Imaging Geographic Atrophy in Age-Related Macular Degeneration

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Key Words

Age-related macular degeneration · Geographic atrophy · Fundus autofluorescence · Scanning laser ophthalmoscopy

Abstract

Advances in retinal imaging technology have largely contributed to the understanding of the natural history, prognostic markers and disease mechanisms of geographic atrophy (GA) due to age-related macular degeneration. There is still no therapy available to halt or slow the disease process. In order to evaluate potential therapeutic effects in interventional trials, there is a need for precise quantification of the GA progression rate. Fundus autofluorescence imaging allows for accurate identification and segmentation of atrophic areas and currently represents the gold standard for evaluating progressive GA enlargement. By means of high-resolution spectral-domain optical coherence tomography, distinct microstructural alterations related to GA can be visualized.

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Introduction

Geographic atrophy (GA) represents the atrophic late-stage manifestation of ‘dry’ age-related macular degeneration (AMD). It is responsible for approximately 35% of all cases with late AMD [1, 2]. While the overall incidence

of neovascular AMD is still higher than the incidence of GA, atrophic disease has recently been found to occur 4 times as often as neovascular AMD in individuals ≥ 85 years of age, reflecting its important impact on public health in aging populations [1]. Histopathological studies have revealed cell death of the retinal pigment epithelium (RPE), the outer neurosensory retina and the choriocapillaris in areas of GA [3, 4]. The natural course of the disease is characterized by the development of atrophic areas that enlarge continuously over time and that are associated with a corresponding absolute scotoma [3, 5, 6]. Visual acuity highly depends on the extent of foveal involvement. While a breakthrough has been achieved in the therapy of neovascular AMD by intravitreally administered anti-vascular-endothelial-growth-factor [7], there is to date no means to counteract the progression of GA. Recently, various interventional trials have been initiated.

For diagnosing GA different imaging modalities are available: fundus photography, fluorescence angiography, fundus autofluorescence (FAF) and optical coherence tomography (OCT; fig. 1). These not only allow refined phenotyping, but are also indispensable means for documenting the natural history of the disease and for identifying possible therapeutic effects in ongoing trials. Novel imaging technologies such as FAF imaging have also been helpful in identifying prognostic factors for disease progression and moreover may contribute to the understanding of the pathogenetic pathways in GA.

Fig. 1. Multimodal imaging in GA due to AMD: fundus photography, fluorescein angiography, FAF and high-resolution spectral-domain OCT (from left to right); from Fleckenstein et al. [62], with kind permission from Springer Science+Business Media.

Fundus Photography

Fundus photography can be used to detect the presence of GA. In clinical trials, based on conventional fundus photographs, GA has been defined as a sharply demarcated area of apparent absence of the RPE, larger than 175 μm in diameter, with visible choroidal vessels and no neovascular AMD [5, 8, 9]. Reduction of retinal thickness due to loss of outer neurosensory cell layers can be visualized by means of stereo fundus photography.

Until recently, fundus photography has been regarded as standard method for the assessment of the slowly progressive disease process [10]. Indeed, the majority of epidemiological and natural history studies addressing the enlargement of GA areas over time have used color fundus photographs [5, 10–13]. However, graders at reading centers have reported difficulty in reproducibly measuring atrophic patches due to intersubject variability of fundus pigmentation, media opacities and the presence of drusen or small satellites of atrophy [14–16]. Low contrast between the hypopigmented atrophic areas and the surrounding nonatrophic retina may in addition lead to difficulties in exact and reliable delineation.

Fluorescence Angiography

In fluorescein angiography, atrophic patches appear as well-demarcated, hyperfluorescent areas resulting from increased visualization of the normal choroidal fluorescence due to loss of RPE cells, which would otherwise weaken the transmission of fluorescein fluorescence (window defect; fig. 1). As compared to fundus photography, this signal exhibits higher contrast levels for delineation.

However, neovascular or fibrotic alterations as well as drusen or pigmentary changes may also lead to an increased fluorescence signal and hence may interfere with GA delineation.

In indocyanine green angiography (ICG-A), atrophic patches appear as discrete hypofluorescent areas [17] with loss of background fluorescence due to accompanying atrophy of the choriocapillaris. Large and deep choroidal vessels may still be visible. Outlining the area of atrophy appears to be more difficult in ICG-A than it is in fluorescein angiography.

Fluorescence angiography is an invasive technique associated with several risks for the examined patient. Furthermore, it is relatively time consuming and less appropriate for GA quantification than the noninvasive techniques as described below. However, fluorescence – and especially fluorescein – angiography can be useful in identifying choroidal neovascularization that may develop in the course of the disease process.

