FUNDUS AUTOFLUORESCENCE OF CHOROIDAL MELANOCYTIC LESIONS AND THE EFFECT OF TREATMENT

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ABSTRACT

Purpose: To correlate fundus autofluorescence (FAF) patterns in choroidal melanocytic lesions with changes present on the surface of such lesions, including lipofuscin, hyperpigmentation, drusen, and fibrous metaplasia, and to describe the effect of treatment on FAF.

Methods: Retrospective chart review of 23 consecutive patients with choroidal nevi and melanoma who underwent FAF photography. The correlation between increased FAF patterns and foci of lipofuscin, hyperpigmentation, drusen, or fibrous metaplasia was defined as a complete correlation, partial correlation, or no correlation. The posttreatment FAF photographs of 6 patients with choroidal melanoma who were managed with plaque radiotherapy or plaque radiotherapy and transpupillary thermotherapy were also analyzed.

Results: Lipofuscin was present in 13 tumors, hyperpigmentation in 9 tumors, drusen in 6 tumors, and fibrous metaplasia in 4 tumors. A complete correlation between increased FAF and lipofuscin was found in 8 tumors (61.5%), a partial correlation in 3 tumors (23.1%), and no correlation in 2 tumors (15.4%). A complete correlation between hyperpigmentation and increased FAF was found in 5 tumors (55.6%), a partial correlation in 3 tumors (33.3%), and no correlation in 1 tumor (11.1%). A partial correlation was found between drusen and increased FAF in all 4 tumors. A partial correlation was found between fibrous metaplasia and increased FAF in all 3 tumors. Following treatment, increased FAF was observed in 6 choroidal melanomas owing to an increase in lipofuscin and hyperpigmentation.

Conclusions: Choroidal melanocytic lesions with overlying lipofuscin and hyperpigmentation are associated with increased FAF in about 90% of cases. Fundus autofluorescence photography may be helpful in evaluating small melanocytic tumors, since lipofuscin is a risk factor for growth. Following treatment, choroidal melanomas may show increased FAF.

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INTRODUCTION

Fundus autofluorescence (FAF) photography relies on the stimulated emission of light from naturally occurring fluorophores, the most significant being lipofuscin.[1-3] Lipofuscin pigment may accumulate in retinal pigment epithelial cells and macrophages over several types of benign and malignant choroidal tumors. Patterns of increased or decreased FAF have been described in many retinal and choroidal diseases and have been extensively studied in age-related macular degeneration (ARMD). Previous studies have described changes in FAF in eyes with dry ARMD. Increased FAF was found to correlate with areas of hyperpigmentation, soft drusen, hard drusen, or normal fundus appearance.[4-7] Of note, drusen may have an increased, normal, or decreased FAF signal.[5] Overall, larger drusen were more often associated with increased FAF than smaller ones.[5] Several studies concluded that FAF imaging in dry ARMD does not necessarily correspond to the fundus changes observed and gives information over and above the normal fundus photography methods used.[5,8]

Fundus autofluorescence studies in acute central serous retinopathy reveal increased FAF in the active stages, corresponding to increased metabolic activity of the retinal pigment epithelium (RPE), and decreased FAF in chronic central serous retinopathy, indicating reduced metabolic activity of the RPE due to photoreceptor cell loss.[9] Hereditary retinal dystrophies are generally associated with increased FAF, although exceptions exist.[10,11] Retinal pigment epithelium atrophy and cell loss, as in geographic atrophy or pseudoxanthoma elasticum, have been associated with decreased FAF.[12,13] Fundus autofluorescence has also been used to study the success of selective RPE destruction by laser treatment. Initial hypoautofluorescence observed after RPE destruction by laser treatment was found to change to hyperautofluorescence after 1 week owing to the proliferation of RPE.[14]

The aims of this study were to correlate increased FAF patterns in choroidal melanocytic lesions with changes present on the surface of such lesions, including lipofuscin (orange pigment), hyperpigmentation, drusen, and fibrous metaplasia, and to evaluate the FAF findings after treatment.

METHODS

After approval was obtained from the institutional review board of the Mayo Clinic, a retrospective review of records of patients with choroidal melanocytic lesions, including choroidal nevus and choroidal melanoma, who underwent FAF photography was performed. All patients were examined at the Mayo Clinic between October 2005 and May 2006. Patients with choroidal melanocytic lesions underwent complete ocular examination, including Snellen visual acuity, intraocular pressure measurement, slit-lamp biomicroscopy, and funduscopy. Further evaluation included A and B scan ultrasonography, optical coherence tomography, color fundus photographs,

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fluorescein and indocyanine green angiography, in addition to FAF photography. Patients with media opacities, including cataract greater than 2+ nuclear sclerosis and/or 1+ cortical cataract, opacified posterior capsule in the presence of a posterior chamber intraocular lens, asteroid hyalosis, vitreous debris, and vitreous hemorrhage, were excluded from the study. Patients who had received prior ocular treatment were excluded.

The FAF images of patients with choroidal melanoma who had treatment including plaque radiotherapy or combined treatment including plaque radiotherapy and transpupillary thermotherapy were also retrieved. Patients who had treatment were followed up at regular intervals of 1 month, 3 months, and at 3-month intervals thereafter for the first year. The ocular examination and ancillary testing methods, as outlined above, were repeated as necessary at follow-up examinations.

