Early Detection of Incipient Retinal Pigment Epithelium Atrophy Overlying Drusen with Fundus Autofluorescence *vs* Spectral Domain Optical Coherence Tomography

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Abstract

Purpose. This study aims to compare fundus autofluorescence (FAF) and spectral domain optical coherence tomography (SD-OCT) for the early detection of retinal pigment epithelium (RPE) demise overlying drusen.

Methods. Single-site retrospective, observational, longitudinal study. Patients with intermediateAMD (iAMD) [large (> 125 µm) or intermediate (63-125 µm) drusen with hyper/hypopigmentation] with a minimum follow-up of 18 months were included. Drusen with overlying incipient RPE atrophy were identified on SD-OCT in the context of choroidal hyper-transmission or nascent geographic atrophy (nGA). These selected drusen were then traced backwards in time to determine if incipient RPE atrophy overlying drusen was present in FAF (well-demarcated region of absence of autofluorescence) before, simultaneously or after the first signs of incipient RPE atrophy on SD-OCT.

Results. One hundred and thirty-three drusen in 22 eyes of 22 patients were included. Of these, 112 (84.2%) showed choroidal hyper-transmission and 21(15.8%) nGA. Early signs of atrophy overlying drusen were found simultaneously on SD-OCT and FAF in 52 cases (39.1%, 95% CI 30.8 -47.9%), first on FAF in 51 (38.3%, 95% CI 30.0-47.2%) and first on SD-OCT in 30 (22.6%, 95% CI 15.8-30.6%; $p<0.05$). FAF detected signs of incipient atrophy earlier than SD-OCT ($p=0.005$). When RPE atrophy was found first on FAF, median time to diagnosis with SD-OCT was 6.6 months (95% CI 5.5 to 8.6), while if detection occurred earlier on SD-OCT, median time until identification with FAF was 12.6 months (95% CI 6.0 to 23.4; $p=0.0003$).

Conclusions. In those iAMD cases in which early atrophy overlying drusen is not detected simultaneously in FAF and SD-OCT, FAF was significantly more sensitive, although a multimodal approach is required.

1. Introduction

The hallmark of the intermediate stages of age-related macular degeneration (AMD) is the presence of drusen [1]. Drusen are deposits of extracellular material that are located between the basement membrane of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane [2]. In early stages, drusen can produce mild metamorphopsia and decreased sensitivity in microperimetry or dark adaptation, among others. As drusen increase in number or size, the disease progresses from the early to the intermediate stages and the risk of vision loss increases. In the late stages, the disease shows the greatest visual deterioration when evolving towards neovascular AMD or geographic atrophy (GA) [3,4].

In the last decades, new imaging techniques have been incorporated to study macular pathology, among them fundus autofluorescence imaging (FAF), which provides information of RPE integrity in a non-invasive way. In different studies it has been shown that, in early or intermediate stages, the FAF image has the capacity to show RPE alterations in normal-appearing fundus regions [5-8]. On FAF, GA appears as a well-demarcated region of marked hypoautofluorescence due to the absence of the fluorophore lipofuscin contained within the RPE [7-11]. GA usually appears in the central or parafoveal macula and may extend centrifugally [8,12]. New areas of atrophy may show a relatively lower intensity of hypoautofluorescence. FAF imaging is especially valuable in GA because it delineates the areas of discrete or small GA in comparison with other imaging modalities [11,13].

On the other hand, spectral domain optical coherence tomography (SD-OCT) facilitates *in vivo* high resolution evaluation of the retina [14]. GA has been extensively studied with SD-OCT, and alterations within the atrophic area and its borders have been described in detail [14-18].

Guymer *et al* used SD-OCT to visualize precursors of GA. They defined nascent geographic atrophy (nGA) by the presence of either the subsidence of the outer plexiform layer (OPL) and the inner nuclear layer (INL) and/or a wedge-shaped band within the boundaries of the OPL [18-20].

