Autofluorescence Imaging of Choroidal Neovascularization Due to Age-Related Macular Degeneration

Samantha S. Dandekar, MRCOphth; Sharon A. Jenkins, MSc; Tunde Peto, PhD; Hendrik P. N. Scholl, MD, MA; Kulwant S. Sehmi, FRPS; Fred W. Fitzke, PhD; Alan C. Bird, MD; Andrew R. Webster, MD

Objective: To describe the autofluorescence (AF) characteristics of choroidal neovascularization (CNV) in patients with age-related macular degeneration.

Methods: Autofluorescence images of 65 consecutive eyes with CNV at various stages of evolution were analyzed. Twenty images were of recent-onset CNV (group 1), 8 were of eyes 1 to 6 months after CNV diagnosis (group 2), and 37 were late-stage CNV (group 3). Autofluorescence images from groups 1 and 2 were compared with fundus fluorescein angiographic images.

Results: Group 1 showed areas of hyperfluorescence on fundus fluorescein angiography corresponding to areas of normal AF in 16 of 20 cases, with adjacent areas of increased AF in 13 cases. The main areas of abnormal AF were larger than the main areas of abnormal fluorescence on fundus fluorescein angiography in 18 of the 20 cases. Groups 2 and 3 showed areas of decreased AF corresponding to areas of previous leakage on fundus fluorescein angiography (in group 2) or atrophy.

Conclusions: Preserved AF in group 1 indicates viable retinal pigment epithelium initially, which has implications for visual prognosis. Decreased AF in groups 2 and 3 indicates loss of retinal pigment epithelium and photoreceptors. Autofluorescence imaging may increase our understanding of CNV in age-related macular degeneration.

Arch Ophthalmol. 2005;123:1507-1513

Choroidal neovascularization (CNV) is a common cause of visual loss in patients with age-related macular degeneration (AMD). It occurs in the setting of early age-related maculopathy that consists of changes in the retinal pigment epithelium (RPE) and Bruch membrane.1-3 The process starts with inward growth of vessels from the choroid that extend through Bruch membrane into the subretinal pigment epithelium (sub-RPE) area.1 Occasionally, these may extend into the subretinal space.1,5 Choroidal neovascularization may then lead to serous or hemorrhagic detachment of the RPE or retina. As the lesion develops, RPE and photoreceptor degeneration occurs over the disciform scar.4

In the clinical setting, fundus fluorescein angiography (FFA) is routinely used to assess the location, extent, and nature of lesions in AMD. Neovascular complexes are classified as classic or occult lesions based on the definitions by the Macular Photocoagulation Study Group.6 Autofluorescence (AF) imaging has been developed as a tool to evaluate RPE during aging and in ocular disease,7,8 based on initial observations by Delori et al.9 It allows the assessment of AF derived from lipofuscin in RPE.7,9 Excessive accumulation of this compound precedes photoreceptor degeneration.10-12

The ability to assess the integrity of RPE may be important for 2 reasons. First, RPE may affect the behavior of the choroidal new vessel complex.13,14 Second, visual outcome may be determined by whether RPE maintains its physiological function. In geographic atrophy, AF is decreased because either there is RPE loss or AF depends on metabolic activity of RPE that is driven by the need for photoreceptor outer segment renewal, and areas of increased AF are observed at the junctional zone.15

Autofluorescence imaging in CNV is less well studied and may provide further information about the involvement of the RPE during the course of neovascular AMD. In this study, we evaluated AF images in patients with different stages of CNV and compared them with FFA images in patients with recent-onset CNV.
ment [PED]) at various stages of disease were analyzed from an ongoing cross-sectional clinical study and collection of DNA from patients with AMD. Eligibility criteria for this study included (1) patients with CNV or PED due to AMD in at least 1 eye; (2) absence of other retinal changes causing visual loss; (3) age older than 50 years at the onset of visual symptoms; and (4) AF, color, and FFA imaging in the patients with recent-onset CNV. The study was approved by the local ethics committee, and informed consent was obtained for participation in the study.