Autofluorescent fundus photography was performed using the Heidelberg confocal scanning laser ophthalmoscope (SLO) system (Heidelberg Retina Angiograph [HRA]; Heidelberg Engineering, Dossenheim, Germany). The HRA is a confocal SLO equipped with a laser source able to emit laser light with 4 different wavelengths for different acquisition modes. Autofluorescence is excited by the argon blue wavelength (488 nm), and the emitted light above 500 nm is detected with a barrier filter. The illumination beam is 3 mm in diameter, and the aperture of the dilated eye is used to collect light from the posterior pole. Maximal retinal irradiation at 30° is 0.2 mW/cm^2, well below the limits established by the American National Standards Institute.[15] The confocal detection unit employs a 400-μm pinhole aperture to suppress the light from below or above the confocal plane. The digital images are saved for processing and where an average image from the original image series is created to reduce noise and to produce more detailed images for the spatial distribution of the FAF.

Using Adobe Photoshop CS 2, the areas of orange pigment, hyperpigmentation, drusen, and fibrous metaplasia were marked on the color fundus pictures. The markings were then overlayed on the composite FAF-color fundus pictures to see the correlation between the clinically observable variable and increased FAF signal. The correlation between clinical photographs and FAF images was evaluated as a complete correlation, partial correlation, or no correlation separately for each of the 4 variables, whichever were present in a particular lesion.

A complete correlation was defined as the presence of increased FAF corresponding to all areas of a clinically observed variable. A partial correlation was defined as the presence of at least one area of focally increased FAF corresponding to at least one area of a clinically observed variable in the absence of a complete correlation. No correlation was defined as a complete lack of correspondence between any of the clinically observed features and FAF images for a given variable.

RESULTS

Twenty-three patients were included in this study. Patient demographics and tumor features are shown in Table 1. Of 23 patients, 16 were female and 7 were male. The mean age was 63.1 years, ranging from 41 to 82 years. The mean largest tumor base was 9.6 mm, ranging from 5.0 to 15.0 mm. The mean tumor thickness was 3.2 mm, ranging from 0.9 mm to 9.0 mm. Twelve of 23 tumors were diagnosed as choroidal melanoma, and the remaining 11 were choroidal nevi. Of 23 tumors, 8 were pigmented, 8 were amelanotic, and 7 were partially pigmented. Lipofuscin was found to be present in 13 tumors, hyperpigmentation in 9 tumors, drusen in 6 tumors, and fibrous metaplasia in 4 tumors. The presence of subretinal or intraretinal fluid was documented in 18 of 23 eyes (78.2%) by optical coherence tomography scanning.

Table 2 shows the correlation scores between increased FAF patterns and lipofuscin, hyperpigmentation, drusen, and fibrous metaplasia. A complete correlation between increased FAF and lipofuscin was found in 8 tumors (61.5%) (Figures 1a,b), a partial correlation in 3 tumors (23.1%), and no correlation in 2 tumors (15.4%). A complete correlation between hyperpigmentation and increased FAF was found in 5 tumors (55.6%) (Figures 2a,b); a partial correlation in 3 tumors (33.3%), and no correlation in 1 tumor (11.1%). A partial correlation was found between drusen and increased FAF in 4 tumors (66.7%) (Figures 3a,b) and no correlation in 2 tumors (33.3%). A partial correlation between fibrous metaplasia and increased FAF was found in 3 tumors (75%) and no correlation in 1 tumor (25%). In 11 (48%) of 23 eyes (2, 5, 6, 8, 12, 13, 14, 15, 18, 20, 21), there were more hyperautofluorescent areas in the lesion than would be predicted by the clinical variables being considered.

Of the 8 pigmented tumors, a complete correlation between increased FAF and lipofuscin was found in 4 tumors (50%) and a partial correlation in 1 tumor (12.5%) (Tables 1 and 2). Of the 8 amelanotic tumors, a complete correlation between increased FAF and hyperpigmentation was found in 4 tumors (50%) and a partial correlation in 2 tumors (25%).

Seven of 8 eyes with a complete correlation for increased FAF and lipofuscin had either subretinal or intraretinal fluid (Tables 1 and 2). Two of 3 eyes with a partial correlation for increased FAF and lipofuscin had subretinal fluid, and 1 eye with no correlation had subretinal fluid. Four of five eyes with a complete correlation for increased FAF and hyperpigmentation had either subretinal or intraretinal fluid; 2 of 3 eyes with a partial correlation had intraretinal fluid, and 1 eye with no correlation had subretinal fluid (Tables 1 and 2). Three of four eyes with partial correlation for drusen had subretinal fluid (one eye had subretinal fluid originating probably from coexisting ARMD choroidal neovascularization) and 2 of 3 eyes with partial correlation for fibrous metaplasia had either subretinal or intraretinal fluid (Tables 1 and 2).

Of 6 patients who had treatment, 2 patients had plaque radiotherapy alone and 4 patients had plaque radiotherapy in combination with transpupillary thermotherapy. The mean follow-up after treatment was 5 months (range, 2-9 months). All treated tumors had an increase in lipofuscin, and 4 had hyperpigmentation over the lesion surface after treatment. A complete correlation between increased FAF and lipofuscin was found in all 6 tumors after treatment (Figure 4a,b). A partial correlation between hyperpigmentation and increased FAF was found in 4 tumors. There was subretinal fluid in 3 of 6 patients (50%) after treatment at the short-term follow-up of our study.

