



## Fundus autofluorescence in exudative age-related macular degeneration

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**ABSTRACT.** The aim of this study was to investigate the characteristics of fundus autofluorescence (FAF) in patients with wet (exudative) age-related macular degeneration (AMD). Color fundus photographs, fundus fluorescein angiograms, indocyanine green angiograms, and FAF images were obtained from 61 patients (72 eyes) with exudative AMD. The FAF results for different patterns of exudative AMD were compared to those revealed by other fundus images. Of the 72 eyes evaluated, which were classified into three patterns based on the results of fundus fluorescein angiography, 68 had abnormal FAF. Forty-six eyes (63.9%) had classic wet AMD with abnormal FAF. Among these, 10 exhibited a slightly decreased FAF with near-normal or background FAF signal at the center of the lesion area; 36 demonstrated not only decreased FAF at the center of the lesion but also an increased FAF signal toward the lesion edge. Sixteen eyes (22.2%) had occult wet AMD, of which 12 exhibited heterogeneous fluorescence at the lesion site; 4 yielded normal FAF images. Ten eyes (13.9%) had a mixed pattern of wet AMD with abnormal FAF. FAF imaging suggested that the areas

of blood and exudates decreased; however, fluorescence angiography revealed that lesions with hyperfluorescence had background or slightly increased FAF. These results showed that various patterns of wet AMD exhibit different autofluorescence characteristics. These represent the functional and metabolic features of retinal pigment epithelial cells. Therefore, FAF can be used to monitor disease development and evaluate the severity and prognosis of AMD.

**Key words:** Exudative; Age-related macular degeneration; Autofluorescence

## INTRODUCTION

Age-related macular degeneration (AMD) is one of the most common ocular diseases causing blindness in people aged 50 years or older. The prevalence of AMD increases dramatically with age. Research has shown that the prevalence of AMD in people aged 55 to 64 years *vs* those aged 85 years or older are 0.20 *vs* 13%, respectively (Mukesh et al., 2004). AMD is classified into two patterns: wet (exudative) and dry (nonexudative). Exudative AMD accounts for 10 to 20% of all AMD cases and leads to several visual disturbances among patients with AMD. The main fundus features of wet AMD are hemorrhage under the neuroepithelial layer of the retina or retinal pigment epithelium (RPE) of the posterior pole of the fundus, yellow-white fatty exudate, and drusen within or around the lesion area. Macular hemorrhage in the late stage leads to cicatrix formation and central vision loss. Therefore, wet AMD is the leading cause of blindness among people aged 60 years or older (Ma and Chen, 2006; Yah and Wang, 2008). Early discovery and early diagnosis are essential for treatment success. Fundus fluorescein angiography (FFA), indocyanine green angiography (ICGA), and three-dimensional optical coherence tomography (3-D OCT) are conventionally used to diagnose AMD; however, recent advances in diagnostic ophthalmological techniques have allowed for the development of fundus autofluorescence (FAF) imaging, a simple and noninvasive diagnostic technique that can be used to diagnose different patterns of AMD early in the course of disease. Lipofuscin is a type of fluorescence matter that mainly exists in RPE cells. It is the accumulation of matter generated from the outer segments of photoreceptors phagocytosed by RPE cells. RPE cell metabolism and dysfunction lead to large amounts of lipofuscin accumulating in RPE cells, which can result in abnormal FAF results. Therefore, the function, activity, distribution, and metabolic status of RPE cells, which play an important role in the occurrence and development of AMD, can be determined using FAF imaging (McBain et al., 2007; Zhao and Peng, 2008). Different patterns of autofluorescence (AF) can be used to visualize the extent of damage and the stage of disease progression. Therefore, FAF represents a potential prognostic marker (Delori et al., 1995; Barry, 2007). Numerous studies have reported on the fluorescein angiography (FA) patterns in patients with dry AMD (Spaide, 2003; Bui et al., 2006); however, the nature of FA images obtained in patients with wet AMD remains largely unexplored. This study evaluated 61 patients (72 eyes) who were initially diagnosed with exudative AMD during the period from April 2010 to March 2012. Patients with classic wet AMD, those with occult AMD, and those with mixed wet AMD exhibited different FAF patterns.

