Fundus autofluorescence applications in retinal imaging

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Fundus autofluorescence (FAF) is a relatively new imaging technique that can be used to study retinal diseases. It provides information on retinal metabolism and health. Several different pathologies can be detected. Peculiar AF alterations can help the clinician to monitor disease progression and to better understand its pathogenesis. In the present article, we review FAF principles and clinical applications.

Key words: Fundus autofluorescence, lipofuscin, retina, retinal imaging

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Fundus autofluorescence (FAF) is a novel noninvasive imaging technique providing *in-vivo* information on retinal status. It is commonly employed in clinical practice to diagnose and study several pathologies.[1,2] Lipofuscin (LF) and melanolipofuscin are the main sources of retinal AF. These fluorophores are endogenous, thereafter there is no need to inject any dye to acquire FAF images. Because of age-related or pathologic accumulation/depletion of fluorophores within the retinal pigment epithelium (RPE) cells and retinal tissue, FAF can show changes in the integrity and metabolism of retinal cells.[3-5] In this review, we summarize the basic principles and clinical applications of FAF.

Retinal Pigment Epithelium and Lipofuscin

Retinal pigment epithelium is a monolayer of approximately hexagonal cells located between the neurosensory retina and the choroid. Its functions include rod's outer segment (OS) phagocytosis. Each RPE cell supports approximately 45 photoreceptors and phagocytes approximately 3 billion OSs over a lifetime. The byproducts of this process are stored in lysosome residual bodies as LF.[6,7] LFs are ubiquitous lipoproteic pigments accumulating in postmitotic cells in nervous, myocardial and retinal cells during ageing. LF occupies approximately one-third of the RPE cells cytoplasm over the age of 70 and emits AF when excited by specific wavelengths. N-retynilidene-N-retynilethanolamine (A2E) represents the LF's major fluorophore.[8,9] It accumulates in the lysosomes because it is not recognized by lytic enzymes as a consequence of photo-oxidative alterations.[10] Its chemical structure is responsible for the detergent-like action on the RPE cells membranes, and its conjugated double bonds promote light absorption and fluorescence emission.[11]

Fundus Autofluorescence Imaging Techniques and Instrumentation

In-vivo FAF was observed for the first time during vitreous fluorophotometry.[5] Subsequently von Rückmann *et al*. introduced the confocal scanning laser ophthalmoscope (cSLO) that elicits retinal AF by scanning the retina with a low-powered laser beam.[12-14] By adopting confocal optics, this technology overcomes the interference of autofluorescent preretinal structures, such as the lens. Confocal optics ensure that the reflectance of the scanning laser and the retinal fluorescence are derived from the same optical plane. The exciting and emission filters of standard confocal ophthalmoscopes are 488 nm (blue light), and 500–520 nm respectively so that cSLO-AF is called also blue-AF or short-wavelength (SW)-AF. Near infra-red (NIR)-AF also uses confocal optics, but with longer exciting wavelength (790 nm). The emission is above 800 nm and its signal is 60–100 times weaker than what seen in blue light AF. Melanin is the main fluorophore in NIR-AF, so fluorescence is more intense in choroidal tissue and RPE cells due to higher melanin density.[15]

Besides cSLO, fundus cameras can also be adapted to provide FAF images projecting a single flashlight on the entire retina at 1 time. Differently from cSLO-AF, fundus cameras don't have a confocal optics system, so the AF gets elicited also from preretinal structures, such as crystalline lens. Potential interferences may be overcome using specific filters with longer wavelength (excitation filter 535–580 nm, barrier filter 615–715 nm) developed by Schmitz-Valckenberg *et al*.[16] This type of FAF is also called green AF.

Fundus Autofluorescence Appearance and Distribution

In the healthy eye, cSLO-AF shows a hypofluorescent area in the foveal region due to the high concentration of xanthophylls

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and melanin. Xanthophylls (lutein and zeaxanthin) protect the foveal photoreceptors and RPE cells filtering blue light, scavenging free-radicals and masking the natural AF of the subfoveal RPE cells.[17-19] AF in the macular area is more intense between 5° and 15° from the fovea, but it still less intense than in the peripheral retina. This is due to a lower LF content and higher melanin deposition in the macular RPE cells when compared to the peripheral ones.[5,20] Optic disc and retinal vessels appear dark, because of the absence of RPE in the optic disc and the blood blocking AF where vessels lie [Fig. 1]. Normal FAF appears slightly different with green-AF showing a less evident AF blockage by the macular pigments and less signal decreasing over the optic disc and blood vessels.[16] The high foveal autofuorescence with NIR-AF imaging in the normal retina corresponds to the higher concentration of melanin in that area, due to the higher and more cylindric shape of RPE cells [Fig. 2].[15]