TABLE 1. PATIENT DEMOGRAPHICS AND TUMOR FEATURES IN 23 PATIENTS WITH CHOROIDAL MELANOCYTIC LESIONS UNDERGOING FUNDUS AUTOFLUORESCENCE STUDIES

PATIENT	AGE (YR)	SEX	LOCATION	TUMOR BASE (mm)	TUMOR HEIGHT (mm)	COLOR	LIPO-FUSCIN	HYPER-PIG	DRUSEN	FIB META	SRF CLIN	SRF OCT
1	56	F	Sup, peripap	12×9	1.5	Amel	-	+	-	-	+	IR
2	49	F	Inferior	8×5	1.8	Par pig	-	+	-	-	+	IR
3	50	F	Suptemp	7×5	2.1	Par pig	+	+	-	-	+	IR
4	41	F	Temp	10×9	4.1	Par pig	+	-	-	+	+	SR
5	81	F	Macular	5×4	1.2	Amel	-	+	-	+	-	IR
6	64	F	Suptemp	8×5	1.3	Amel	-	+	-	+	-	-
7	61	F	Sup	10×9	3.4	Pig	+	-	-	-	+	SR
8	45	M	Macular	4×4	1.2	Pig	-	-	+	-	+	SR+IR
9	75	M	Macular	10×8	2.4	Pig	+	-	-	-	-	SR
10	72	F	Inftemp	10×8	1.8	Pig	+	-	-	-	+	SR
11	56	M	Suptemp	10×9	6.5	Par pig	+	-	-	-	+	SR
12	68	F	Infnasal	15×10	6.4	Par pig	+	-	-	-	-	NA
13	61	F	Temp	15×9	5.4	Amel	-	+	-	+	+	SR
14	56	M	Temp	5×5	1.0	Amel	-	+	-	-	+	SR
15	71	M	Inftemp	15×10	7.1	Par pig	+	-	-	-	+	NA
16	55	F	Nasal	10×7	4.0	Amel	+	-	+	-	+	SR+IR
17	78	F	Suptemp	15×10	1.7	Pig	-	-	+	-	-	NA
18	74	F	Temp	9×9	3.1	Pig	+	-	+	-	-	SR
19	74	F	Temp	6×4	0.9	Pig	-	-	+	-	-	SR (from ARMD)
20	62	M	Infmac	8×7	2.0	Par pig	+	-	-	-	-	SR
21	82	F	Macular	5×4	2.0	Pig	+	-	+	-	+	SR
22	62	F	Sup	14×14	9.0	Amel	-	+	-	-	+	SR
23	58	M	Inftemp	11×9	2.8	Amel	+	+	-	-	-	-

Amel, amelanotic; ARMD, age-related macular degeneration; Fib meta, fibrous metaplasia; Hyperpig, hyperpigmentation; Infmac, inferior to macula; Infnasal, inferonasal; Inftemp, inferotemporal; IR, intraretinal; NA, not available; Par pig, partially pigmented; Peripap, peripapillary; Pig, pigmented; SR, subretinal; SRF OCT, subretinal fluid present on OCT; SRF clin, subretinal fluid present on clinical exam; Sup, superior; Suptemp, superotemporal; Temp, temporal; +, present; -, absent.

TABLE 2. CORRELATION OF INCREASED FUNDUS AUTOFLUORESCENCE (FAF) PATTERNS WITH LIPOFUSCIN, HYPERPIGMENTATION, DRUSEN, AND FIBROUS METAPLASIA IN PATIENTS WITH CHOROIDAL MELANOCYTIC LESIONS

PATIENT	INCREASED FAF/ LIPOFUSCIN CORRELATION	INCREASED FAF/ HYPERPIGMENTATION CORRELATION	INCREASED FAF/ DRUSEN CORRELATION	INCREASED FAF/ |FIBROUS METAPLASIA CORRELATION
1	...	Complete	…	…
2	...	Partial	…	...
3	Complete	Complete	…	...
4	Complete	…	...	None
5	…	Partial	...	Partial
6	…	Partial	...	Partial
7	Partial	…	...	…
8	...	…	Partial	...
9	Complete	…	...	…
10	Complete	...	…	...
11	Complete	…	...	…
12	Partial	...	…	...
13	…	Complete	...	Partial
14	…	None	...	…
15	None	...	…	...

TABLE 2. (continued) CORRELATION OF INCREASED FUNDUS AUTOFLUORESCENCE (FAF) PATTERNS WITH LIPOFUSCIN, HYPERPIGMENTATION, DRUSEN, AND FIBROUS METAPLASIA IN PATIENTS WITH CHOROIDAL MELANOCYTIC LESIONS

PATIENT	INCREASED FAF/ LIPOFUSCIN CORRELATION	INCREASED FAF/ HYPERPIGMENTATION CORRELATION	INCREASED FAF/ DRUSEN CORRELATION	INCREASED FAF/ |FIBROUS METAPLASIA CORRELATION
16	None	…	Partial	...
17	…	...	Partial	…
18	Complete	...	None	…
19	...	…	Partial	...
20	Partial	…	...	…
21	Complete	...	None	…
22	...	Complete	…	...
23	Complete	Complete	…	...