## MATERIAL AND METHODS

### Patients

Sixty-one patients (72 eyes) aged 45 to 86 years old who were initially diagnosed with exudative AMD during the period from April 2010 to March 2012 were included in this study. All procedures were performed in accordance with the Declaration of Helsinki. Detailed examinations of visual acuity, intraocular pressure, as well as fundus, FFA, ICGA, 3-D OCT, and FAF imaging were performed. Other fundus diseases, refractive media turbidity, and previous treatment were causes for exclusion from the study. Of the 61 patients, 35 were males, 26 were females, 11 of 61 had bilateral vision problems, the remaining 50 had unilateral problems. Their visual acuity varied from counting fingers to 0.4. Disease duration ranged from 0.5 to 11 months. This study was conducted with approval from the Ethics Committee of Xinhua Hospital of Shanghai JiaoTong University. Written informed consent was obtained from all participants.

### Imaging

The Kowa nonmyd 7 fundus camera (Kowa Company, Ltd., Japan) was used to take color fundus photographs of each patient. The 3-D scans of the posterior pole were taken with frequency-domain OCT (OCT-1000; Topcon Company, Japan) using a 512 x 128 protocol; 128 horizontal scans of 6 mm were obtained in a 6 x 6 mm area around the center of the fovea; then scans of 512 Å were obtained. A data cube of 6-mm<sup>2</sup> was generated. Photographs of FAF in the macular area as well as FFA and ICGA images were taken with a Heidelberg Retina Angiograph2-Confocal Scanning Laser Ophthalmoscopy (HRA2-cSLO, Heidelberg Engineering, Dossenheim, Germany). A 488-nm argon laser and an emitter with a wavelength of 514 nm from HRA2-cSLO were used for FAF imaging. The recording scope of the fundus posterior pole was 30°. A pinhole aperture of 400 µm was used for confocal detection to prevent interference from a reflective light source. Twenty-five frames of standard FAF images for each eye were automatically superimposed, arranged, aligned, and analyzed with a computer program, and the data were saved accordingly.

## RESULTS

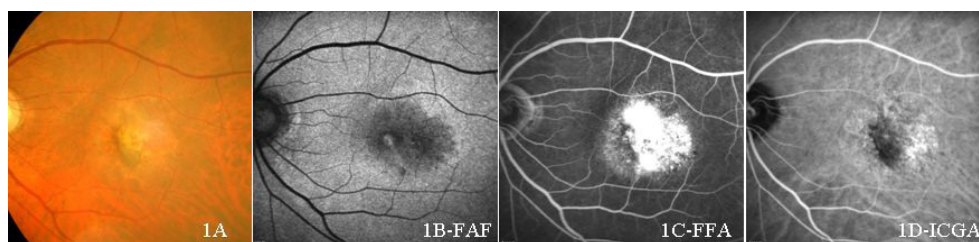
### Clinical characteristics

Of the 72 eyes evaluated, 68 exhibited abnormal FAF. All 72 eyes were classified into three patterns using FFA: 46 eyes (63.9%) had classic wet AMD with abnormal FAF; 16 eyes (22.2%) had occult wet AMD, of which only 12 had abnormal FAF; and 10 eyes (13.9%) had a mixed pattern of wet AMD with abnormal FAF. The specific FAF characteristics of each subtype are described below.

