Retina

Increased Fundus Autofluorescence Associated with Outer Segment Shortening in Macular Translocation Model of Neovascular Age-Related Macular Degeneration

Fred K. Chen,1,2,3 Prateen J. Patel,2,3,4 Peter J. Coffey,2,5 Adnan Tufail,2,3,4 and Lyndon Da Cruz1,2,3

PURPOSE. To report the frequency and origins of increased fundus autofluorescence (AF) in age-related macular degeneration using the model of macular translocation.

METHODS. In this retrospective observational case series, postoperative serial fundus AF images from 40 consecutive patients were examined. The origin of well-delineated increased AF changes was explored by examining simultaneous spectral-domain optical coherence tomography (SD-OCT) scans and coregistered microperimetry.

RESULTS. AF images were taken between a mean of 13 and 36 months. Seven patients were excluded from analysis because of lack of postoperative AF imaging or extensive macular RPE atrophy. Of the remaining patients, 9 had masking pattern of foveal AF, 21 had small, round increased AF lesions in the fovea, and 3 had a normal pattern of foveal hypo-AF. Parafoveal increased AF was seen in all 33 patients in 1 of 3 patterns: well-delineated homogenous increased AF patches (17), curvilinear increased AF bands (4), and speckled increased AF (12). Simultaneous SD-OCT showed loss of signal from the interface of the inner and outer segments of the photoreceptor cell layer with variable loss of outer nuclear layer thickness. Microperimetry showed subnormal retinal sensitivity in regions with increased AF. Parafoveal increased AF size remained stable for 2 to 5 years of follow-up.

CONCLUSIONS. SD-OCT and microperimetry changes observed after translocation may be attributed to shortening of the outer segments. A corresponding reduction of visual pigment in the shortened outer segments may lead to an unmasking effect. Increased AF in some macular diseases may be attributed to unmasking of AF rather than to increased fluorophores within abnormal retina. (Invest Ophthalmol Vis Sci. 2010;51:4207–4212) DOI:10.1167/iovs.09-4728

Fundus autofluorescence (AF) imaging is a clinical tool that allows evaluation of the interaction between photoreceptor cells and the retinal pigment epithelium (RPE) in macular disease. The predominant fluorophores arising from the fundus have been shown to be located within the RPE lipofuscin. This hypothesis has been supported by macular disease models such as full-thickness macular hole and RPE rip. Increased fundus AF has been shown to occur in age-related macular degeneration (AMD), central serous retinopathy, and various types of inherited retinal or macular dystrophies. In these disease models, the increased AF is thought to be caused by increased fluorophores within the RPE, subretinal space or outer retina, resulting from the accumulation of RPE lipofuscin or the interruption of normal RPE phagocytosis of the outer segments. Increased AF has also been observed after macular translocation for myopic choroidal neovascularization (CNV). This disease model provides a unique opportunity for the study of the source of increased AF because the neuroretina has been separated from the diseased RPE and reattached to relatively normal paramacular RPE. In contrast to excision of the CNV alone, as shown in the outcomes of the Submacular Surgery Trials, macular translocation can restore near normal visual acuity. However, the relocated macula does not exhibit normal retinal sensitivity or electrophysiological responses. Although normal macular architecture after translocation has been demonstrated by time-domain optical coherence tomography (OCT), Terasaki et al. showed angiographic cystoid leakage in 70% of eyes despite normal foveal thickness. Sawa et al. showed 74% of eyes had well-defined regions of increased fundus AF after macular translocation in myopic CNV. They hypothesized that the increased AF was caused by the neurosensory retina carrying fluorophores from its interaction with the CNV during macular translocation. We and others (Tollot et al. IOVS 2009;50:ARVO E-Abstract 347) have observed similarly increased AF changes after macular translocation in patients with neovascular AMD. In this study, we report the frequency of the various patterns of increased AF changes within the foveal and parafoveal regions after translocation. Simultaneous spectral domain (SD) OCT scans and fundus-controlled microperimetry were also examined in a subset of patients with increased parafoveal AF to further explore the origin and implications of these unusual AF changes in this macular disease model.