Fundus Autofluorescence Images Interpretation

By evaluating abnormalities in FAF images, several retinal alterations can be identified. A lower AF signal can express a reduction in the RPE cells number and/or low LF concentration. Areas, where RPE is atrophic, will appear hypoautofluorescent.[21,22] Fibrosis, the presence of intraretinal fluid, the accumulation of pigment and blood can reduce the AF as well. Conversely, areas of LF accumulation (such as in Stargardt's disease, Best's disease, and other dystrophies) correspond to an increased AF signal. A FAF enhancement can also be observed in the presence of some types of drusen and macular edema.[16]

Clinical Applications of Fundus Autofluorescence Imaging

Retinal dystrophies

Autofluorescent material deposition characterizes several retinal dystrophies. An increased background AF and focal hyper-AF are common in these disorders.

Autosomal recessive Stargardt disease

Autosomal recessive Stargardt disease-fundus flavimaculatus (STGD1) is caused by mutation occurring in the ABCA4 gene on the chromosome 1 that leads to an excessive LF formation. By irradiating the juxtafoveal retina of STGD1 patients with a 510 nm wavelength, Delori *et al*. found a 3 times higher AF signal than controls.[23] By using SW-AF, Boon *et al*. observed focal hyper-AF in patients affected by STGD1, not necessarily corresponding to ophthalmoscopically evident flecks and associated to nearby areas of normal or reduced macular AF. In other cases, they found a diffusely increased FAF with hypoautofluorescent speckles and macular areas showing a reduced AF.[24] Recently Burke *et al*. found STGD1 hyper-AF (488 nm excitation) to characterize certain phenotypes. They observed the G1916E and G851D genetic mutations to be associated with a slower LF accumulation and less intense AF as compared to other genotypes.[25,26] These evidences indicate how FAF findings may be used to detect candidates for the genetic screening of ABCA4 mutations. The presence of peripapillary sparing, that is present in most cases and was considered pathognomonic of Stargardt disease in the past[27] can be assessed using FAF. This finding correlates with better general retinal function as observed by electroretinogram (ERG). Conversely peripapillary atrophy and hypo-AF are associated with lower photopic and scotopic response on ERG[28] and may indicate a worst prognosis. Furthermore, NIR-AF can help to diagnose STGD1.[27] Hence, FAF is useful to study STGD1 and generally shows focal hyper-AF in the affected eyes, although other FAF abnormalities can also be found.

Bull's eye maculopathy

Typically, Bull's eye maculopathy (BEM) shows concentric parafoveal rings of enhanced and diminished AF. Duncker *et al*. observed that in eyes with BEM studied with SW-AF, AF was higher in the presence of ABCA4 mutations.[28] For this reason, FAF can be helpful in deciding whether or not to prescribe genetic investigations.

Retinitis pigmentosa

The term retinitis pigmentosa (RP) encompasses a heterogeneous group of progressive retinal degenerations leading to a gradual loss of photoreceptors and vision.[29,30] RP presents with a ring or arc of high AF enclosing a zone of normal fluorescence where photoreceptors are preserved.[31-33] A correlation between the ring diameter and the inner segment/OS junction length and between the diameter and the retinal

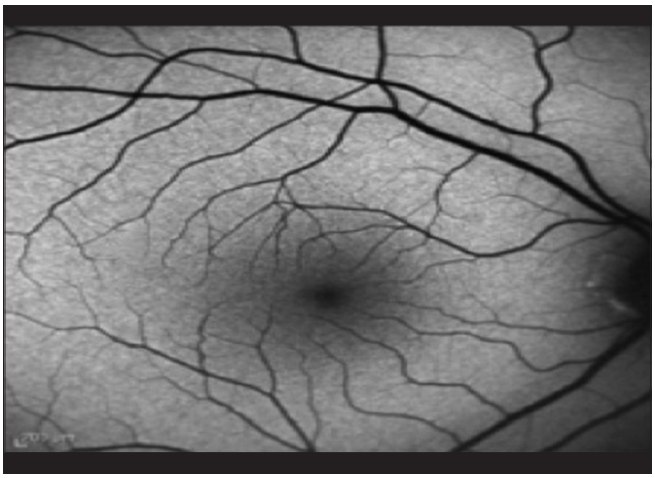

Figure 1: Confocal scanning laser ophthalmoscope short-wavelength-autofluorescence image of a normal right eye