A previous study that investigated FAF in areas of nGA and areas of drusen-associated atrophy concluded that areas of nGA were most commonly characterized by both hyper and hypoautofluorescent changes, while in drusen associated atrophy, most often appeared as areas of hypoautofluorescence [19]. Currently, GA has no treatment, so deciphering its earliest signs can help understand the natural course of the disease, which in turn could help to develop new preventive or therapeutic strategies.

In the present study, we aimed to compare two imaging techniques, SD-OCT and FAF, to determine which one is more sensitive to detect incipient RPE atrophy overlying drusen in patients with iAMD.

2. Materials and Methods

2.1 Design and participants

This was a retrospective, observational, longitudinal study conducted at the Institut de la Màcula (Hospital Quirón Teknon; Barcelona, Spain). The study adhered to the tenets of the Declaration of Helsinki and was approved by the Fundación Quirón Salud Ethics Committee. All patients signed an informed consent.

Charts of patients with a diagnosis of iAMD visited between January 2010 and October 2014 were reviewed, and the last date of follow-up was July 2017. The drusen type of interest were soft drusen.

All patients met the following inclusion criteria: males or females aged 50 years or older, diagnosed with iAMD (AREDS stage 2 or 3: large [> 125 μm] or intermediate [63-125 μm] drusen and associated hyper/hypo pigmentation), with a minimum follow-up of 18 months after diagnosis. Patients were excluded if the study eye included any prior history of neovascular AMD, areas of RPE atrophy> 0.5 area discs (1.27 mm^2), other concomitant macular diseases (macular edema, retinal dystrophies, etc.), spherical equivalent greater than ± 6'00 D, previous history of intraocular treatment (laser photocoagulation, intravitreal injections) or surgery (with the exception of phacoemulsification), concomitant use of medications known to be toxic to the retina (chloroquine, hydroxychloroquine, tamoxifen, etc.) or OCT image quality< 20. The presence of reticular drusen in the study eye was not considered a reason for exclusion.

2.2 Procedures

All patients received a complete ophthalmic exam by an experienced retina specialist. This examination included: medical history, best-corrected visual acuity, intraocular pressure with Goldmann applanation tonometry and indirect fundus ophthalmoscopy. Imaging consisted of infrared, FAF (excitation 488 nm, absorption >500 nm) and SD-OCT imaging with the Heidelberg Spectralis HRA+OCT® (Heidelberg Engineering, Heidelberg, Germany) and fovea-centered, non-stereoscopic 30º colour fundus photography (TRC-50DX, Topcon Medical Systems, Tokyo, Japan). The SD-OCT examination consisted of volume scans performed using 19 or 37 horizontal high-resolution B-scans centered in the fovea. FAF was acquired in 30º with the high resolution (1536x1536 pixels) mode and centered in the fovea.

Upon review of the Heidelberg Spectralis database of the Institut de la Màcula, an experienced observer (AR) selected and classified the patients with iAMD and meeting the aforementioned eligibility criteria, looking for individual soft drusen showing early signs of RPE atrophy overlying them, as defined by either of the following:

1. Hyper-transmission: drusen that show increased hyper-transmission into the choroid on SD-OCT than in adjacent structures, which indicates a loss of overlying RPE (Figure 1a); or
2. nGA: defined by the presence of subsidence of the OPL/INL and/or a hyporeflective wedge-shaped band within the boundaries of the OPL (Figure 1b).