The patients were divided into 3 groups. Group 1 consisted of 20 patients who had AF imaging undertaken at the time of CNV diagnosis using FFA. Group 2 consisted of 8 patients who had AF imaging performed 1 to 6 months after the diagnosis of CNV. Group 3 consisted of the remaining 37 patients, who had AF imaging of late-stage CNV with established disciform scars. Fundus fluorescein angiography was not performed in group 3.

In patients with recent-onset CNV (group 1), the FFA images were compared with the AF images using Topcon IMAGEnet 2000 software (GB Ltd, Newbury, United Kingdom). This allowed areas of hyperfluorescence on FFA to be mapped exactly to corresponding areas on the AF images. Measurements between vessel bifurcations were made as an internal control to ensure adequate adjustment of magnification differences between images. Patterns of AF within and around the lesions were analyzed, and overall areas of abnormality on AF imaging and FFA were measured (described in the next subsection). In group 1, the proportion of classic occult CNV and avascular PED was estimated from the early and late FFA images according to guidelines by the Macular Photocoagulation Study. In group 2, in which the AF images were taken a few months after the diagnosis of CNV, the proportion of classic occult CNV and avascular PED was estimated from the early and late FFA images. Group 3 consisted of the remaining 37 patients, who had AF imaging of late-stage CNV with established disciform scars. Fundus fluorescein angiography was not performed in group 3.

AF IMAGING AND FFA

Autofluorescence imaging was recorded using a Heidelberg retinal angiograph (HRA) (30° field of view; Heidelberg Engineering, Heidelberg, Germany) or a Zeiss scanning laser ophthalmoscope camera (40° field of view; Carl Zeiss, Jena, Germany). To enhance the image contrast, a confocal aperture was used to suppress light originating from outside the focal plane. Both instruments have an ametropic corrector to correct refractive error to focus on the structure of interest. An argon blue laser (488 nm) was used for illumination and excitation in both instruments, and emitted light was detected above the wavelength of the barrier filter (500 nm for the HRA and 521 nm for the Zeiss camera). This cutoff filter suppresses light originating from outside the focal plane. Both instruments have an ametropic corrector to correct refractive error to focus on the structure of interest. An argon blue laser (488 nm) was used for illumination and excitation in both instruments, and emitted light was detected above the wavelength of the barrier filter (500 nm for the HRA and 521 nm for the Zeiss camera). This cutoff filter suppresses light originating from outside the focal plane. Both instruments have an ametropic corrector to correct refractive error. The AF images were then digitized, aligned, and averaged (9 with the HRA and 32 with the Zeiss camera) using image analysis software to produce a single image per eye. The HRA uses software provided by Heidelberg Engineering (Heidelberg Eye Explorer, version 2.1.0, 11/2002 for Windows 98/NT/2000/XP), and the Zeiss camera uses an in-house frame grabber program written in the C programming language using functions from the Matrox Image Library (Matrox Electronic Systems Ltd, Quebec City, Quebec).

Fundus fluorescein angiographic images were obtained using a digital fundus camera system (Topcon IMAGEnet 2000). Five milliliters of 20% fluorescein dye was injected into the antecubital vein, and images were recorded according to standard techniques.

IMAGE ANALYSIS SOFTWARE AND AREA MEASUREMENT

For all patients in group 1, a mid to late venous phase FFA image was selected so that the site of leakage could be identified and compared with the AF image. In each case, the AF image was taken just before FFA to avoid artifactual increased AF. Both images were imported into the Topcon IMAGEnet 2000 program to compare images, and at least 6 reference points at vessel bifurcations were selected to allow comparison and compensation for varying magnification. Using the area measurement feature, the extent of abnormal fluorescence on AF and FFA images and vessel-to-vessel reference measurements between bifurcations were measured by one of us (S.S.D.) (Figure 1A and B). For each measurement, the mean of 3 measures was determined. The main areas of abnormality or leakage were measured because these were considered to be better delineated and more easily identifiable than the entire lesion border, which was often difficult to determine.