PATIENTS AND METHODS

This is an observational exploratory study consisting of a retrospective review of all consecutive patients with neovascular AMD who underwent full macular translocation with 360° retinotomy at Moorfields Eye Hospital. All procedures were performed by one of the authors (LDC), and the surgical technique used was similar to that described by Eckardt et al. and Mruthyunjaya et al. Specifically, all translocations were performed using the same perfluorooctane heavy liquid (Perfluoron; Alcon Laboratories Inc., Fort Worth, TX). At the end of the operation, all patients received the same 1300 cS silicone oil (Oxane; Bausch and Lomb, Rochester, NY) for endotamponade. The median
duration of endotanoponade was 11 weeks; perfluorohexyloclotane (F6G8H) was not used in any patient. The study was approved by the institutional review board (Moorfields Research Governance Committee), and the research followed the tenets of the Declaration of Helsinki. All patients gave informed consent for macular translocation and subsequent clinical investigations.

Forty patients (16 men, 24 women) underwent macular translocation during the 6-year period (2003–2008). Mean age was 77 years, and median duration of symptoms was 7 weeks of visual loss. The indications for surgery included definite RPE rip (7 patients), predominantly submacular hemorrhage (21 patients), and predominantly CNV lesions (12 patients).

All patients underwent preoperative and postoperative best-corrected visual acuity (BCVA) testing using the Early Treatment of Diabetic Retinopathy Study charts after standardized refraction, dilated clinical examination, color fundus photography, and fluorescein angiography (TRC-50 IA/IMAGEnet H1024 system; Topcon, Tokyo, Japan). Fundus AF and OCT were also performed routinely every 6 to 12 months to detect subtle changes in RPE and subclinical recurrence of CNV.

Preoperative and postoperative fundus AF images were acquired through dilated pupil with a confocal scanning laser ophthalmoscope (HRA2 or Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). These instruments scan the fundus with a low-power optically pumped semiconductor laser (λ = 488 nm) to elicit autofluorescence, which is detected through a barrier filter (λ > 500 nm) and captured at a rate of 16 frames per second over a 30° × 30° or a 55° × 55° field (768 × 768 pixels). To enhance the signal-to-noise ratio of the fundus AF images, 9 to 15 frames were averaged.

The foveal and parafoveal AF patterns were reviewed independently by two of the authors (FKC, PJP). Disagreements were arbitrated by a third author (LDC). Fundus AF images were examined in conjunction with color fundus photographs to identify eyes with abnormal foveal or parafoveal hypo-AF and to determine whether this was caused by masking effects from dense epiretinal membrane (ERM), isolated foveal RPE defect, enlargement of parafoveal RPE defect, or CNV recurrence at the edge of the RPE defect. Those with relatively preserved AF in the foveomacular region were further examined for the presence or absence of increased round AF lesions in the fovea and regions of increased AF in the parafoveal region. The relationship between AF pattern in the parafoveal region and AMD lesion subtypes was examined using the χ² test.

A subset of patients with increased AF changes also underwent simultaneous fundus AF imaging and SD-OCT scanning (Spectralis; Heidelberg Engineering). Integrated eye tracking capability with a tracking laser tomograph (TruTrack within the Spectralis; Heidelberg Engineering) enables pixel-to-pixel registration between the fundus AF image and the SD-OCT line scan acquired simultaneously. The SD-OCT scans are acquired at a rate of 40,000 A-scans per second using a superfuminescent diode source (λ = 870 nm) and spectral-domain technology. The optical axial and transverse resolutions are 7 and 14 μm, respectively. With a maximum horizontal span and vertical depth of 30° and 1.8 mm, respectively, the corresponding digital transverse and axial resolutions are 5 and 3.5 μm/pixel for each OCT line scan image. To enhance the signal-to-noise ratio, 30 to 100 single-line OCT frames were averaged during simultaneous fundus AF and SD-OCT imaging. In each eye, several OCT line scans through separate regions with increased AF were obtained to examine the retinal architectural correlates to these increased AF regions. The 3 highly reflective layers in the outer retina are interpreted as suggested by Drexler et al.11,12

Preoperative and postoperative fundus-controlled perimeter (micropentometry) was performed in a subset of patients with a microperimeter (MP1; NAVIS software version 1.7.2; Nidek Technologies, Padova, Italy), which enables continuous registration (at 25 Hz) between test loci and fundus landmarks (on infrared) during examination. Micropentmetry was performed using a 1.27 cd/m² background luminance, Goldmann III size stimulus of 200-ms duration, stimuli luminance of 1.27 to 127 cd/m² (20-dB range), 4–2 staircase strategy, and a 76 loci test grid covering the central 20°, centered at the fovea. Retinal sensitivity maps were overlaid on color fundus photographs and coregistered with fundus AF images taken within 3 months of the microperimetry to determine whether regions with increased AF had reduced retinal sensitivity. Normal retinal sensitivity using Goldman III size stimulus is approximately 18 to 20 dB.