Fundus Autofluorescence

Multiple lines of experimental and clinical evidence indicate that excessive accumulation of lipofuscin (LF) in postmitotic RPE cells together with its toxic components such as N-retinylidene-N-retinylethanolamine plays a role in the pathogenesis of GA associated with AMD [18–20]. LF is thought to mainly derive from the incomplete digestion of photoreceptor outer segments. It accumulates in the lysosomal compartment of RPE cells with age [21] and also in various complex and monogenetic retinal diseases including Best's disease, Stargardt's disease and AMD [19]. As yet it has not been completely understood

Fig. 2. Progression of GA illustrated by FAF imaging and GA quantification over time. Areas are segmented (white lines) for quantification of total GA area. During a follow-up period of about 9 years, the atrophic area increased to about 4 times the size

at baseline. Note that enlargement of GA dominantly takes place in areas where there is a high FAF intensity in the perilesional zone at baseline; from Scholl et al. [37], with kind permission from PLoS One.

why physiological LF accumulation with age leads to pathological findings and onset of retinal diseases in only some individuals.

FAF represents an imaging modality capable of reflecting the morphological changes associated with this common downstream pathogenetic pathway. It allows for topographic in vivo imaging of intrinsic fluorophores in the retina in a non-time-consuming and noninvasive way [22]. Spectrophotometric analysis by Delori et al. [23] has shown that the in vivo FAF signal is mainly derived from RPE LF.

As opposed to the invasive fluorescein angiography and ICGA, the FAF signal intensity is much lower. Hence, for achieving good image quality with a sufficient signal-to-noise ratio, camera systems are required that (1) reduce autofluorescence (AF) signals from planes other than the RPE, (2) use high sensitivity levels and (3) are capable of averaging multiple single images during acquisition. With the advent of confocal scanning laser ophthalmoscopy (cSLO), it has been possible to combine these properties in one imaging device [22, 24–27].

Using an excitation wavelength of 488 nm and an emission bandwidth of 500–700 nm, areas of GA exhibit very low to extinguished FAF signals (dark) as RPE cell death is accompanied by loss of LF, leading to high contrast between atrophy and the perilesional retina (fig. 1). Contrast levels rise even further by the commonly observed finding that the perilesional zone shows high FAF intensities. Perilesional FAF changes in particular appear to have pathogenetic relevance: areas of increased FAF and hence LF accumulation outside GA may be associated with variable degrees of retinal sensitivity loss and

precede the development and enlargement of outer retinal atrophy (fig. 2) [28–30]. These findings correlate with experimental data suggesting that certain molecular compounds of LF such as N-retinylidene-N-retinylethanolamine possess toxic properties and may interfere with normal cell function [31–33].

High contrast to the perilesional nonatrophic retina allows for exact delineation and segmentation of atrophic areas using gray-value-based region-growing algorithms in semiautomated software [6, 34]. Further development has recently resulted in the RegionFinder software by Heidelberg Engineering, Germany, for the existing Heidelberg Eye Explorer software (fig. 3). This novel image-processing tool allows for reproducible and rapid semiautomated detection and measurement of the size and progression of atrophic GA areas [35]. Its functions comprise direct export and alignment of recorded images, semiautomated identification of atrophic patches after selecting a seeding point and adjusting tolerance levels, shadow correction, semiautomated vessel detection and creation of a report form displaying the results of the analysis. Its application is not only suitable for analysis of progression rates in natural history studies or interventional trials aiming to slow down the disease process, but also for routine use in daily life, documenting disease progression in each individual patient.

However, quantification of GA based on FAF images alone may be challenging. This is mainly due to low FAF intensities in the fovea because of absorption of short-wavelength light by foveal pigment. Utilizing corresponding blue reflectance (488 nm) and near-infrared re-

Fig. 3. Semiautomated quantification of GA by means of image analysis software (RegionFinder, Heidelberg Engineering, Germany). Areas of atrophy can be quantified in a precise, reproducible and time-effective way. In this example, the difference image

on the right shows the enlargement of GA between baseline (left) and the follow-up visit (middle); from Fleckenstein et al. [63], with kind permission from Springer Science+Business Media.

flectance (820 nm) images may help to improve discrimination of foveal lesion boundaries.

In various longitudinal natural history studies, high interindividual variation of atrophy progression has been independently reported, which cannot be explained by any of the demographic factors analyzed so far [10, 12, 13, 27]. There is evidence that more specific individual or eye characteristics may at least in part be able to explain interindividual variability in the spread of atrophy. The FAM (Fundus Autofluorescence in Age-related Macular Degeneration, www.clinicaltrials.gov, NCT00393692) study group has identified different phenotypic patterns of abnormal FAF in the junctional zone of GA based on

cSLO FAF imaging and described a step-wise classification approach (fig. 4) [27, 36]. Analysis on longitudinal FAF imaging data on 195 eyes of 129 patients has shown prognostic relevance of these patterns for GA enlargement over time [27].