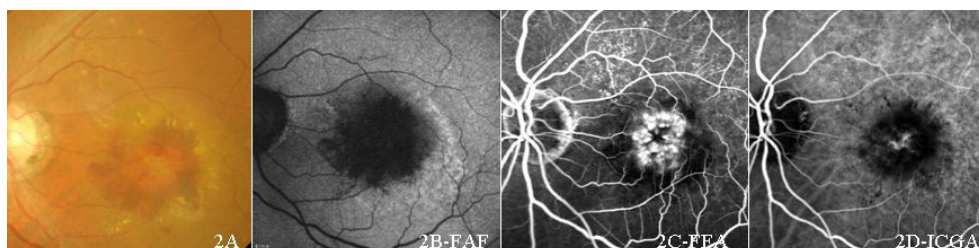
### FAF in classic wet AMD

Slightly decreased FAF with near-normal or background FAF signal was detected at the center of the lesion areas in 10 of the 46 eyes with classic wet AMD, indicating that RPE function had been preserved. These lesions did not show increased FAF in the surrounding areas

(Figure 1A and B). They appeared hyperfluorescent on FFA and demonstrated heterogeneous fluorescence on ICGA due to the leakage of dye during FA, and their abnormal areas were almost the same as those revealed by FAF (Figure 1C and D). The other 36 eyes with classic wet AMD demonstrated not only decreased FAF at the center of the lesion but also an increased FAF signal toward the lesion edge. Increased FAF signals may represent macrophages or the proliferation of RPE cells (Figure 2A and B). The lesion areas appeared hyperfluorescent due to the leakage of dye in FA, but on ICGA, these registered as dark, except at the center of the lesion. The abnormal areas with leakage appeared to be significantly smaller than those seen on FAF imaging. This finding implied that abnormal FAF may represent not only dead RPE and photoreceptor cells but also cells in the process of dying, which would explain why lesions appeared larger with FAF vs FFA (Figure 2C and D).



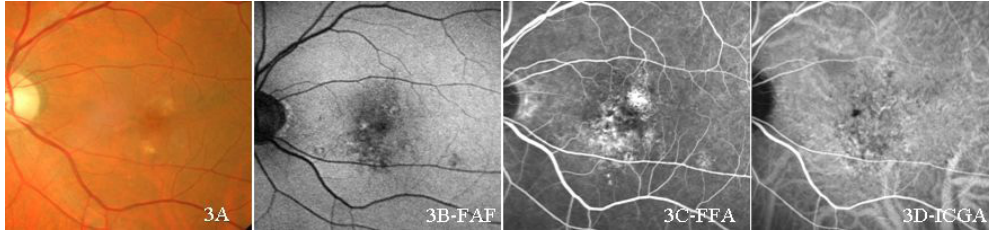
**Figure 1.** Fundus color, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA), and indocyanine green angiography (ICGA) images of an eye with classic wet AMD. The lesion area shows slightly decreased FAF with near “normal” or background FAF signal. The lesion appeared heterogeneously hyperfluorescent on ICGA. Note that the area of leakage appears to be slightly larger than that of increased FAF.



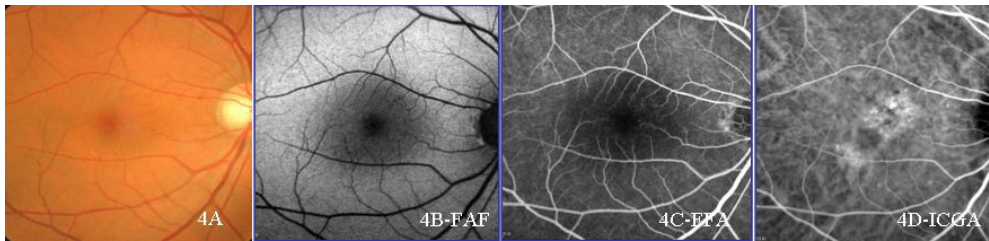
**Figure 2.** Fundus color, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA), and indocyanine green angiography (ICGA) images of a separate eye with classic wet AMD. Decreased FAF was observed at the lesion, with an increased FAF signal towards the edge of the lesion. The lesion area appeared hyperfluorescent on fluorescein angiography, but the lesion area was dark on ICGA images, except for the lesion center. Note that the area of leakage appears to be smaller than that of increased FAF.