RESULTS

Preoperative median (range) BCVA was 0.80 (0.18–1.98) log-MAR. At the most recent follow-up (mean, 38 months; range, 12–67 months), the median BCVA was 0.78 (0.18 to hand motions) log-MAR. Fundus AF images from 5 patients were excluded from further analysis in this study given that the macula was not translocated because of technical issues (3 patients) or postoperative total retinal detachment precluded fundus AF imaging (2 patients). From the remaining 35 patients, 133 fundus AF images were reviewed. Each patient had an average of 4 (range, 1–8) postoperative AF images. These fundus AF images were taken between a mean of 15 and 36 months after surgery; the earliest was at 2 months, and the latest was at 67 months.

Among the 35 patients, 2 patients had extensive RPE atrophy precluding further analysis of increased AF changes within the foveal or parafoveal region. In these 2 patients, there was no typical increased AF at the rim of the extensive atrophy, as seen in geographic atrophy.

Foveal AF was absent because of dense ERM (3 patients), extension of parafoveal RD defect (3 patients), and isolated small foveal RD defect (3 patients). The remaining 24 eyes had either small, round increased AF lesions at the fovea (21 patients; Fig. 1) or relatively normal foveal hypo-AF caused by masking by the macular luteal pigments (3 patients). Subsequently, in some of these patients, foveal AF intensity declined because of subfoveal expansion of recurrent CNV or parafoveal RD defect (5 patients) or development of de novo subfoveal CNV or RPE atrophy (3 patients).

Increased AF change was also noted in parafoveal regions in the 33 patients without extensive RPE atrophy (Fig. 1). These could be categorized into 3 patterns of change: well-delineated homogenous increased AF patches (17 patients; Fig. 2), curvilinear increased AF bands (4 patients; Fig. 3), and poorly demarcated speckled increased AF (12 patients), which can be an isolated macular change or one associated with cystoid macular edema and recurrent CNV. The location and distribution of increased AF did not change at 2 to 5 years of follow-up (Fig. 2).

Among the 21 hemorrhagic lesions, 7 patients had the homogenous increased AF regions, 1 had curvilinear increased AF band, 1 had extensive atrophy, 9 had speckled AF pattern, and 3 were excluded because of total retinal detachment or unsuccessful translocation. Among the 19 CNV or RPE rips, 10 had homogenous increased AF regions, 3 had curvilinear increased AF band, 1 had extensive atrophy, 3 had speckled AF pattern, and 2 were excluded because of unsuccessful translocation. Testing (χ²) showed that the difference in the distribution of the various patterns of AF was not statistically significant between the groups of patients with hemorrhagic and CNV/rip subtypes (P = 0.20).

Simultaneous fundus AF with SD-OCT was available for review in 20 patients. Foveal round increased AF lesions were noted in 14 eyes, while 6 did not have elevated AF, and 1 had no macular detachment. Both homogenous patches and curvilinear bands of increased AF were observed with varying degrees of loss of the outer retinal architecture on SD-OCT. They all shared a common feature: loss of the line that corresponds to the so-called interface of the inner and outer segment of the photoreceptor cell layer (IPLR) with or without
absence of the external limiting membrane and reduction of the outer nuclear layer thickness (Fig. 4). Speckled increased AF lesions coregistered with variable features, including small intraretinal cystic change at the level of outer nuclear layer, loss of IPRL, or even intact outer retinal structures.

Postoperative microperimetry was available for coregistration with fundus AF images in 20 patients. In general, there was retinal sensitivity over increased AF regions. However, sensitivity did not reach normal levels (18–20 dB) in any of these patients, as seen in regions with relatively normal AF intensity (Figs. 2, 3).