STATISTICAL ANALYSIS

To determine whether there was a significant difference between the main areas of abnormality imaged using AF imaging vs FFA, a paired t test was performed. Graphically, the difference in area was plotted against the mean area using a Bland-Altman plot.

RESULTS

Of the 65 patients analyzed, 27 were men and 38 were women. The median age was 78 years (age range, 58-90 years). The median ages of groups 1, 2, and 3 were 78, 75, and 78 years, respectively. One eye from each patient was analyzed, including 35 left eyes and 30 right eyes. In group 2, the AF images were taken a few months after the diagnosis of CNV (mean interval, 4 months; range, 1-6 months). In group 3, all the patients had established disciform scars and had lost vision in the affected eye more than 1 year before the initial examination. The median Snellen visual acuities in groups 1, 2, and 3 were 20/60, 20/100, and 20/600, respectively.

VESSEL-TO-VEssel MEASUREMENTS

Vessel-to-vessel measurements were comparable between the AF and FFA images in all cases, and the difference was not statistically significant (mean difference, −0.008 mm; 95% confidence interval, −0.056 to 0.040 mm; P = .73). This indicated that magnification differences were adequately adjusted for using the Topcon IMAGEnet 2000 software.
In group 1, the main area of abnormal AF was more widespread on AF imaging than the main area of abnormal fluorescence on FFA in 18 of the 20 cases (mean difference in size, 1.63 mm²; 95% confidence interval, 0.58-2.68 mm²; \( P = .004 \)). This is demonstrated by the Bland-Altman plot (Figure 2). In 3 cases in which the main abnormal area extended beyond the edges of the AF image, the area was only measured up to the limits of the image. Therefore, we would expect that the difference in total areas between the AF and FFA images would be even larger in these 3 cases.

**SPATIAL CORRELATION OF LEAKAGE ON FFA**

In groups 1 and 2, the areas of hyperfluorescence on FFA were mapped to the AF images. In group 1, these mapped to an area of normal AF in 16 cases (Figure 1A-H). The "a" indicates areas of decreased AF where leakage is seen on the FFA image. The "b" indicates a widespread area of increased AF (thought to be a gravitational effect) surrounding the lesion and not corresponding to anything on the FFA image. The "c" indicates normal patches of AF within the lesion, with increased AF toward the edge of the lesion. The "d" marks the optic disc.
with an adjacent area of increased AF in 13 cases (Figure 1A-F, K, and L). In 4 cases, the hyperfluorescence corresponded to an area of decreased AF (Figure 1A, B, and I-L). In group 2, the areas of previous leakage on FFA mapped to areas of decreased AF in all 8 cases (Figure 3A-D). In 4 of 8, an area of increased AF was present adjacent to fluoresecin hyperfluorescence (Figure 3C and D). Fundus fluorescein angiography was not performed in group 3.

PATTERNS OF AF

Eighteen of 20 eyes in group 1 demonstrated patches of normal AF that could be seen within the lesions. In 13 of these, normal AF could be seen at the fovea, and 3 had a Snellen visual acuity of 20/30. Five of 20 eyes were classified as having lesions with 0% to 50% classic components (Figure 1C), 6 had greater than 50% classic lesions (Figure 1A and C), 7 had 100% occult lesions (Figure 1K), and 2 had avascular PEDs (Figure 1G). Of the 7 eyes without a focal increase in AF adjacent to FFA hyperfluorescence, 3 were 100% occult, 2 had avascular PEDs, and 2 were 50% or less classic lesions.

Seven of 8 eyes in group 2 demonstrated some areas of normal AF within the lesions, with 4 including the fovea (Figure 3B). One patient with normal AF at the fovea had a visual acuity of 20/30.

In group 3, focal increases of AF were seen in 27 of 37 cases (Figure 4A and B). Twenty-nine cases consisted of fibrous scar, and 8 were graded as exudative lesions with a serous detachment. In this group, 31 cases had no normal AF (Figure 4A-D), and 6 cases showed patches of normal AF within the lesions but none at the fovea. No patient had a visual acuity better than 20/50.