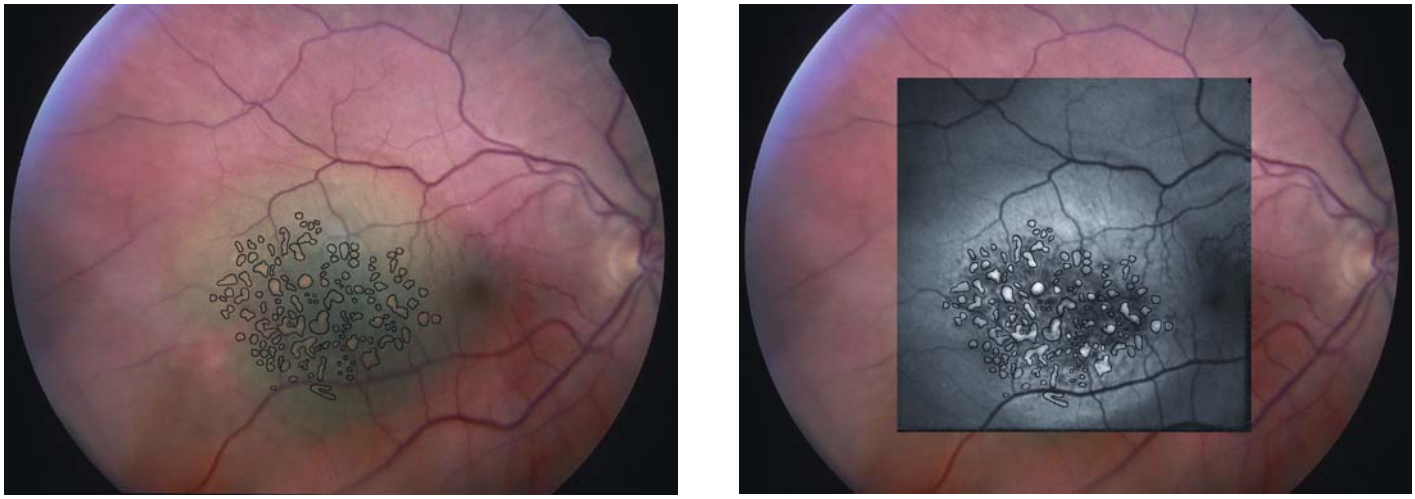

FİGURE 1

Left, Color fundus photograph of a pigmented choroidal melanoma demonstrating several foci of lipofuscin overlying the lesion (black outlines). Right, The composite FAF-color fundus picture of the same eye demonstrating increased fundus autofluorescence patterns corresponding to the location of lipofuscin (black outlines).

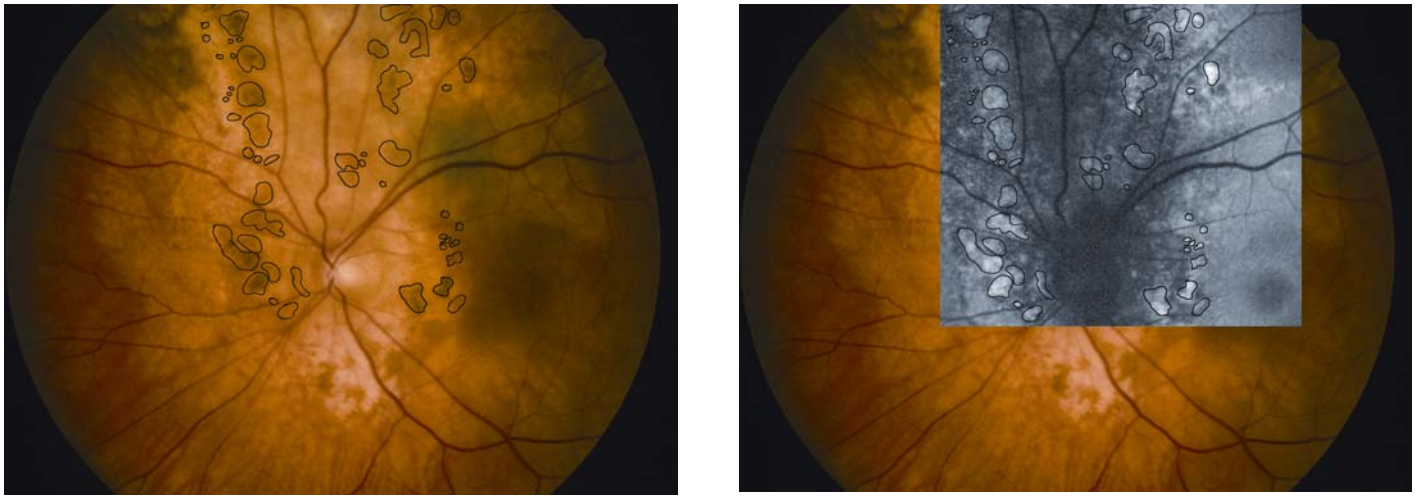

FİGURE 2

Left, Color fundus photograph of an amelanotic choroidal melanoma demonstrating several foci of hyperpigmentation on the lesion surface (black outlines). Right, The composite FAF-color fundus picture of the same eye showing increased fundus autofluorescence patterns at the sites of hyperpigmentation (black outlines).