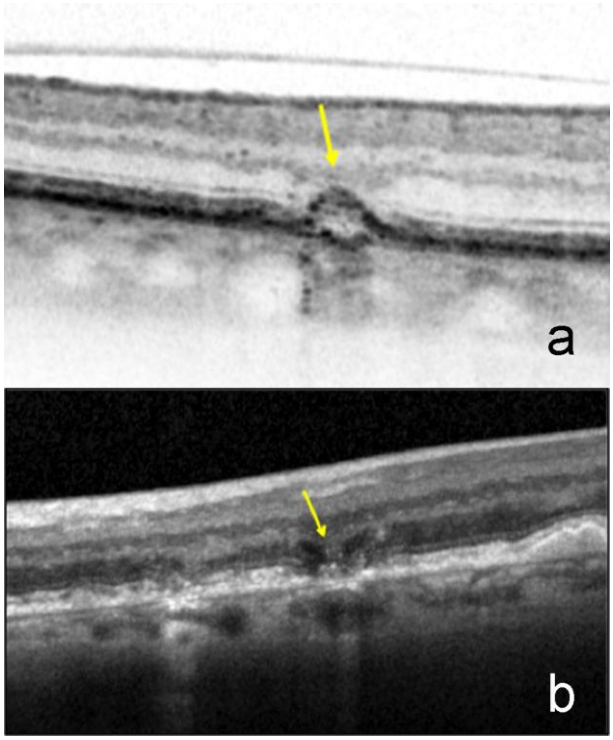

Figure 1. (a) B-scan of SD-OCT where observe a drusen with hyper-transmission; (b) B-scan of SD-OCT with nascent geographic atrophy.

RPE atrophy on FAF was defined as a well-demarcated region of absence of autofluorescence (Figure 2).

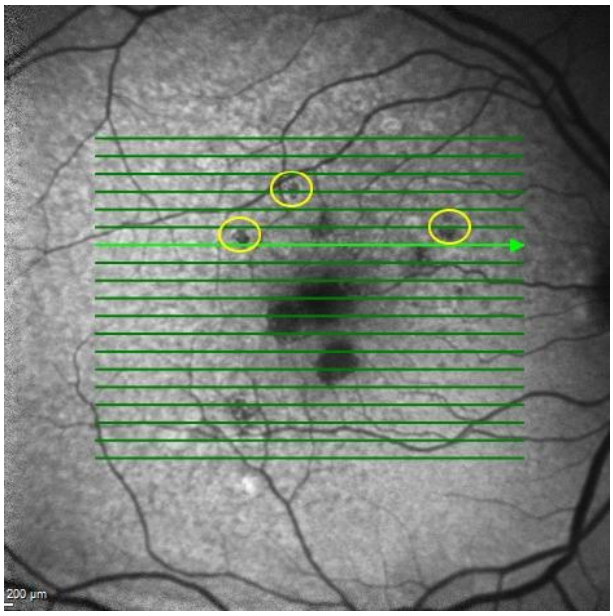

Figure 2. An example of retinal pigment epithelium atrophy by fundus autofluorescence. The yellow circle shows a region of absence of autofluorescence.

It was verified with the colour fundus photography that the absence of autofluorescence was not caused by pigment clumping over drusen. Drusenoid pigment epithelium detachments (defined

in the AREDS studies as a well-defined, pale yellow or white, large mound consisting of many large drusen or confluent drusen with ≥350 µm in the narrowest diameter) [21] were excluded. When a drusen presented hyper-transmission or nGA, previous and subsequent SD-OCT and FAF images were reviewed until the first time that absence of autofluorescence on FAF was observed. There were three possible scenarios:

- If the first time that absence of autofluorescence on FAF was observed coincided with the first time atrophy of the RPE was detected by SD-OCT, the diagnosis of incipient RPE atrophy overlying drusen was simultaneous with both imaging methods.
- If absence of autofluorescence on FAF was present before RPE atrophy was detected by SD-OCT, FAF identified incipient RPE atrophy overlying drusen earlier than SD-OCT.
- If absence of autofluorescence on FAF was present after RPE atrophy was detected by SD-OCT, SD-OCT identified incipient RPE atrophy overlying drusen earlier than FAF.

When in doubt, a second experienced observer (AS) reevaluated the case and the decision of the second evaluator was taken as the outcome for the analysis.

2.3 Main Outcome measures

The primary endpoint was the comparison of the number of drusen in which there was incipient RPE atrophy overlying drusen detected earlier by FAF than by SD-OCT *vs* the number of drusen in which this occurred earlier on SD-OCT than on FAF. The number of drusen in which RPE atrophy was detected simultaneously on both imaging modalities was also recorded.

