from lipofuscin in FAF images.[24,59–61] Because of its cross-sectional character, we cannot assign the increased peripheral FAF to a certain time period prior to or after the clinical onset of the disease.

However, the detection of peripheral FAF using the novel Optomap Panoramic 200Tx allows for obtaining information about presumed pan-retinal lipofuscin accumulation in the RPE layer. This technique may provide new, additional information to help detect and monitor development and progression of AMD. In addition, it may be a more reliable method than detection of central FAF alone, which can be affected by hemorrhage, scars, choroidal neovascular membranes, exudations, or RPE detachment, common complications occurring in eyes suffering from AMD. Additional studies are needed to substantiate our findings and to further evaluate the prognostic and diagnostic value of peripheral FAF in AMD.

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