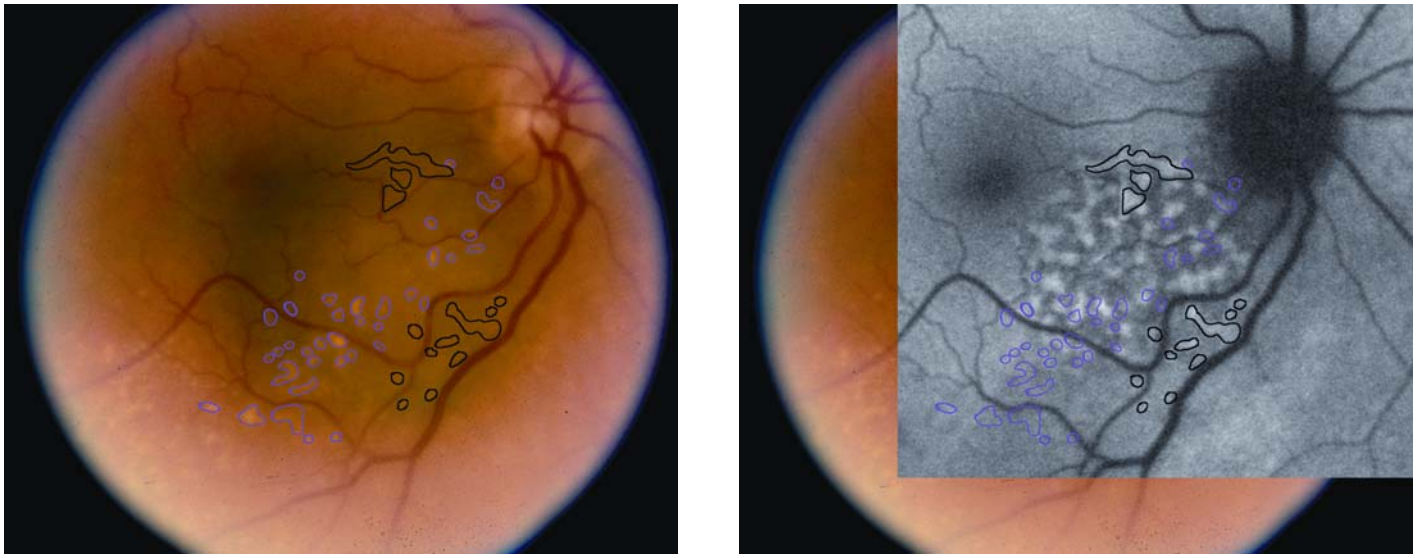

FİGURE 3

Left, Color fundus photograph of a pigmented choroidal melanoma showing lipofuscin (black outlines) and drusen (violet outlines) over the lesion surface. Right, The composite FAF-color fundus picture of the same eye showing complete correlation between lipofuscin and increased fundus autofluorescence pattern (black outlines) over the lesion and partial correlation between the drusen and increased fundus autofluorescence pattern (violet outlines). There is a greater area of FAF than would be suggested by lipofuscin and drusen.

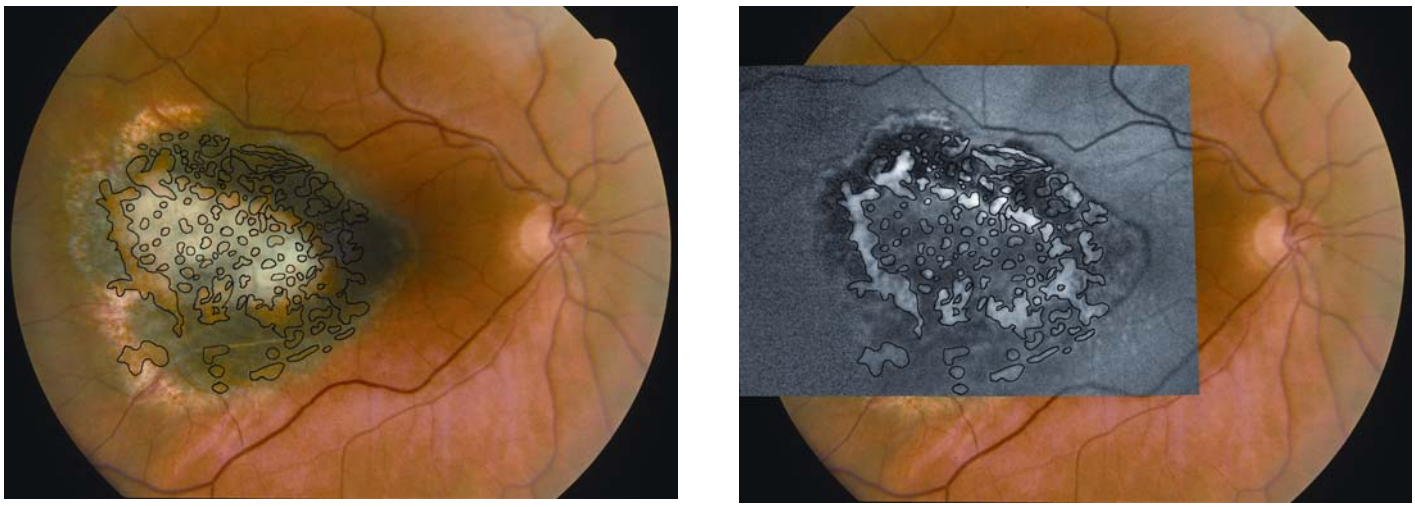

FİGURE 4

Left, Color fundus photograph of the pigmented choroidal melanoma depicted in Figure 1, left, 3 months after treatment. Extensive lipofuscin accumulation (black outlines) overlying the lesion can be seen. Right, The composite FAF-color fundus picture of the same eye showing complete correlation between lipofuscin and increased autofluorescence pattern (black outlines).