### FAF in occult wet AMD

Twelve of the 16 eyes with occult wet AMD showed dark white spotty and heterogeneous fluorescence at the lesion areas (Figure 3A and B). Dye leakage during FA resulted in patchy hyperfluorescence. Choroidal vessel dilation and spotty hyperfluorescence were observed on ICGA images (Figure 3C and D). The other 4 eyes exhibited minimal, diffuse leakage in the macular region but did not exhibit abnormal FAF in FFA images. One eye showed normal colored, FAF, and FFA images, but the results of ICGA revealed vascular abnormality, with the distortion and dilation of choroidal vessels and patchy hyperfluorescence (Figure 4A-D).



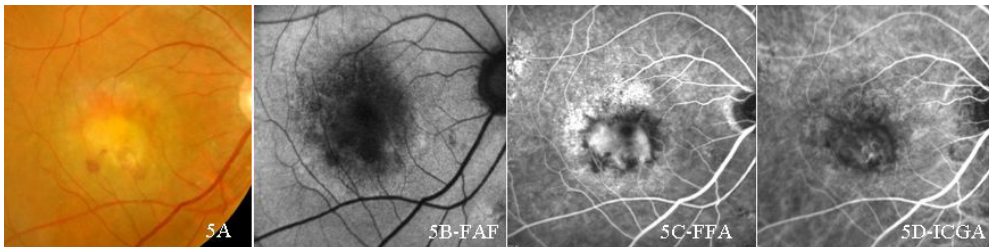
**Figure 3.** A. Fundus color, B. fundus autofluorescence (FAF), C. fundus fluorescein angiography (FFA), and D. indocyanine green angiography (ICGA) images of one eye with occult wet AMD. 3B shows that the lesion area had a decreased FAF with little patchy increased FAF signal. The lesion area appeared mottled fluorescence on FFA and scattered hypofluorescent on ICGA.



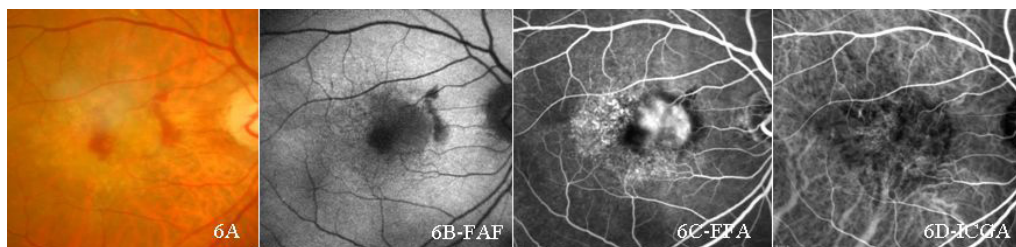
**Figure 4.** Fundus color, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA), and indocyanine green angiography (ICGA) images of another eye with occult wet AMD. A.-C. show no abnormal FAF or FFA but abnormal ICGA with obvious distortion and dilation of the choroidal vessels and patchy hyperfluorescence (D).

### FAF in mixed-pattern wet AMD

In 10 eyes with a mixed pattern of wet AMD, areas of blood and exudate appeared dark on FAF imaging. Based on fluorescence angiography, lesions that exhibited hyperfluorescence appeared to have background FAF intensity or slightly increased FAF intensity (Figures 5A, B and 6A, B). Patchy and spotty hyperfluorescence was detected by FA; the dark signals at the lesion edge and center corresponded to blood. The ICGA images also revealed distorted, dilated choroidal vessels and large, patchy hyperfluorescence (Figures 5C, D and 6C, D). Thus, the mixed pattern of wet AMD exhibited FAF characteristics of both occult and classic wet AMD.