Atrophy progression rates were slowest in eyes without abnormal FAF in the perilesional zone (median 0.38 mm²/year) followed by eyes with focal increased FAF (median 0.81 mm²/year). Enlargement rates were higher in eyes with a more pronounced increase in FAF such as the banded (median 1.81 mm²/year) or diffuse (median 1.77 mm²/year) patterns. The comparison of progression rates between the groups 'none' or 'focal' on the one hand

Fig. 4. Dominant phenotypic patterns of abnormal FAF in the junctional zone of GA based on cSLO FAF imaging. These patterns have prognostic relevance for the disease progression in terms of atrophy enlargement over time. While those patterns with only little change in FAF intensities surrounding GA predis-

pose for slow progression, the banded and all diffuse patterns representing a more pronounced increase in FAF are frequently accompanied with high progression rates; GPS = fine granular with peripheral punctate spots; from Fleckenstein et al. [63], with kind permission from Springer Science+Business Media.

and 'banded' or 'diffuse' on the other hand revealed a statistically significant difference ($p < 0.0001$). Although these patterns may reflect heterogeneity on a molecular, cellular or genetic level, significant risk loci for susceptibility of GA in AMD appear to have no significant influence on GA progression [37]. Regarding the data retrieved by Schmitz-Valckenberg et al. [38] and Bearely et al. [39] on the amount of increased FAF around atrophy and its influence on atrophy enlargement, all these findings underline the hypothesis of LF accumulation causing RPE cell death. Other prognostic factors for GA progression have also been described, i.e. the knowledge of prior enlargement rates [10] or the enlargement rate of the fellow eye [10, 40], multifocal configuration of GA [13], baseline GA size [10, 27] and the diagnosis of the fellow eye [Goebel AP, et al.: Invest Ophthalmol Vis Sci 2011, vol. 52, ARVO E-abstract 1682].

Natural history data and identification of high-risk characteristics have already and will further help to test novel interventions in clinical trials aiming to counteract

the slowly progressive disease process. Based on the natural history data in the previous studies, it appears that the GA progression rate has been approved by the Food and Drug Administration as primary outcome measure in clinical trials on GA [41].

Recently, FAF imaging using a different wavelength has been described as near-infrared AF using the ICGA mode of the cSLO without dye injection (excitation: 790 nm, emission >810 nm) [42, 43]. Herein, melanin in the RPE seems to be the dominant source of the signal besides a varying signal from the choroid. However, contribution of other fluorophores cannot be excluded [42, 44]. Comparative imaging with both modalities (AF 488 nm and AF 790 nm) exhibited a decreased signal over atrophic areas in both cases. The contrast to non-atrophic surrounding retina was more pronounced, however, in images of AF 488 than in AF 790 nm [44]. Also, the two imaging techniques reveal distinct AF changes in the perilesional zone of atrophy, but these abnormalities differ between both modes [42, 44, 45]. Further longitudinal

Fig. 5. Progression of GA illustrated with high-resolution SD-OCT over a time period of 14 months. Progressive loss of the RPE layer (inner part of band 4), interface of the inner and outer segments of the photoreceptor layer (IPRL; band 2) and external limiting membrane (ELM; band 1) as well as thinning of the outer nuclear layer (ONL) at the border of atrophy are accompanying GA enlargement. Within the atrophic lesion, the remaining part of band 4 becomes more homogeneous, and the ONL progressively thins. Finally the outer plexiform layer (OPL) seems to lie directly adjacent to the outer part of band 4; BM = Bruch's membrane; from Fleckenstein et al. [51], with kind permission from the Association for Research in Vision and Ophthalmology.

Fig. 6. Quantification of lateral spread of GA in an SD-OCT scan. Combined imaging with cSLO and SD-OCT allows for follow-up examinations exactly at the same location as the baseline examination. Longitudinal analysis reveals spread of atrophy measured by documenting GA border position; from Fleckenstein et al. [63], with kind permission from Springer Science+Business Media.

analysis is needed to evaluate whether near-infrared AF is able to identify additional high-risk markers for disease progression.

An alternative method to acquire FAF images is the use of a fundus camera (FC). In order to reduce its disadvantage of not using confocal optics and hence being prone to detection of fluorescence from all tissue levels in the light beam, e.g. the lens, a modified FC with adjusted excitation and barrier filters has been proposed by Spaide (excitation 500–610 nm, emission 675–715 nm) [46]. Comparing FAF images recorded with the modified FC and with the cSLO suggests that the agreements for atrophy quantification are similar with both techniques.