DISCUSSION

Autofluorescence and angiography images were obtained using the HRA confocal scanning laser ophthalmoscope (cSLO). The confocal imaging mode ensures that light from the retinal plane comes to a focus on the image sensor. Consequently, light from retinal locations outside the focal plane does not come to a focus on the image sensor, but rather it focuses in front of or behind the image sensor. Confocal imaging typically has a depth of field of 300 μm or more, depending on the wavelength and the optics of the equipment and other factors.[16] Therefore, light originating from out-of-focus planes is blocked by the aperture of the confocal light

detection unit. Based on this property, there are special features of cSLO to consider when imaging studies are performed over elevated fundus lesions. Retinal images out of the depth of acquisition are suppressed. Therefore, out-of-focus regions will not be visible or the image quality will be poor. The barrier filter used in HRA is appropriate for detecting the autofluorescence of lipofuscin. Other autofluorescent material, including drusen and vitamin A, may contribute to autofluorescence, but previous work has shown that lipofuscin at this wavelength is the most autofluorescent material in the fundus.[3]

There are certain limiting factors for FAF imaging that should be recognized. First, media opacities, including lens opacification, decrease the FAF image contrast so that analysis of the image is not possible. Second, camera adjustment is crucial and motion artifacts should be excluded, since this may result in uneven background FAF and may lead to misinterpretation. Third, an absolute quantification of FAF images is not possible. This is probably due to the fact that macular pigments vary with age and a normative FAF imaging database is not available. Furthermore, different types of cSLO equipment have different outputs. Therefore, only different FAF intensities within one FAF image can be used for classification. The lack of reproducibility and consistency remains a major problem in many FAF studies.[17]

In this study, nearly 90% of the tumors showed at least 1 focus of increased autofluorescence signal corresponding to the locations of the lipofuscin and hyperpigmentation over the lesion. Some 50% of the tumors showed a complete correlation between increased FAF and these features. In 6 (75%) of 8 amelanotic choroidal melanocytic lesions, increased FAF intensities were observed in areas of hyperpigmentation. This could be due to an accumulation of lipofuscin in areas of hyperpigmentation, as alluded to before in studies on ARMD.[7,8]

The reasons for a partial or poor correlation between the clinical findings and FAF imaging may be attributed to several factors, including increased retinal thickness, intraretinal edema, presence of subretinal fluid, and motion artifacts. It is possible that fluorophores within the fluid may interfere with autofluorescence imaging or that dispersed RPE cells within the fluid may contribute to different autofluorescence patterns.[8,18] Despite the evidence in the literature, a breakdown analysis showed that many patients with a good or partial correlation between the presence of lipofuscin or hyperpigmentation and increased FAF indeed had subretinal or intraretinal fluid in our series. Because of the confocal planar characteristics of the cSLO equipment, increased tumor thickness may also adversely affect the accuracy of the FAF signal. By way of altering the FAF signal, some of these factors may also be responsible for the more extensive than expected hyperautofluorescence that was observed in 48% of the eyes in our study. Alternatively, FAF may be a more sensitive method of detecting lipofuscin compared to the clinical examination.

Drusen may have variable autofluorescence characteristics, presumably related to the nature of the RPE change overlying them. Some drusen may present with normal or near normal AF, some others may have decreased AF, whereas large soft foveal drusen have increased AF. A previous study[19] found that there is a central area of decreased autofluorescence, surrounded by a ring of AF. The investigators postulated that the decreased central AF in drusen may be related in many cases to the RPE being stretched over the drusen, with a thinner layer of lipofuscin granules over the drusen and with overall conservation of the amount of lipofuscin. In our study, we found a partial correlation between increased FAF and drusen.

The use of FAF imaging may have a significant impact on clinical decision making. The decision to treat choroidal melanocytic lesions depends on the presence of risks for growth, including lipofuscin, subretinal fluid, tumor thickness, proximity to optic nerve, and visual symptoms.[20] Most ocular oncologists will treat a choroidal lesion if 2 or more of these 5 risk factors for growth are present, as the risk for growth in 5 years is over 50%.[20] Furthermore, documented growth is a risk factor for metastasis.[21] Under these circumstances, the assessment of lipofuscin becomes a crucial issue. Clinically, lipofuscin pigment is seen as orange-colored patches over a pigmented choroidal melanocytic lesion. The presence of subtle lipofuscin pigment in choroidal melanocytic lesions can be more difficult to assess. FAF imaging can be particularly helpful in these situations to demonstrate the lipofuscin. Furthermore, the determination of lipofuscin over amelanotic lesions can even be more challenging. Our study shows that hyperpigmentation over amelanotic choroidal lesions may actually represent accumulations of lipofuscin or melanolipofuscin and therefore should be considered an important factor in the decision-making process for treatment.

Foci of lipofuscin and hyperpigmentation became larger and more numerous after treatment with plaque radiotherapy or combined plaque radiotherapy and transpupillary thermotherapy. The increase of lipofuscin observed in melanomas after treatment might be secondary to the damage suffered by the RPE cells in the necrosing tumor. The increase in hyperpigmentation may be secondary to the increased metabolic activity of the RPE during the proliferation that follows destruction, similar to that seen after laser treatment.[14] FAF photography after treatment showed complete or partial correlation with orange pigment or hyperpigmentation in all patients.

In conclusion, this pilot study demonstrates that choroidal melanocytic lesions with overlying lipofuscin and hyperpigmentation are associated with an increased FAF pattern in about 90% of cases. The increased FAF pattern may demonstrate a complete or partial topographic correspondence with these clinically observed features. Fundus autofluorescence studies may be especially useful to evaluate subtle lipofuscin pigment over pigmented choroidal melanocytic lesions and in the evaluation of hyperpigmentation over amelanotic choroidal lesions. Following treatment, choroidal melanomas may show increased FAF due to an increase in lipofuscin and hyperpigmentation.