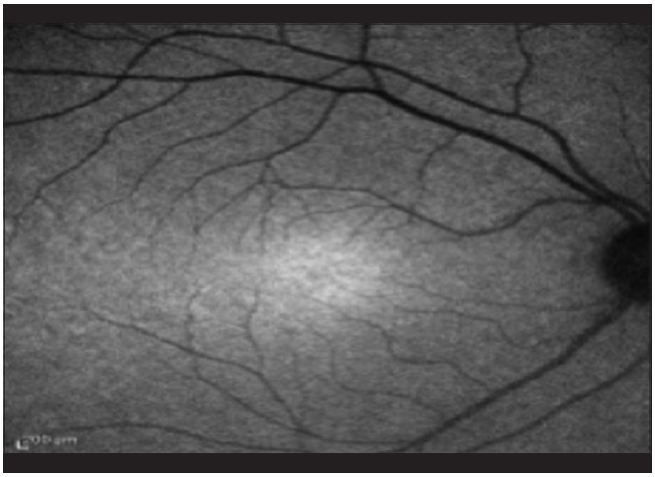

Figure 2: Near infra-red-autofluorescence image of a normal right eye

sensitivity has been reported.[33-41] Greenstein *et al*. found that in 12 out of 21 eyes affected by RP, SW-AF was normal outside the ring/arc.[40] According to published evidences, FAF in RP not only shows the LF distribution in the RPE, but also the presence of other fluorophores in the photoreceptors layer.[40,41] Hence, the hyper-AF could also correspond to degenerating photoreceptors with subsequent increased production of LF.[42] In RP NIR-AF appearance resemble a ring or arc probably due to the separation of melanosomes by the interspersion of increased numbers of LF granules.[43,44] Therefore, NIR-AF may reveal pathological processes earlier than SW-AF [Fig. 3].

Best vitelliform macular dystrophy

Lipofuscin deposition in the subretinal space is a typical sign of best vitelliform macular dystrophy. This early-onset autosomal dominant dystrophy is caused by a mutation in the BEST1 gene expressing the protein called bestrophin.[45,46] Clinically the disease presents with large LF-like subretinal deposits in both eyes, progressing to atrophy in advanced stages.[47,48] Several studies showed that SW-AF patterns in Best dystrophy are variable, ranging from an increased AF in the early phases of the disease to hypo-AF in the later stages [Fig. 4].[24,48-52] By using NIR-AF, some authors found early signs of the disease at a preclinical stage. They observed central hypo-AF surrounded by a hyperautofluorescent ring in patients presenting BEST1 mutation with preserved visual acuity and lack of fundus alterations at biomicroscopy. The authors speculate that bestrophin, a calcium-activated chloride channel, mutation may cause electrolytic imbalances in the RPE, with calcium binding melanin and altering the normal AF.[53-55] This could explain the central low-intensity signal on NIR-AF. A classification of the auofluorescence patterns in Best vitelliform macular dystrophy has been proposed by Parodi *et al*. They observed six patterns at SW-AF and NIR-AF: Normal, multifocal, hyper- autofluorescent, hypoautofluorescent, patchy, spoke-like and multifocal. The patchy was the most frequent and could be observed in various disease stages.[53]

Cone-rod dystrophies

Oishi *et al*. examined patients affected by cone dystrophy and cone-rod dystrophy. By using ultra-wild field FAF (excitation 532 nm, absorption 570–580 nm) they found that the extent of areas of hyper or hypo-AF reflected the severity of functional loss in patients affected by cone-dominant retinal dystrophies. The scotoma they observed on the visual field examination corresponded to areas of altered AF.[56] A relationship was also observed between FAF and ERG findings under dark and light-adapted conditions. The rod function showed a stronger association with areas of abnormal FAF, confirming that AF matches the distribution of this type of photoreceptors.[56,57]

Age-related macular degeneration

Age-related macular degeneration (AMD) represents the most frequent cause of legal blindness in the developed countries. Early AMD is characterized by drusen and approximately one patient out of five progresses to geographic atrophy (GA) and/or to neovascular AMD (nAMD).[58-60]

Lipofuscin accumulation may play an important role in AMD pathogenesis, but the exact mechanisms of this process have not been completely established yet. In general lysosomal dysfunction due to lipid accumulation and protein peroxidation in the RPE cells may accelerate the LF formation in AMD.[61,62] A2E, the major component of LF, impairs the lysosomal and mithocondrial function in aged RPE cells. In particular A2E seems to obstacle the degradation processes inside the lysosomes and to increase the formation of mithocondrial oxygen reactive species subsequently compromising the autophagic processes and the energy supply that are essential for the cellular homeostasis and survival.[9]