**Figure 5.** Fundus color, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA), and indocyanine green angiography (ICGA) images of one eye with mixed wet AMD. Dark fluorescence can be seen at the areas of blood and exudate on FAF images (A, B). The lesion itself appeared as hyperfluorescent on FFA, but the edge of the lesion appeared as slightly increased FAF with background intensity. The area of FAF was larger than that of FFA. ICGA revealed distortion and dilation of the choroidal vessels and patchy hyperfluorescence (C, D).



**Figure 6.** Fundus color, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA), and indocyanine green angiography (ICGA) images of another eye with mixed wet AMD. Dark fluorescence shows areas of blood on FAF images (A). The edge of the lesion shows slightly increased FAF with background intensity (B). The center of the lesion shows patchy hyperfluorescence with heterogeneous fluorescence toward the edge on FFA (C). Distortion and dilation of choroidal vessels and large areas of patchy hyperfluorescence were also noted on ICGA images. These lesions were larger than those revealed by FFA and FAF (D).

## DISCUSSION

Visual loss in patients with wet AMD is primarily attributed to choroidal neovascularization (CNV), which is characterized by retinal neovascularization in the macular area. The bleeding and exudation associated with neovascularization lead to a sharp decrease in visual acuity and abnormal FAF results. Most scholars think that CNV is caused by damage to the RPE. The structural integrity and composition of Bruch's membrane along with the RPE and RPE cell layers are damaged. Neovascularization originates from the choroidal vasculature and goes through Bruch's membrane to the sub-retinal space. Therefore, the early diagnosis of RPE lesions and evaluation of the prognosis are of great clinical significance to the evaluation and curative efficacy of exudative AMD. The development, severity, and prognosis of AMD are predictable because the structural integrity and function of RPE cells can be determined accurately by studying FAF images.

Sixty-one cases (72 eyes) were included in this study. Both increased and decreased FAF were observed in all but four patients with occult wet AMD. The causes of the reduction in FAF included reduced lipofuscin levels, atrophy, and pigment epithelium dropout. Hypofluorescence, which can represent fresh bleeding, exudate, or thick vitreous opacity, was also commonly detected. Various causes of increased fluorescence, including diseases contributing to RPE dysbolism, such as Stargardt's disease, dry AMD, were also identified. These factors lead to the maldistribution and accumulation of lipofuscin, which results in increased AF. In the present study, cases with refractive media opacity were excluded and only eyes with fundus AMD were evaluated. Although changes in RPE structure and function presented in both exudative and nonexudative AMD, their imaging manifestations differed. Exudative AMD involved retinal neovascularization bleeding or a change in the RPE, which complicated our observations of the RPE.

In the early stage of wet AMD, the FAF signal appeared normal because the activity of RPE cells was preserved. Four cases of occult AMD that did not exhibit abnormal FAF were identified, with color fundus photography and FFA revealing no abnormalities in one case. These findings are consistent with the report of Vaclavik et al. (2008), who found a large region of hypofluorescence caused by a photoreceptor defect as well as scar formation and RPE atrophy in patients with advanced AMD. Dandekar (2005) compared the results of FAF