Two patterns of AF were seen in this group. In the first pattern, 23 of 37 cases had widespread areas of decreased homogeneous AF throughout the lesions (Figure 4D). In 9 of these, surrounding secondary atrophy of the RPE was demonstrated on the color images (Figure 4C). The second pattern was more heterogeneous and mottled, with areas of increased and decreased AF (Figure 4B). This pattern was seen in 14 cases, and 6 had areas of atrophy on the color images.

In all 3 groups, areas of decreased AF corresponded to areas of atrophy (Figure 4C and D) (n=21), subretinal blood (n=6) (Figure 3C and D), or exudates (n=12) (Figure 3A-D). In 1 case in which the blood was predominantly sub-RPE, the intensity of AF appeared preserved.

PIGMENT EPITHELIAL DETACHMENTS

Two cases were graded as having avascular PEDs. On AF imaging, the sub-RPE fluid appeared to show normal AF corresponding to the funduscopic visible area of PED, with the typical normal appearance of decay of AF intensity toward the foveal center (Figure 1H). In 1 case, a dark rim was seen surrounding the elevated area (Figure 1G and H).

This study demonstrated and characterized the inherent retinal AF in eyes with CNV. There is good evidence to support the assumption that a continuous area of AF represents surviving RPE.13 Eyes in groups 1 and 2 often had preservation of AF in the RPE, in contrast to eyes in group 3 in which patches of decreased AF were seen. These results may indicate conservation of RPE viability at least initially in CNV, which may have implications for treatment interventions and long-term visual prognosis. The fact that the median visual acuity in group 1 was better than that in group 3 suggests that visual outcome may be determined by maintenance of RPE function, and this is supported by the good visual acuity in patients with intact AF at the fovea. The time frame to RPE dysfunction and cell death is unknown but is likely to occur in the first few weeks or months after the onset of leakage from new vessel complexes, as shown by the results of this study.

Figure 1J shows an area of increased AF intensity inferior to the actual leakage. This is a common AF finding in eyes with other exudative changes such as central serous retinopathy and is thought to be a result of a gravitational effect.19

Evidence that the RPE may determine the behavior of CNV comes from work by Yamagishi et al13 in which sodium iodate was used to damage RPE cells in primates while inducing CNV with photocoagulation. At least initially, viable RPE was required to induce new vessel growth, and later in the course of the disease, it suppressed the neovascular process. In another study,14 the RPE was thought to produce diffusible factors that then affected the integrity and permeability of the choroid. Experiments in rodents have demonstrated that overex-
pression of vascular endothelial growth factor in the RPE can induce intrachoroidal neovascularization, and high concentrations of vascular endothelial growth factor and its receptors have been detected in choroidal neovascular membranes and in the RPE. This hypothesis is supported by the fact that RPE is present in the early stages of CNV development and overlies the area of maximum leakage. Subsequently, there may be up-regulation of pigment epithelium–derived factor expression, which is believed to be antiangiogenic and may cause the lesion to form an inactive scar.

As measured on AF imaging in group 1, the extent of the main abnormal areas was larger than that on FFA in 18 of 20 cases. Although the difference was small, it was statistically significant and has been interpreted as implying that the RPE abnormality is more extensive than the main area of FFA hyperfluorescence. Therefore, AF imaging may more accurately delineate areas of CNV. In support of this, a histological study by Bynoe et al of subfoveal neovascular membranes from 6 patients (4 with AMD) showed that excised CNV complexes were significantly larger than expected from FFA. Moreover, the authors demonstrated that the distribution of blood vessels was irregular and that large areas of the complex periphery were avascular and would not be visible on FFA. If this interpretation is correct, it has implications for the recurrence of CNV following laser treatment based on FFA findings and supports the need to include a surrounding area of apparently normal retina in any treatment.