Early age-related macular degeneration

The clinical features of early AMD include pigmentary RPE alterations and drusen. FAF abnormalities not always correspond to funduscopically visible lesions and not all visible lesions correspond to notable AF alterations. However, hyperpigmentated areas tend to show increased SW-FAF signals due to melanolipofuscin accumulation, whereas hypopigmentation is often associated to a reduced AF expressing a degenerated or lacking RPE.[16,17] Drusen may present as intrinsically hyperautofluorescent lesions. Actually, autofluorescent elements have been observed within drusen in postmortem specimens.[16] However, a normal FAF appearance

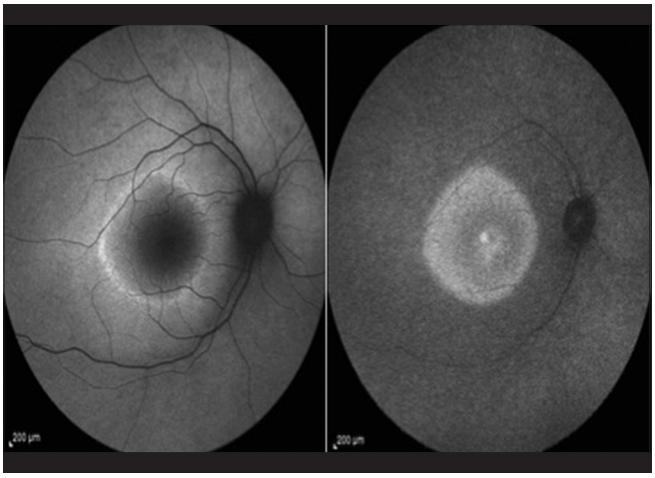

Figure 3: Short-wavelength-autofluorescence (AF) (on the left) and near infra-red-AF (on the right) images showing a ring shaped AF in retinitis pigmentosa

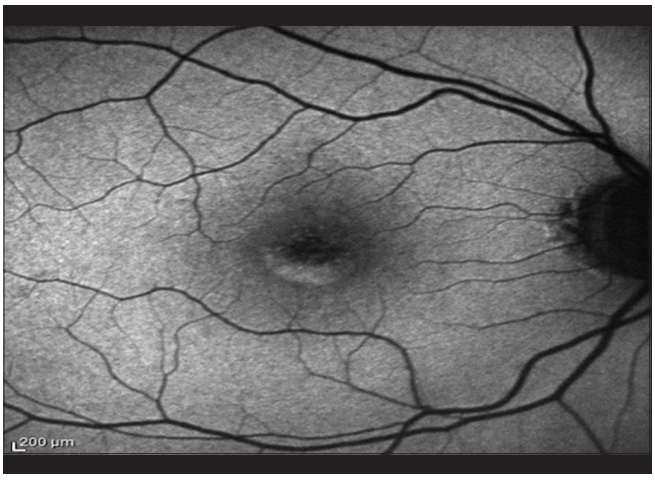

Figure 4: Short-wavelength-autofluorescence image of a hyperautofluorescent juxtafoveal lipofuscin accumulation in Best dystrophy

can be observed in patients with small drusen due to low image resolution or when FAF alterations are masked by macular pigments. Overall, large drusen are more frequently associated with abnormal AF. With cSLO-AF large confluent drusen and drusenoid retinal pigment epithelium detachments (PEDs) present an increased AF areas whereas, when using fundus cameras large drusen show a dark center surrounded by a faint hyperautofluorescent area. Crystalline drusen are often seen as decreased AF spots.[16] Delori *et al*. observed that the rarefaction of RPE cells at the center of the drusen and the increased LF concentration at their edges can appear as a ring-shaped hyper-AF surrounding an hypoautofluorescent space.[18] Specific types of drusen demonstrate peculiar FAF patterns: Cuticular drusen are hyperautofluorescent, reticular drusen appear as elongated, roundish hypoautofluorescent areas included in a network of normal AF.[16,63] The retina surrounding drusen can present increased FAF due to melanolipofuscin accumulation.[16]

Bindewald *et al*. proposed a classification of the abnormal AF patterns in early AMD, classifying them as normal, minimal change, focal increased, patchy, linear, lace like, reticular and speckled.[64]

Interestingly, Midena *et al*. observed a correlation between altered AF and reduction in retinal sensitivity in early AMD.[65] In summary, there are various possible FAF changes in early AMD. FAF alterations can precede the appearance of visible lesions. Therefore, FAF may represent a valuable imaging technique to diagnose early AMD and to monitor its progression.