The secondary endpoint included the time elapsed from the identification with the more sensitive method to identification with the other in cases of non-simultaneous identification of early atrophy overlying drusen.

2.4 Statistical Analysis

Univariate statistics were used to describe the sample using mean and standard deviation (SD) for quantitative and number and percentage for categorical variables. The unit of analysis was each soft drusen contained in the 20º x 30º, fovea-centered SD-OCT macular grid. The percent of time in which RPE atrophy overlying drusen was detected earlier by SD-OCT, FAF or simultaneously was determined and compared using Fisher's exact test.

Secondary endpoints

When one imaging method detected incident RPE atrophy overlying drusen earlier than the other, Kaplan-Meyer plots were used to estimate median the time to detection with the second, less sensitive method. The time when one method detected RPE atrophy for the first time (either FAF or SD-OCT) was considered time 0. The logrank test was used to compare both curves.

In cases were FAF detected RPE atrophy earlier than SD-OCT, then the time to detection of RPE atrophy with SD-OCT using either the definition of hyper-transmission or nGA was determined, and results were again compared using the logrank test.

The intra and interobserver agreement for FAF detection of RPE atrophy was determined using the kappa index (κ) in a randomly selected sample of 35 drusen. The κ measures agreement between categorical observations adjusted for chance.

Data analysis was conducted using Stata IC, version 15.1 (StataCorp, Texas, USA). A two-tailed p-value<0.05 was considered statistically significant.

3. Results

One hundred fifty-one drusen from 22 eyes in 22 patients with iAMD showed hyper-transmission or nGA after a minimum follow-up of ≥18 months and were initially enrolled. Eighteen of these drusen (seven patients) were excluded: eight could not be assessed by being present in a region with dense macular pigment and ten by lack of previous visit information. Therefore, 133 drusen from 22 patients were finally included in the analysis. The median age of these patients was 71.1 (6.9) years, 93.3% were female and all were Caucasian. The number of drusen included per eye ranged from 1 to 27, with a mean of 13.9 (8.8). The mean baseline visual acuity of study eyes was 0.11 (0.13) logMAR, equivalent to approximately 20/25 in Snellen notation. The interobserver agreement in the determination of incipient atrophy with FAF was 91,4%, with a kappa of 0.62.

Incipient RPE atrophy overlying drusen was observed simultaneously on both tests in 52/133 drusen (39.1%, 95% CI 30.8% to 47.9%), while early RPE loss was detected first by FAF in 51/133 (38.40 %, 30.1% to 47.2%) and first by SD-OCT in 30/133 (22.6%, 95% CI 15.8% to 30.6%), as seen in Table 1 and Figure 3. The difference between early detection with FAF and with SD-OCT was statistically significant (p=0.005).

Imaging method	**n**	**Percentage (95% CI)**
Fundus autofluorescence	51/133	38.4 (30.1 to 47.2)
SD-OCT	30/133	22.6 (15.8 to 30.6)
Simultaneously on both tests	52/133	39.1 (30.8 to 47.9)

Table 1. Percentage of cases of incipient atrophy of the retinal pigment epithelium detected on each imaging method. The percentages do not add to 100% due to rounding. CI: confidence interval; SD-OCT: spectral domain optical coherence tomography.