**DISCUSSION**

The intensity of fundus AF is not always related to the concentration of fluorophores within the RPE. Increased AF may be caused by other fluorophores located anterior or posterior to the RPE or by unmasking of the RPE AF through reduced absorption of excitation light by pigments located anterior to the RPE. Therefore, the study of fundus AF in a posttranslocation disease model, in which the neuroretina is separated from the diseased RPE, may help us to examine the consequences of interaction between macular photoreceptor cells and paramacular RPE and the potential unmasking effects resulting from changes in pigments within the neuroretina.

We demonstrated that the normal fundus AF pattern is rare after macular translocation. A review of AF images of 35 patients revealed that 31% did not have an identifiable foveal AF pattern because of extensive atrophy, masking by ERM, or foveal atrophy caused by de novo foveal RPE loss or encroachment of an expanding lesion from the edge of the CNV excision site. Of the remaining 69%, most had small, round increased AF lesions and only 3 patients had normal foveal AF patterns. In almost all patients, increased AF was present in the parafoveal region. There were 3 patterns of increased AF; most (55%) had well-delineated patches of increased AF. The difference in parafoveal AF pattern was unrelated to endotamponade because all patients received the same silicone oil and none had F6H8. There was also no statistically significant relationship between lesion subtype and pattern of parafoveal AF. Together with a lack of topographic correlation between subretinal blood or fluid and regions of increased AF, our data suggest that blood degradation product or fibrin deposit is unlikely to contribute to this increased AF. However, simultaneous SD-OCT with AF and overlay of microperimetry on AF showed a loss of IPRL and subnormal retinal sensitivity over these well-delineated patches of increased AF.

Our results contrast with those reported by Sawa et al., who showed that 16% of their patients had almost normal fundus AF patterns and only 10% had decreased AF intensity. These differences are likely to be related to the definitions of their classification of AF features, lesion characteristics (smaller type 2 myopic CNV rather than our larger CNV lesions in AMD) and patient age (mean of 60 years rather than 77 years in our cohort). In addition, they did not specifically examine round increased AF lesions within the fovea. They observed that the size of parafoveal increased AF was the same as or larger than the preoperative CNV lesion. From this observation and the relatively early onset of the increased AF change, they concluded that the increased AF was caused by fluorophores arising from interaction between the neuroretina and the CNV, which had since been retained within the neuroretina even after translocation. However, they did not have high-definition SD-OCT images or microperimetry with which to further analyze these regions. We propose other explanations for the parafoveal increased AF changes in our cohort of patients.

Increased AF may be related to increased concentrations of fluorophores within the RPE resulting from excessive meta-

**FIGURE 1.** Abnormal patterns of fundus autofluorescence (AF) in posttranslocation model. (A, B) Normal foveal and parafoveal patterns of AF for comparison with abnormal patterns of AF seen in posttranslocation eyes. (C, D) Small, round increased AF within the foveal region. (E, F) Well-delineated region or patch of increased AF in the parafovea. (G, H) Curvilinear bands of increased AF in the parafovea. (I, J) Speckled increased AF in the parafovea.
bolic demand of the foveomacular photoreceptors on paramacular RPE, analogous to that seen in the junctional zones of geographic atrophy. This is supported by the rare observation in our study of intact IPRL on SD-OCT, in the region of speckled patterns of increased AF surrounding areas of reduced AF. However, a more common observation in this study was the exact coregistration between missing IPRL and speckled or homogeneous patterns of increased AF (Fig. 4) on simultaneous SD-OCT with AF imaging.