Geographic atrophy

Geographic atrophy results from the death of RPE cells. The loss of EPR cells and their LF content results in a dramatic decrease in AF. SW-AF is able to more distinctly discriminate areas where RPE is atrophic than color fundus photography (CFP). These areas appear as sharply defined regions with no AF. Although CFP and FAF findings demonstrate a strong intraobserver agreement, FAF gives more reproducible images and is more accurate in detecting smaller atrophic areas.[66,67] On the downside, media opacities, such as advanced cataract, and AF absorption from macular xanthophylls may be obstacles in FAF imaging in the central macula.[68] NIR-AF can help to overcome some limitations of conventional FAF such as the overestimation of atrophy in the foveal area, allowing a better distinction among true atrophy, drusen, and RPE hypopigmentation.[69]

Fundus AF also provides valuable help to predict GA progression. An increased AF surrounding the GA has been reported to precede its extension, and more hyper-autofluorescent GA borders seem to correlate with a higher extension rate [Fig. 5].[59,70,71] Schmitz-Valckenberg *et al*. observed a diminished retinal sensitivity where the AF is increased on the borders of GA.[60] Different FAF classifications of GA have been proposed. Lois *et al*. classified GA as focal, increased, reticular, combined and homogeneous.[72] Subsequently, a classification of GA patterns based on the SW-AF appearance around the atrophy was proposed by the FAM study group. The authors defined the different patterns as focal, banded, patchy or diffused. Some of them showed a higher risk of GA progression.[73] In the presence of diffuse and banded AF pattern atrophy seems to progress more rapidly as compared to other patterns, with the diffuse trickling pattern being the rapidest.[64]

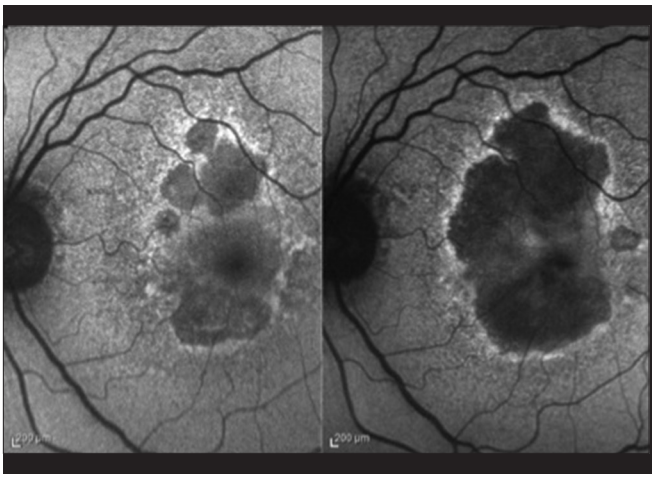

Figure 5: Geographic retinal pigment epithelium atrophy extension in 2 years (from left to right). Hyperautofluorescence at the atrophy margins indicates the direction in which it will extend

Neovascular age-related macular degeneration

Specific FAF alterations can be observed in nAMD, adding specific information to that offered by other imaging techniques like optical coherence tomography (OCT), fluorescein angiography (FA) and indocyanine green angiography (ICGA). LF granules were identified around the lumen of choroidal neovessels in bioptic specimens and AF alterations in correspondence of choroidal neovascularization (CNV) have been reported.[74-77] In nAMD eyes treated with anti-vascular endothelial growth factor Heimes *et al*. found a poorer functional response in the presence of higher pretreatment central AF, by using a cSLO-AF system.[78] According to McBain *et al*. FAF can be reduced in classic CNVs due to the AF blockade by growing neovessels.[75] Occult CNVs aspect at FAF is variable depending on the co-existence of heterogenous RPE alterations and atrophy. Both classic and occult CNVs can show enhanced AF next to the CNV expressing the presence of phagocytized RPE remnants and chronic accumulation of sub-retinal fluid.[79] Retinal PED may present with different AF patterns. In most cases, PED presents as a hyperautofluorescent area surrounded by a less autofluorescent halo. Within the PED, subretinal fluid and atrophy usually result in hypo-AF signal. RPE tears appear as an area of absent AF where the RPE has been displaced and hyper-AF in correspondence of folded RPE at the edge of the tear. Scarring in the late phase nAMD is usually hypoautofluorescent.[80] A SW-AF enhancement can be observed around the scar, probably due to an irregular pigment deposition within a multilayered RPE.[76]