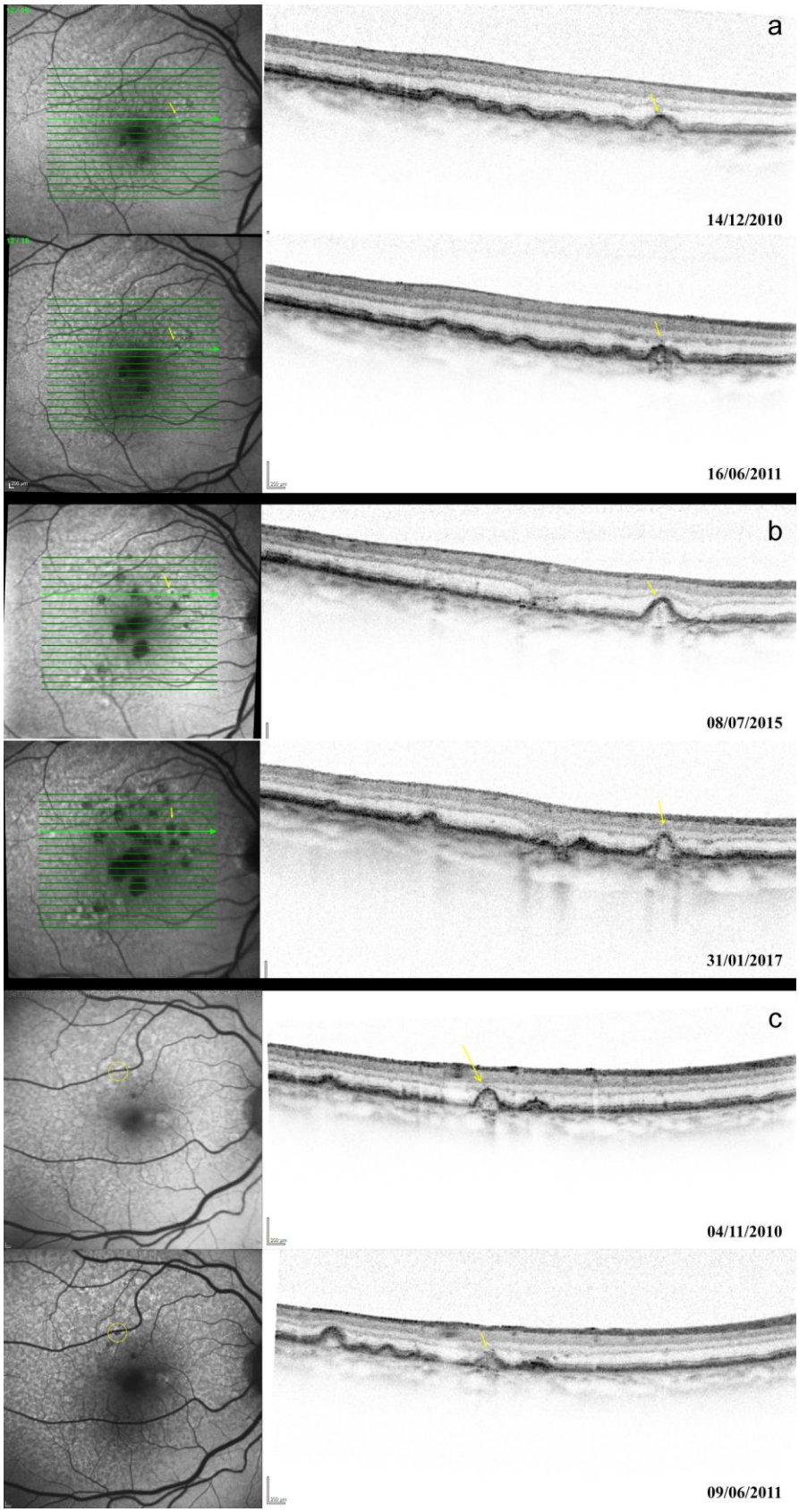

Figure 3. Example of simultaneous detection, earlier detection with FAF and earlier detection with SD-OCT. (a) A case of simultaneous detection, where the incipient RPE atrophy occurs at the same time with FAF and with SD-OCT. In the top image (14/Dec/2010), the selected druse

(yellow arrow) showed normal autofluorescence on FAF and no hyper-transmission on SD-OCT. In the next visit (16/Jun/2011), incipient atrophy of the RPE by both imaging techniques is observed. (b) Earlier detection with FAF. RPE atrophy overlying drusen is detected earlier on FAF than on SD-OCT. The top image (08/Jul/2015) shows that while a marked area of absence of autofluorescence appears on FAF, no signs of RPE atrophy are detected with SD-OCT. In the bottom image (31/Jan/2017), after 18 months, atrophy on SD-OCT can be observed and the area of atrophy on FAF also increased. (c) Earlier detection with SD-OCT. In the top image (04/Nov/2010) there is SD-OCT hyper-transmission (yellow arrow), a feature that defined as incipient RPE atrophy on SD-OCT, but normal autofluorescence on FAF; seven months later (09/Jun/2011), absence of autofluorescence appeared. FAF: fundus autofluorescence; RPE: retinal pigment epithelium; SD-OCT: spectral domain-optical coherence tomography.