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REFERENCES

1. Rovati L, Docchio F. Autofluorescence methods in ophthalmology. *J Biomed Optics* 2004;9:9-21.
2. Eldred GE, Katz ML. Fluorophores of the retinal pigment epithelium. *Exp Eye Res* 1988;47:71-86.
3. Delori FC, Dorey CK, Staurenghi G, Arend O, Goger DG, Weiter JJ. In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. *Invest Ophthalmol Vis Sci* 1995;36:718-729.
4. von Rückmann A, Fitzke FW, Bird AC. Fundus autofluorescence in age-related macular disease imaged with a scanning laser ophthalmoscope. *Invest Ophthalmol Vis Sci* 1997;38:478-486.
5. Lois N, Owens SL, Coco R, Hopkins J, Fitzke FW, Bird AC. Fundus autofluorescence in patients with age-related macular degeneration and high risk of visual loss. *Am J Ophthalmol* 2002;133:341-349.
6. Einbock W, Moessner A, Schnurrbusch UEK, et al; FAM Study Group. Changes in fundus autofluorescence in patients with age-related maculopathy. Correlation to visual function: a prospective study. *Graefes Arch Clin Exp Ophthalmol* 2005;243:300-305.
7. Solbach U, Keilhauer C, Knabben H, Wolf S. Imaging of retinal autofluorescence in patients with age-related macular degeneration. *Retina* 1997;17:385-389.
8. Bindewald A, Bird A, Dandekar SS, et al. Classification of fundus autofluorescence patterns in early age-related macular disease. *Invest Ophthalmol Vis Sci* 2005;46:3309-3314.
9. von Rückmann A, Fitzke FW, Fan J, Halfyard A, Bird AC. Abnormalities of fundus autofluorescence in central serous retinopathy. *Am J Ophthalmol* 2002;133:780-786.
10. von Rückmann A, Fitzke FW, Bird AC. In vivo fundus autofluorescence in macular dystrophies. *Arch Ophthalmol* 1997;115:609-615.
11. Lois N, Halfyard AS, Bird AC, Holder GE, Fitzke FW. Fundus autofluorescence in Stargardt macular dystrophy-fundus flavimaculatus. *Am J Ophthalmol* 2004;138:55-63.
12. Sawa M, Ober MD, Freund KB, Spaide RF. Fundus autofluorescence in patients with pseudoxanthoma elasticum. *Ophthalmology* 2006;113:814-820.
13. Sunness JS, Ziegler MD, Applegate CA. Issues in quantifying atrophic macular disease using retinal autofluorescence. *Retina* 2006; 26:666-672.
14. Framme C, Brinkmann R, Birngruber R, Roider J. Autofluorescence imaging after selective RPE laser treatment in macular diseases and clinical outcome: a pilot study. *Br J Ophthalmol* 2002;86:1099-1106.
15. American National Standards Institute. *American National Standards for the Safe Use of Lasers*. Orlando, FL: Laser Institute of America; 1993: ANSI Z136.1.1993.
16. Bartsch DG, Freeman WR. Scanning laser ophthalmoscopy. In: Ciulla TA, Regillo CD, Harris A, eds. *Retina and Optic Nerve Imaging*. Philadelphia: Lippincott Williams & Wilkins; 2003:59-76.
17. Hopkins J, Walsh A, Chakravarthy U. Fundus autofluorescence in age-related macular degeneration: an epiphenomenon? *Invest Ophthalmol Vis Sci* 2006;47:2269-2271.
18. Karadimas P, Bouzas EA. Fundus autofluorescence imaging in serous and drusenoid pigment epithelial detachments associated with age-related macular degeneration. *Am J Ophthalmol* 2005;140:1163-1165.
19. Delori FC, Fleckner MR, Goger DG, Weiter JJ, Dorey CK. Autofluorescence distribution associated with drusen in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;41:496-504.
20. Shields CL, Cater J, Shields JA, Singh AD, Santos MC, Carvalho C. Combination of clinical factors predictive of growth of small choroidal melanocytic tumors. *Arch Ophthalmol* 2000;118:360-364.
21. Shields CL, Shields JA, Kiratli H, De Potter P, Cater JR. Risk factors for growth and metastasis of small choroidal melanocytic lesions. *Ophthalmology* 1995;102:1351-1361.

PEER DISCUSSION

DR ALAN M. LATIES: The search for improved methods of diagnosis never ends. In the present instance, we have the application of a recently developed technology, fundus autofluorescence, to an old problem—the clinical assessment of choroidal nevi and choroidal melanoma. We have just heard the results of a careful analysis of the findings in 23 patients whose clinical examinations were supplemented by fundus autofluorescence and optical coherence tomography measurements.

1. I restrict my remarks to three topics:
2. 1. When we see fundus autofluorescence, what are we looking at?
3. 2. Does an increase of fluorescence intensity indicate a true increase of lipofuscin?
4. 3. If lipofuscin intensity does increase, what does this mean?