Diabetic retinopathy

Lipofuscin accumulation in diabetic retinopathy (DR) occurs more in the microglia than in the RPE.[81] Currently, studies on FAF imaging in patients with DR are scant and mostly focused on diabetic macular edema (DME). Cystoid macular edema locates in the outer plexiform layer and the inner nuclear layer where it displaces the macular pigments that normally block the AF. This could explain the increased macular AF in the presence of cystoid DME.[82-84] Vujosevic *et al*. observed SW-AF

alterations that correlate with OCT and microperimetry.[85] In a study conducted by Yoshitake *et al*. on NIR-AF negative correlations between central subfield retinal thickness and AF intensity, and between the latter and visual acuity were reported.[86] By using cSLO-AF Pece *et al*. observed that patients with hyperautofluorescent edema had a worse visual acuity as compared to those presenting a single-lobulated pattern. They also reported hyper-AF to increase with the retinal thickness.[84] A relation between SW-AF, OCT and visual acuity in DME was also described by Chung *et al*.[87]

Central serous chorioretinopathy

Fundus AF can complement other imaging techniques such as FA, ICGA and OCT in showing alterations in eyes affected by central serous chorioretinopathy (CSCR).[88-90] CSCR is characterized by an idiopathic subretinal leakage leading to serous retinal detachment with an accumulation of material derived from the photoreceptors catabolism. It typically shows high AF (excitation 500–610 nm, absorption 675–715 nm) with a punctate diffuse distribution.[91]

Teke *et al*. found a reduced AF in correspondence of the focal leakage site detected by FFA in nearly 90% of acute and chronic CSCR patients. This finding may express a reduced retinal metabolism in that area. Differently, by using SW-AF, in the serous detachment area, they observed hypo-AF in acute disease, subsequently to blockage of AF by subretinal effusion, and hyper-AF in the chronic cases, due to the accumulation of dispersed chromophores and OSs of photoreceptors.[92] Hypo-AF at the focal leakage site was also reported by other authors using SW-AF and may correspond to disrupted RPE cells and/or to AF blockade by subretinal fluid.[93] Differently, von Rückmann *et al*. noticed an increased AF at the leakage point possibly reflecting the accumulation of autofluorescent phagosomal material.[94] To summarize, CSCR can present with various FAF patterns reflecting the metabolic state of the RPE, accumulation of photoreceptor-derived material and masking by serous detachment [Fig. 6].

Chorioretinal inflammatory and infective diseases

Lipofuscin accumulation resulting from lysosomal oxidative damage could be an indicator of the disease activity in chorioretinal inflammatory conditions.[95] Generally, FAF shows increased signal in the presence of an active inflammatory response, whereas quiescent phases and final chorioretinal scarring or atrophy are hypoautofluorescent.[96] Specific AF pattern can help to distinguish among different types of chorioretinitis.

White dots syndromes

White dots syndromes include a group of inflammatory conditions affecting the choroid, RPE and outer retina.[97,98] In most cases, they present ophthalmoscopically with white-yellow spots in the RPE and inner choroid. Differential diagnosis may be clinically challenging due to this common appearance. Several authors reported specific FAF alterations in patients with different types of white dot syndromes.[99-106] Multifocal choroiditis (MFC) and punctate inner choroidopathy (PIC) exhibit raised or low AF within the dots and in the surrounding area.[107,108] Inflammation and photoreceptor phagocytosis causes initial hyper-AF in PIC, which may then be followed by hypo-AF due to RPE atrophy.[107] When PIC patients are treated with immunemodulatory drugs, hyperautofluorescent and active lesions turn hypoautofluorescent in response to therapy, with the persistence of hyper-AF indicating a higher risk of recurrences.[109] Birdshot chorioretinopathy (BSC) shows defined hypoautofluorescent areas that often do not correspond to ophthalmoscopically visible lesions, being larger and more diffused and becoming hypoautofluorescent in the advanced stages.[99,110] The presence of placoid macular hypo-AF in BSC represents an unfavorable prognostic indicator and requires an aggressive immunemodulatory treatment.[99,111] In acute multiple evanescent white dots syndrome FAF shows multiple ill-defined hyperautofluorescent spots corresponding to ophthalmoscopically evident dots [Fig. 7]. These findings disappear as the inflammation resolves.[101] Acute posterior multifocal placoid pigment epitheliopathy also shows a biphasic appearance on AF. In the acute phase, there is hypo-AF due to macular edema masking natural AF. Later, as the edema resolves, placoid areas appear hyperautofluorescent due to LF accumulation.[104]