When atrophy detection was not simultaneous on both imaging modalities, the median time from detection with FAF to detection with SD-OCT was 6.6 months (95% CI, 5.5 to 8.6 months), as compared with 12.6 months (95% CI, 6.0 to 23.3 months) from detection with SD-OCT and then with FAF (p-value=0.0003; Figure 4a).

When detection of incident atrophy was made with SD-OCT, in 112/133 of the cases (84.5%, 95% CI 76.9% to 90.0%) it was made through choroidal hyper-transmission and in 21/133 (15.5%, 95% CI 10.0% to 23.1%) with nGA. Therefore, the odds ratio (OR) of identifying incipient RPE atrophy overlying drusen by choroidal hyper-transmission in comparison with nGA was 5.33 (95% CI 3.16 to 9.01, p-value <0.0001). Detection through choroidal hyper-transmission was made after a median of 6.5 months of detection with FAF (95% CI, 4.9 to 8.6 months), and detection through nGA after a median of 6.7 months (95% CI, 2.5 to 27.3 months; p-value=0.09), as seen in Figure 4b.

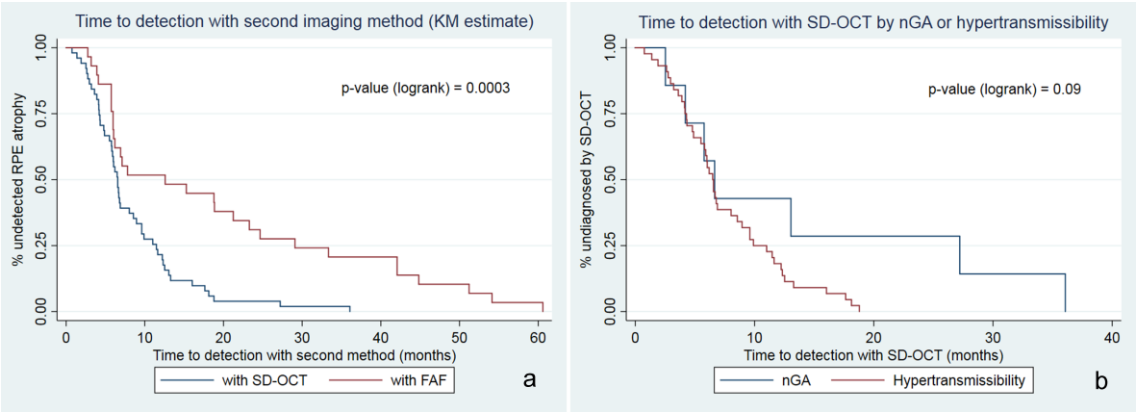

Figure 4. Comparison of time to (secondary) detection. (a) *Left,* Kaplan-Meyer curves of time to detection with SD-OCT when FAF detected atrophy earlier (blue) and with FAF when SD-OCT detected atrophy earlier (red). (b) *Right,* when SD-OCT detected atrophy, the Kaplan-Meyer estimates compare if earlier detection was made by showing choroidal hyper-transmission or nGA. FAF: fundus autofluorescence; nGA: nascent geographic atrophy; SD-OCT: spectral domain optical coherence tomography.

4. Discussion

This study compared FAF and SD-OCT in the detection of incipient RPE atrophy overlying drusen in patients with iAMD. We chose to compare FAF with SD-OCT because they are the standard diagnostic tests in the detection, evaluation and monitoring of GA.

The results of this study show that detection of early atrophy overlying drusen occurred simultaneously on both imaging techniques about 40% of the time (Figure 3a). Therefore, in 60% of the occasions one method detected signs of incident atrophy earlier than the other (Figure 3b-c). This suggests that a multimodal approach that includes FAF and SD-OCT would be recommendable for early detection of atrophy overlying drusen.