In 13 of 20 eyes from group 1 and in 4 of 8 eyes from group 2, areas of increased AF were seen adjacent to areas of FFA hyperfluorescence. These areas appeared to be more prevalent in those eyes with a classic component on FFA. This observation may be explained by the fact that increased AF may be due to phagocytosis of laterally diffused debris derived from the new vessel complex, which would be greater in those with classic new vessels, and would not necessarily only define the area of leakage.

The differences in the anatomical site of neovascular membranes in eyes with classic and occult CNV was investigated in a clinicopathological study of 29 eyes by...
Lafaut et al. They showed that 18 of 19 classic complexes had a predominantly subretinal fibrovascular component, as opposed to occult lesions (n=10), which all contained a sub-RPE fibrovascular component. It is hypothesized that classic membranes originate in the sub-RPE space and then grow through the plane of the RPE to proliferate in the subretinal space. Therefore, we would expect to see more abnormalities on the AF images of classic membranes than on those of occult membranes.

In all 3 groups, areas of decreased AF corresponded to areas of atrophy, subretinal blood, or intraretinal exudate. In 3 eyes, increased AF was seen adjacent to areas of RPE atrophy. This phenomenon is known to occur in primary geographic atrophy in eyes with AMD and represents areas of RPE with abnormal function that are vulnerable to subsequent atrophic change.

The finding of normal AF intensity from RPE overlying avascular PEDs is in accord with the view that these lesions occur because of changes in the structure of the underlying Bruch membrane and that the anatomy and function of the overlying RPE would remain undisturbed. In one of these cases in our study, a rim of decreased AF on AF imaging surrounded the pigment epithelial lesion and corresponded to an area of subretinal fluid seen on biomicroscopic examination (Figure 1G and H).

Autofluorescence imaging, unlike other forms of retinal examination, exclusively examines the RPE layer, and the results of this study indicate that AF imaging provides more information on the integrity of this layer than FFA alone. Our results also support the theory that the RPE is important in modifying the behavior of CNV. Serial AF imaging over time in patients with CNV is required to substantiate these results. Unfortunately, these data were unavailable because of the cross-sectional nature of our study. Further investigation may document the extent of RPE viability during the evolution of these lesions, as well as the effectiveness of treatments such as photodynamic therapy.

Submitted for Publication: July 31, 2003; final revision received February 21, 2005; accepted February 21, 2005.

Correspondence: Samantha S. Dandekar, MRCOphth, Moorfields Eye Hospital and Institute of Ophthalmology, 10 Cromwell Ave, Highgate, London N6 5HL, England (sam@hitbits.co.uk).

Financial Disclosure: None.

Funding/Support: This study was supported by grant G000682 from the Medical Research Council, London, United Kingdom, and The Foundation for Fighting Blindness, London (Dr Dandekar), by Friends of Moorfields,
Moorfields, United Kingdom (Dr Peto), and by fellowships SCH 734/2-1 and DFG SCH 734/1-2 from Deutsche Forschungsgemeinschaft Heisenberg, Heisenberg, Germany (Dr Scholl).

**Acknowledgment:** We are grateful to the Medical Research Council for supporting this work and thank the patients for their participation, Anthony Halfyard, PhD, for his technical assistance with the scanning laser ophthalmoscope, and Catey Bunce, PhD, for statistical advice.

### REFERENCES


## Notice to the Authors of Reports From Clinical Trials

The *Journal of the American Medical Association* (JAMA) and the *Archives of Ophthalmology* function as an editorial consortium. With one submission and one set of reviews, your clinical trial manuscript will be considered for publication in both JAMA and the *Archives of Ophthalmology*.

Submit your paper to the journal of your choice according to the appropriate “Instructions for Authors” and the following guidelines will apply:

1. If your manuscript is accepted by JAMA, it will be considered for an editorial or commentary in JAMA. Your abstract may be published in the *Archives of Ophthalmology* with a commentary or editorial.
2. If your manuscript is accepted by the *Archives of Ophthalmology*, it will be considered for an editorial or commentary in the *Archives of Ophthalmology*. Your abstract will also be considered for publication in JAMA.