Vogt-Koyanagi-Harada disease

Vogt-Koyanagi-Harada (VKH) disease is a bilateral granulomatous autoimmune panuveitis complicated by

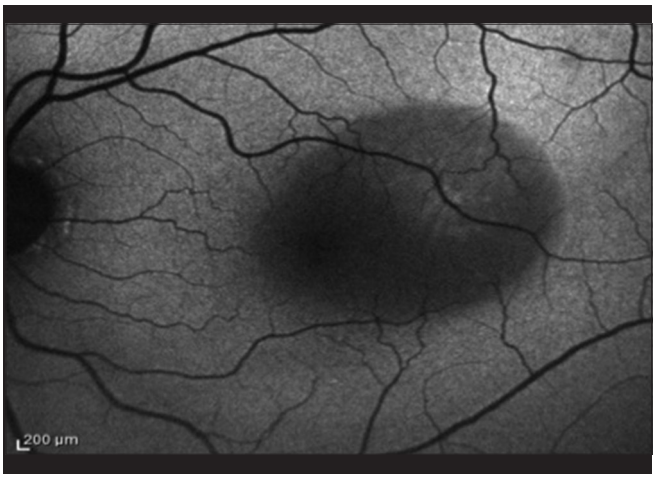

Figure 6: Serous neuroretinal detachment in central serous chorioretinopathy masks the normal retinal autofluorescence

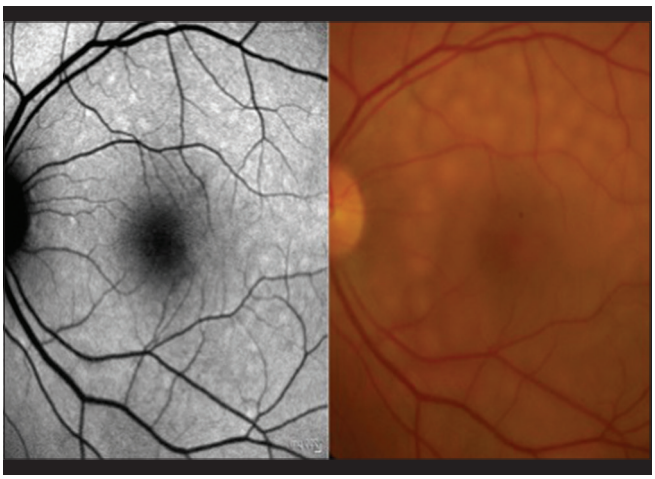

Figure 7: Short-wavelength-autofluorescence (AF) image (on the left) and fundus photograph (on the right) in multiple evanescent white dots syndrome showing the dots mild hyper-AF

exudative retinal detachments. It presents with an acute phase sometimes followed by a chronic stage with choroidal depigmentation and RPE clumping configuring the "sunset glow" fundus appearance.[112-115] Koizumi *et al*. analyzed the FAF appearance in acute VKH patients receiving prompt immunosuppressive treatment and in untreated or late-treated patients. The first group shows a transitory mild hyper-AF, the second group exhibits an initial widespread scattered hyper-AF then progressing to a mixed pattern showing both hyper- and hypo-AF areas.[116] Chronic VKH is not associated with FAF alterations since the typical depigmentation, resulting from the autoimmune process against the choroidal melanocytes, spares the RPE cells.[117,118]

Serpiginous and serpiginous like choroiditis

An association between foveal hypo-AF and visual impairment was demonstrated in patients with serpiginous choroidopathy (SPC) [Fig. 8].[100] Hyper-AF can anticipate the development of CNV in some patients with SPC and MFC.[100,102] Serpiginous like choroiditis is presumed to be caused by tuberculosis and starts with ophthalmoscopically evident placoid lesions that tend to progressively coalesce with a serpiginous appearance.[119,120] FAF typically shows ill-defined predominantly hyperautofluorescent lesions in the acute phase. Later the lesions appear more defined with a subtle hypo-AF at the borders and prevalence of enhanced AF internally. When the lesion evolves, hypofluorescent areas may appear due to the presence of damaged RPE cells.[121,122]