1. It is generally believed that autofluorescence is an emission that derives mainly from the excitation of the complex lipid polymer, lipofuscin, enclosed in a small organelle within the retinal pigmented epithelium. A fraction of the lipofuscin can be embedded in melanin. It is likely that a minor contribution is made by other fluorophors of biological importance. This component is usually ignored.[1]

2. Overall, there is a reasonable argument that an increased intensity of autofluorescence follows an increase in the amount of lipofuscin. However, in an individual instance, this is far from certain: fluorescence yield is a complex composite, influenced by the partial pressure of oxygen, molecular dispersion, percent dissociation, pH, as well as the degree of polymerization.[2] Finally, it is

subject to masking by melanin pigment. When all of these factors are considered, it is little wonder that normative data are lacking or that fluorescence intensity is not specified in clinical reports.

3. If autofluorescence intensity does increase locally or if there is a spread of autofluorescence, even after the aforementioned caveats, it is likely that we are perceiving a true signal of cellular impairment. As noted by Shields and associates,[3] and as mentioned by the authors, the clinical visualization of orange pigment and/or subretinal fluid over a tumor are considered risk factors in the evaluation of choroidal melanocytic tumors. To the degree this is so, they likely represent markers of significant stress by tumor on neighboring cells. More specifically, that the increase in autofluorescence is evidence that retinal pigmented epithelium cells have failed in their major task of digesting cellular components. And instead, that partially digested cellular components, largely lipidic, have polymerized to form lipofuscin. Similarly, much the same reasoning explains subretinal fluid accumulation. Once again, stress-impaired retinal pigment epithelium cells have failed in their essential assignment to transport water outward, resulting in the subretinal fluid visible on optical coherence tomography.

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REFERENCES

1. Delori FC, Dorey CK, Staurenghi G, Arend O, Goger DG, Weiter JJ. In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. *Invest Ophthalmol Vis Sci* 1995;36:718-729.
2. Pringsheim P. *Fluorescence and Phosphorescence.* New York: Interscience Publishers Inc; 1965:327-355.
3. Shields CL, Cater J, Shields JA, Singh AD, Santos MC, Carvalho C. Combination of clinical factors predictive of growth of small choroidal melanocytic tumors. *Arch Ophthalmol* 2000;118:360-364.

DR. CAROL L. SHIELDS: I have no conflict of interest; however, we are also studying autofluorescence. I would like to make a comment, give a suggestion, and ask a question. The authors have made a beautiful correlation of the orange pigment visualized clinically with the orange pigment detected by autofluorescence. As we have seen in age related macular degeneration (AMD), autofluorescence is going to pick up more than you can see with a fundoscopic examination. I recall that Dr. Gunduz showed one clinical picture where they saw localized orange pigment, but the autofluorescence demonstrated a larger area of diffuse orange pigment. We like to call autofluorescence our "orange pigment detector". I suggest that they might change the name of this paper entitled "Fundus autofluorescence in choroidal melanocytic lesions and changes after treatment" to "Fundus autofluorescence in related retinal pigment epithelium (RPE) alterations associated with choroidal melanoma", because most autofluorescence comes from the RPE. You nicely addressed the RPE alterations, but what are the changes of autofloursecence in the melanoma? Dermatologists have used autofluorescence in the diagnosis and management of cutaneous melanomas for more than a decade. They determined that wavelengths in the near infrared range can differentiate melanomas from nevi in the skin with some sensitivity. I do not know if the Heidelberg instrument has that ability.

DR. SANFORD M. MEYERS: I have no financial interest, even though I am working on a project on retinal autofluorescence with Dr. Robert Bonner, a biophysicist at the National Institutes of Health. Recent work from Sparrow's group at Columbia and Travis' group at UCLA investigated A2E related fluorophores, A2E, and its epoxides, which are major components of lipofuscin. We are attempting to differentiate them with a noninvasive imaging technique in vivo using different spectral emission filters in relation to age related maculopathy (AMD). You have an excellent opportunity to correlate the preoperative clinical findings of autofluorescence with histopathologic and chemical analysis in enucleated eyes. Have you had been able to do that correlation?

DR. KAAN GUNDUZ: We would like to thank Dr. Laties and everyone in the audience for their relevant comments regarding our paper. We agree with Dr. Laties that orange pigment is a sign of retinal pigment epithelial distress and, as such, can be a sign of early malignant transformation of indeterminate choroidal melanocytic lesion into melanoma. Dr. Shields mentioned that autofluoresent imaging identifies more orange pigment than is clinically visualized, and we completely agree with her. We believe that this form of imaging will be more useful in the future and will detect subtle orange pigment that we are unable to see clinically. With respect to the change of the title of the paper she has proposed, we certainly welcome her suggestion. In the last part of her question, she asks to how to differentiate a nevus from a melanoma. I think fundus autofluourscent imaging is most useful in small choroidal melanocytic lesions in which we are able to detect the changes on the surface of the lesion more easily than within the substance of the thicker lesions. In that sense, I believe that the presence of orange pigment on the surface of the lesion is a strong indicator that the lesion is evolving into a melanoma. With respect to the second question, several eyes in our study were enucleated. In some cases, the decision to perform the enucleation was related to the desire of the patient to have their eye removed. In others, enucleation was performed because the tumors were thick and they were not suitable for plaque radiation treatment. As you know, the determination of whether the clinical findings correlate with pathology is difficult to assess. I reviewed the pathology reports and, although lipofuscin and hyperpigmentation were described, I am not sure how these pigment changes correlate with the specific sites of autofluorescence demonstrated by imaging. There will likely be some positive degree of correlation with the findings of fundus autofluorescence imaging and pathologic changes.