However, the FC seems to be inferior in capturing distinct FAF changes in the perilesional zone and hence in detecting prognostic markers for GA progression [47].

Optical Coherence Tomography

High-resolution spectral-domain OCT (SD-OCT) has provided new insights into various microstructural alterations associated with GA [48–55]. Anatomical sites of particular interest are the atrophic area itself as well as the perilesional retinal tissue. SD-OCT imaging revealed a wide spectrum of morphological abnormalities at both sites. As a common finding, within the atrophic lesions, SD-OCT bands 1, 2, 3 and 4 (corresponding to anatomical layers including the external limiting membrane, the inner-segment/outer-segment layer, and the RPE/Bruch's membrane complex) as well as the assumed outer nuclear layer are usually absent [50].

Polarization-sensitive SD-OCT seems to be able to give even more detailed insights into the integrity of the RPE [56, 57]. Ahlers et al. [58] illustrated that the intrinsic tissue property of the RPE to depolarize backscattered light can be used to clearly identify this cell layer even in the presence of severe distortions of the physiological retinal structure. In areas of GA they observed loss of polarization, along with patterns of depolarizing signals in deeper layers.

Simultaneous recording of cSLO and SD-OCT images in one instrument with an exact topographic overlay during image acquisition allows for correlation of pathological findings in different imaging modalities and accurate orientation of cross-sectional SD-OCT scans at anatomical sites of interest. Furthermore, serial examinations at the same anatomical location over time can be acquired [51, 52, 55]. Comparing FAF changes in GA eyes with simultaneously acquired SD-OCT scans, Schmitz-Valckenberg et al. [54] could show that the mean length of an atrophic lesion measured in the FAF image showed closest agreement with the disruption of choroidal hyperreflectivity as recorded in the SD-OCT scan, demonstrating that the severe reduction of the FAF signal is spatially correlated with the abrupt transition from a hyporeflective to hyperreflective area in the choriocapillaris below Bruch's membrane on the SD-OCT scan presumably due to the loss of the RPE layer. Recently, there have been promising approaches to planimetrically measure the area of GA by detecting pathological findings in the correspondent SD-OCT-scans. However, it needs to be further evaluated which of the multiple pathological

changes in B scans of GA eyes should be graded to obtain equivalent planimetric measurements in FAF. Sayegh et al. [59] showed that the area of complete choroidal signal enhancement in the SD-OCT B scans correlated best with the hypofluorescent area measured in FAF.

Using SD-OCT technology, the dynamic nature of development and progression of atrophy can be longitudinally analyzed in the same eye on a 3-dimensional level. Microstructural changes such as the advancing loss of the RPE and photoreceptor bands at the GA border or the subsequent apposition of the outer plexiform layer with Bruch's membrane within the atrophic lesion have recently been described (fig. 5) [51]. Quantification of the lateral spread of GA by documenting the change in GA border position over time in longitudinal B scans at the same anatomical site showed a median spread of 106.90 $\mu\text{m}/\text{year}$ (fig. 6) [51]. However, there was high variability not only between different patients, but also between different scan directions in the same eye. By this it can be assumed that specific local factors may influence GA growth [51]. This approach may be helpful both for monitoring the natural course of the disease and to elucidate pathogenetic mechanisms. In particular, the identification of structural risk factors reflecting disease activity and future GA progression may be important for prognosis and visual function.

There have also been different approaches using the SD-OCT to document GA area and progression by creating an en face fundus image similar to that produced by the cSLO on the basis of multiple side-by-side B scans [53, 60–62]. First results are promising, but further analysis appears mandatory. Using OCT en face fundus images, Yehoshua et al. [61] examined 86 eyes of 64 patients and found a mean total GA area at baseline of 4.59 mm^2 . They reported high reproducibility and intergrader agreement. In contrast to the acquisition of FAF images with the cSLO, here only one type of scan is needed for documenting both en face and cross-sectional images.

Disclosure Statement

A.P. Göbel, Heidelberg Engineering, F; M. Fleckenstein, Heidelberg Engineering, Optos Ltd., F; Heidelberg Engineering, R; S. Schmitz-Valckenberg, Heidelberg Engineering, Optos Ltd, Topcon UK, F; Heidelberg Engineering, R; C.K. Brinkmann, Heidelberg Engineering, F; F.G. Holz, Heidelberg Engineering, Optos Ltd., F; Heidelberg Engineering, Zeiss Meditec AG, C; Heidelberg Engineering, R (F = Financial Support; R = Recipient; C = Consultant).

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