Primary vitreoretinal lymphoma

Non-Hodgkin's lymphomas arising in the eye are commonly B-cell derived and known as primary vitreoretinal lymphoma (PVRL) or primary intraocular lymphoma.[123-125] A granular AF was reported in PVRL affected eyes with hyper-AF resulting from the accumulation of LF next to the tumoral infiltration under the RPE and hypo-AF from the blockage by the invading tumor or due to the RPE damage and atrophy.[126]

Choroidal melanocytic lesions

Lipofuscin accumulation and RPE alterations are often present in choroidal melanocytic lesions. Therefore FAF is useful in documenting their presence. Hyperautofluorescent pigment and LF were found in nearly 90% of choroidal melanocytic lesions by Gündüz *et al*. using SW-FAF.[127] FAF can also help to characterize malignant lesions. According to Shields *et al*. choroidal melanomas show a variably increased AF with a typical granular pattern by using the standard FAF technique (580 nm excitation and 695 nm barrier filters). They observed higher AF in larger tumors, pigmented tumors and tumors with disrupted overlying RPE.[128] The sources of hyper-AF within malignant melanomas include remnants of LF granules and macrophages.[129] RPE alterations such as hyperplasia, fibrous metaplasia and atrophy overlying the tumors can be seen as an hypoautofluorescent area.[128] Chronic alterations of the RPE over a choroidal melanoma usually let the intrinsic AF of the lesion to appear.[128,130,131] The orange pigment, representing LF within the macrophages in the subretinal space is a subclinical sign of malignancy and appears hyperautofluorescent.[132]

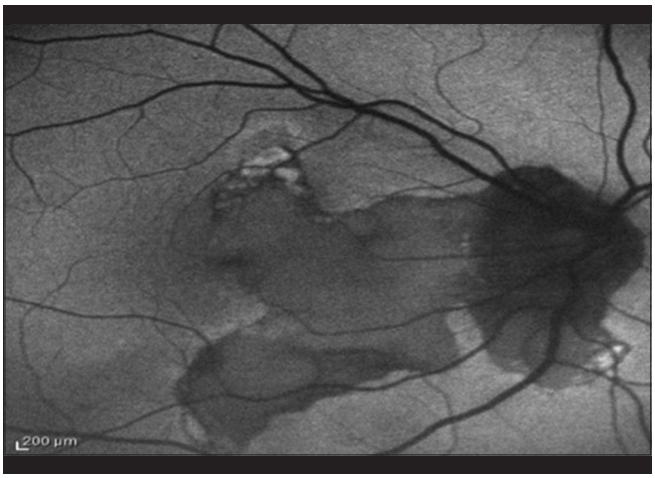

Figure 8: Short-wavelength-autofluorescence image of an eye affected by serpiginous choroidopathy

Clinical Limitations and Future Directions of Fundus Autofluorescence

Fundus AF represents a rapid and noninvasive imaging technique. Thanks to FAF, it is possible to expand the comprehension of retinal diseases' pathogenesis and to monitor their course. Fluorescence reference systems have been developed to compare objectively FAF images taken in different subjects or at different times. For a more precise measurement, AF can also be averaged between different retinal zones or acquired at specific desired points.[133] Optical aberrations may be reduced incorporating adaptive optical systems in the FAF devices allowing to observe the retinal cells with a cellular-level of resolution. Ultra-widefield noncontact imaging system are now available enabling the clinician to visualize the retina with a 200° field of view.[134] Retinal metabolism has been recently investigated with a novel technique named fluorescent lifetime imaging ophthalmoscopy. It employs a modified SLO device that detects the alterations in the lifetime of the AF signal by measuring it with a single-photon counter. The AF lifetime appears to become longer with age and to be shorter in the central retina than in the periphery in healthy eyes. It represents a promising new tool to investigate the retinal metabolism in response to pathological changes.[135] Despite these considerations FAF remains a complementary technique in many retinal conditions where AF is just a pathological ephiphenomenon and shows several limitations that cannot be overstated. At present, there are no reference databases to classify consistently the normal and pathological FAF phenotypes. The interindividual and intraindividual variability of media opacities, refractive error and cellular LF content and genetic expression during the ageing process, make the possibility to develop such database likely challenging.[64,135] Differences in the acquisition system, such as between cSLO and fundus camera, and in other equipment and settings like excitation and emission filters, laser power, laser amplification and photodetectors gain also limit an objective AF measurement and the possibility to compare images from different patients and operators.[13,66] Therefore further technologic advances, the implementation of standard image acquisition procedures and the creation of a comprehensive classification systems of the normal and pathological phenotypes, could largely empower the impact of FAF imaging on the diagnosis and management of retinal diseases.

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