Increased AF may be an unmasking effect. This phenomenon has been described in full-thickness macular holes, cystoid macular edema, and retinal bleaching experiments using short-wavelength fundus AF. Masking occurs because of absorption of the short-wave excitation light (488 nm) within the neuroretina by luteal pigments (i.e., lutein and zeaxanthin within the plexiform and nuclear layers) or visual pigments (i.e., blue cones and rod opsins within the outer segments of the photoreceptor cell layer). Evidence of unmasking effects leading to increased AF were illustrated by the small, round lesions within the foveal region coregistered with cystic change on SD-OCT, consistent with the findings from 2 previous reports on AF features of cystoid macular edema, and well-delineated increased AF patches coregistered with loss of the reflective line that corresponds to the so-called IPRL on SD-OCT (Figs. 2–4). Although the precise histologic correlate corresponding to the interface of inner and outer segments of the photoreceptor cell layer. Note that the external limiting membrane in the region of increased AF is closer to the RPE.
FIGURE 4. Structural correlates of the junction between normal and increased AF in the posttranslocation model. Six examples from 6 patients showing coregistration between isolated loss of IPRL in the OCT section and region of increased AF, supporting the hypothesis that increased AF relates to the loss of visual pigment located in the outer retina. (A) The interface of IPRL dipped into the RPE as the fundus AF increased. (B) The IPRL reappeared as the fundus AF returned to normal intensity. (C) The IPRL dipped into RPE at the junction of increased AF. (D) Band of increased AF corresponded to region of IPRL fusing with the RPE layer. (E) The IPRL reappeared as the fundus AF returned to normal intensity. (F) The IPRL and outer nuclear layer reappeared as the fundus AF returned to normal. The normal architecture of the outer retina on SD-OCT consisted of the following layers: outer nuclear layer (ONL), external limiting membrane (ELM), IPRL, and RPE.
of a missing IRP line is unknown, close examination of the SD-OCT images showed that the IRP line might have merged with the RPE line. If Drexler et al.11,12 were correct in their guide to the analysis of the reflective bands in the outer retina and if this interpretation can be applied to a posttranslocation eye, the fusion between the 2 hyperreflective lines corresponding to the IRP and the RPE may be interpreted as shortening of the outer segment. A corresponding reduction in visual pigment in these regions may explain the relatively increased AF. Further evidence to support possible unmasking effect is the observation of reduced outer nuclear layer thickness in regions of increased AF in some patients and of subnormal retinal sensitivity as demonstrated on microperimetry.

Shortening of the outer segments of photoreceptor cells is a nonspecific response to various genetic and acquired insults to the outer retina.17 Animal models have shown that the development of CNV can lead to shortening of outer segments and loss of outer nuclear layer thickness.18 Similarly, short-term retinal detachment can lead to shortening of the outer segment that persists even after the retina is reattached.19 Detachment, however, cannot explain the observed outer segment shortening because the entire retina was detached during macular translocation surgery whereas the increased AF changes were limited to the parafoveal region. Furthermore, translocation experiments in monkeys showed that reattached retina had a misaligned but normal length of the outer segment.20 Although we did not specifically examine the degree of matching between the distributions of increased AF regions and the preoperative lesion, the parafoveal distribution of increased AF suggests that loss of the outer segment is likely to be related to its interaction with subretinal fluid, blood, or CNV tissue before translocation. Incomplete restoration of outer segment length, even after 5 years, may be attributed to permanent injury to the mechanisms of outer segment renewal as a result of interaction with toxic substances released by the CNV or with a continued unfavorable environment provided by parafoveal RPE for complete outer segment regeneration. These patchy changes in outer segment morphology may explain previous findings of abnormal focal electroretinography and microperimetry within the relocated macula.6,7

Given the descriptive and exploratory nature of this study, we were unable to provide data that quantify the difference in AF signal between normal and posttranslocation eyes. We anticipate that this type of analysis will be confounded by variables, such as image quality, media opacity, and pupil size, that are difficult to control. The sample size was also too small for detailed statistical analysis of the association between lesion subtype and AF patterns. Further investigations using short- and long-wave AF techniques to measure visual pigment optical density may be useful in confirming the hypothesis of an unmasking effect.16 Adaptive optics scanning laser ophthalmoscope imaging may detect changes within the outer segment mosaic in regions with increased AF to support our hypothesis.21

In conclusion, we have shown that the normal AF pattern is rare after macular translocation for the treatment of neovascular AMD. Most patients had foveal increased AF lesions corresponding to intraretinal cystoid change. Many had well-delimited increased AF lesions in the parafoveal region likely caused by the unmasking effect of visual pigment reduction resulting from permanent outer segment shortening rather than fluorophores within the neuroretina, as previously proposed by Sawa et al.9 The distribution and stability of these increased AF changes suggest that shortening of the outer segment may be the result of interaction between the outer retina and subretinal blood, fluid, or CNV before translocation or paramacular RPE after surgery.

References