When atrophy was detected solely by one imaging method, FAF detected it earlier than SD-OCT (38.4% vs 22.6%, p=0.005). True differences favoring FAF may be related to the advantages of using an *en face* modality to visualize changes in the retina as opposed to the cross-sectional nature of SD-OCT, in which protocols with wide interscan distances may miss the point of early atrophy if the B-scan is not located precisely in the location of RPE loss. Given that even very dense protocols have a distance between adjacent B-scans in the range of tenths of microns, combined use of FAF with SD-OCT increases the likelihood of detection of early signs of RPE loss. The exclusion of lesions closer to the foveola due to the impossibility to differentiate the absence of autofluorescence caused by macular pigment absorption from that caused by true early atrophy may have favored an increased sensitivity of detection with FAF.

Also, once atrophy was detected with one imaging modality and not the other, time to detection with the other method differed markedly (p=0.0003). It took approximately 6 months to detect incipient atrophy with SD-OCT after FAF detected it, while detection with FAF once it was detected with SD-OCT took approximately 12 months. These differences are not easily explained taking into account that in almost half of the sample the detection of atrophy was simultaneous with both imaging modalities. Certainly, differences between follow-up times of each patient difficult the estimation of the precise moment of atrophy of individual drusen. On the other hand, it can be speculated that the underlying mechanism leading to RPE loss may differ between distinct drusen, making some imaging modalities to be more readily apt to detect incipient atrophy than others. In fact, using fluorescence lifetime imaging ophthalmoscopy (FLIO) eyes with drusen showed longer autofluorescence lifetimes than healthy controls, and different lifetime values were found in different drusen, suggesting a heterogeneous ultrastructural composition in phenotypically similar lesions [22].

In the vast majority of cases in which atrophy was detected with SD-OCT, choroidal hyper-transmission was observed more frequently than nGA (84% vs 16%, p<0.0001), although the median time to detection with either phenomenon was not different (p=0.09). This suggests that hyper-transmission may be a precursor of nGA. This could be expected since hyper-transmission arises as an immediate consequence of tissue loss, whereas nGA is detected after subsidence of inner retinal tissue and the appearance of the hyporeflective wedge-shaped band, which arise as a consequence (not a primary cause) of a certain amount of tissue loss.

There are limitations that need to be acknowledged. First, although this is a retrospective study, the fact that patients are studied back to where retinal imaging was normal provides a prospective nature to the study. Second, the number of eyes was small, but our units of analysis were the individual drusen. Third, drusen within the foveal area were excluded to avoid luteal pigment absorption; detection of incipient atrophy in this region may be more readily accomplished with SD-OCT because its signal is not as absorbed by luteal pigment as is in the case of FAF. Also, detection with SD-OCT may had been improved by the use of more dense volume protocols (decreased distance between B-scans that may have had an increased chance of crossing a focal area of RPE loss), but this is always a limitation with a cross-sectional device; it remains to be determined if the use of *en face* strategies at different heights could have improved SD-OCT detection rate. Finally, the patients were visited at irregular intervals, and therefore estimates of time to appearance of RPE atrophy may be overestimated.

5. Conclusion

In summary, the detection of incipient atrophy overlying drusen in iAMD was simultaneous on FAF and SD-OCT in 40% of cases. In 60% it was detected first by either imaging method, suggesting that a multimodal approach is recommended to detect the earliest signs of cell loss. In these cases, FAF detected signs of atrophy earlier through absence of autofluorescence than SD-OCT through either choroidal hyper-transmission or nGA.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have not made financial disclosures on the subject of this paper.

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Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anabel Rodríguez, Marc Biarnés and Anna Sala-Puigdollers. The first draft of the manuscript was written by Anabel Rodríguez and all authors commented on previous versions of the manuscript. Rosa M Coco-Martin y Jordi Mones revised the final draft. All authors read and approved the final manuscript.

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