imaging of CNV with those of ocular fundus vascular angiography in 65 patients with AMD and found that the abnormal FAF areas in patients with early CNV were larger. Our results are almost the same as those of Dandekar: in patients with advanced CNV, the decreased FAF area, which only emerged in the areas of former seepage or atrophy, was smaller than the lesion areas detected with fluorescence angiography of the ocular fundus. The function of the RPE in the patients with early CNV was preserved, but it disappeared in those with advanced CNV. The manifestations of FAF were related to various stages of RPE damage, including total death, apoptosis, and early injury. Completely damaged RPE cells were characterized by their low FAF, whereas apoptotic cells were marked by high FAF because of their stimulus-induced compensatory function (Feeney-Burns and Eldred, 1983). At the early stage of injury, FAF appeared normal because lipofuscin levels were only slightly reduced, and the RPE cells had sustained only minimal injuries (Wing et al., 1978). In a study on early CNV, McBain et al. (2007) detected low fluorescence in 43 eyes with exudative AMD and concluded that the low signal area was formed because the CNV generated from the sub-retinal space overshadowed normal FAF, not because of RPE cell damage or death. These findings are consistent with our results for the early stages of classic wet and occult wet AMD. Some of our patients with exudative AMD were treated with photodynamic therapy. One month later, fundus CNV and the low fluorescence area in the center of the lesion became smaller. The surrounding area of high fluorescence was relatively enlarged. These results demonstrated that the RPE cells around the lesions were not directly injured before the therapy and that the original level of fluorescence was attained after treatment. This mechanism differed from that of nonexudative AMD, in which FAF abnormalities were caused by RPE cell death and the disappearance of lipofuscin.

Based on the results of fluorescence angiography of the ocular fundus, CNV was classified into three patterns: classic, occult, or mixed CNV. The characteristics of the three subtypes are known to have different AF manifestations. Classic wet AMD with CNV manifested a decreased FAF at the lesion center and increased FAF at the edge of the lesion; occult wet AMD was characterized by mottled FAF; and the mixed pattern was marked by diffuse hypofluorescent lesions surrounded by heterogeneous regions of AF (Lafaut et al., 2000; Hughes et al., 2005). Research has shown that CNV penetrates the RPE cell layers, extending into the space between the sensory retina and the RPE, resulting in damage to RPE cell layers in classic wet AMD. In occult AMD, these blood vessels grow slowly under the RPE cell layers, resulting in segmental RPE damage or loss and patchy fluorescence (Bressler et al., 1982, 1988; von Rückmann et al., 1995). When the CNV is not severe enough to damage the RPE, abnormal FAF is not detected in the lesion area, which is in agreement with the four cases of exudative wet AMD in our study. Previous studies have also reported that exudative wet AMD manifests with diffuse hypofluorescence, surrounded by areas of high fluorescence. The peri-lesion hyperfluorescence observed in exudative wet AMD is lower than that observed in classic wet AMD. FAF usually increases in various diseases, leading to disordered RPE metabolism and the accumulation of lipofuscin, followed by increased fluorescence (Bindewald et al., 2005a). The high fluorescence signal at the edge of CNV lesions indicates that the accumulation of lipofuscin is significant. This increase is caused by the compensatory proliferation of RPE cells proximal to the CNV in the progressive stage of the disease (Karadimas and Bouzas, 2005). This increase can also be seen in nonexudative AMD atrophy, drusen, and excessive local pigmentation, which are caused by metabolic abnormalities, defects in the external segments of photoreceptors, or disordered phagocytotic and metabolic recycling functions (Bindewald

et al., 2005b; Holz et al., 2007). In summary, the function, activity, distribution, and metabolic status of RPE cells can be demonstrated by FAF imaging. The RPE plays an important role in the generation and development of AMD. FAF imaging can provide supplementary information to color fundus photography and FA, and it is beneficial in histopathological analysis of the RPE (Lois et al., 2002; Spaide, 2003). Although unique AF patterns are associated with many eye diseases, additional information is necessary to conclusively characterize RPE cell metabolism and changes in RPE cell function. RPE cell lesions can currently be observed from HRA2-cSLO only using a special blue laser system with 488 nm wavelength. Moreover, with the fluorescence technique, lesions in other retinal or choroidal layers could not be excluded, which limited researcher conclusions (Spaide, 2003). In clinical practice, combined treatment with the AF technique and other imaging methods, such as FFA, ICGA, 3D-OCT, and microperimetry, can be used to examine patients with exudative AMD and other macular diseases consecutively, monitor disease development and prognosis, guide treatment, and assess clinical outcomes. FAF imaging is clearly a potent, noninvasive, and feasible way to examine patients with AMD.

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