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Hyperreflective Dots: A New Spectral-Domain Optical Coherence Tomography Entity for Follow-Up and Prognosis in Exudative Age-Related Macular Degeneration

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Key Words
Hyperreflective dots · Spectral-domain optical coherence tomography · Exudative age-related macular degeneration · Anti-vascular endothelial growth factor treatment

Abstract
Purpose: Spectral-domain optical coherence tomography (SD-OCT) enables high-resolution analysis of retinal layers and previously unseen hyperreflective dots (HRD). HRD morphological characteristics, evolution, possible origin and prognostic value are discussed. ***Methods:*** We conducted a prospective study of 100 patients with exudative age-related macular degeneration (AMD), who were treated and followed up with monthly imaging examinations. Statistical correlations between visual acuity (VA) and pre-/post- treatment HRD characteristics were evaluated. ***Results:*** HRD were present in all cases, mainly in the outer retinal layers but also elsewhere. After treatment, HRD regressed in a few days, 1 month ($p < 0.04$) and 3 months ($p < 0.01$). Regression was evident in all VA and morphological subsets. Resolution was associated with better final VA ($p < 0.001$). ***Conclusions:*** Presence of initial/recurrent HRD, rapid treatment response and the growing role that early biological inflammatory reaction plays in AMD suggests HRD are activated microglia cells. The correlation between VA and HRD could make HRD a clinical marker for early decisions about treatment and retreatment.

Introduction

During the last decade, optical coherence tomography (OCT) has rapidly become a popular noninvasive optical imaging modality used for the evaluation and follow-up of fluid accumulation in exudative age-related macular degeneration (AMD). Recently, more attention has focused on outer retinal layer changes, and particularly, on a new sign: the hyperreflective dots (HRD) that could be useful for prognosis [1, 2].

AMD is well known as the leading cause of blindness in patients over the age of 60 years in industrialized countries [3]. In the last decade, the role of vascular endothelial growth factor (VEGF) and choroidal neovascularization (CNV) in the pathogenesis of neovascular AMD was emphasized. Consequently, the advent of intravitreal VEGF inhibitors has revolutionized the management of this disease [4].

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Though these therapies reduce intraretinal and subretinal exudation, they do not prevent recurrences. While monthly intravitreal injections have been shown to be the most efficient treatment, suggested scheduled controls and treatments differ, making management extremely challenging [5, 6]. Therefore, any additional test that could, as early as possible, predict potential recurrences would be beneficial to both patients and clinicians.

Recent imaging techniques such as spectral-domain (SD)-OCT have improved the visualization of retinal morphology and provided more information than time-domain OCT for exploring the outer retinal layers. These advances may provide not only a better understanding of pathogenesis and evaluation of treatment efficacy for neovascular AMD, but could also add new findings on OCT (besides central macular thickness) that may be important to guide management strategies in patients affected by macular disease [7–10].

In 2009, Coscas et al. [1] used SD-OCT and reported finding previously unseen intraretinal changes, which they termed HRD. HRD are found scattered throughout all retinal layers, primarily around fluid accumulation in the intraretinal cystoid spaces. Subsequently, they have also been reported in different retinal diseases, such as retinal vein occlusion and diabetic macular edema [11–14].

Different hypotheses about the origin and nature of HRD have been proposed. A number of arguments supports that inflammation may play a possible role in AMD. CNV and vascular hyperpermeability with the release of inflammatory mediators and the recruitment of inflammatory cells induce subretinal and intraretinal fluid accumulation, macular edema and indirect clinical signs: the HRD. The rapid reabsorption of these intraretinal changes has suggested that HRD could represent inflammatory cells and activated microglia [1, 14].

The aim of this paper is to describe the characteristics of HRD in exudative AMD, to report their evolution after anti-VEGF treatment and to discuss a possible origin for these intraretinal features associated with an early inflammatory reaction.

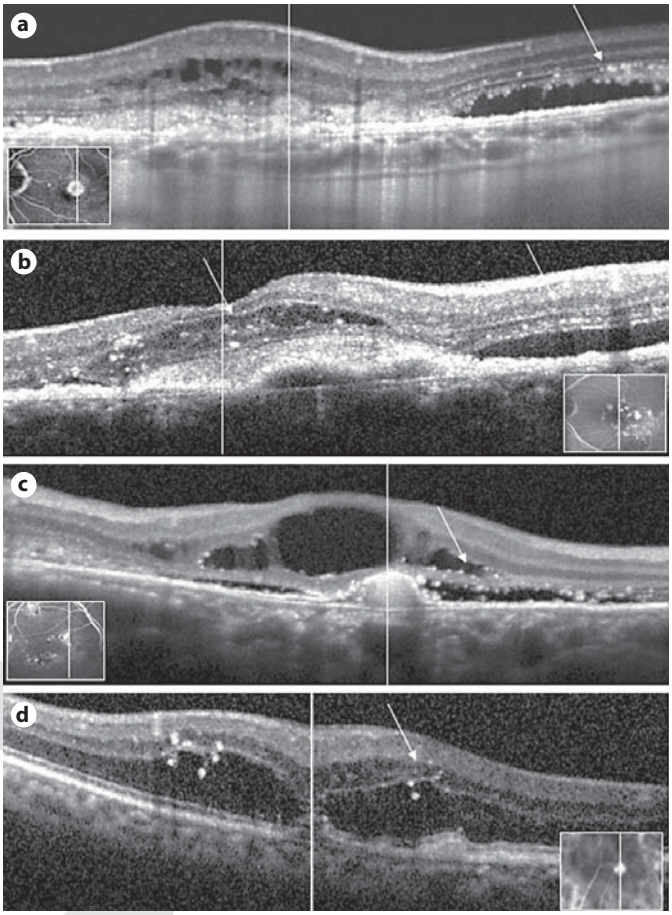

Fig. 1. SD-OCT and fluorescein-angiogram presence of HRD (arrows): small focal hyperreflective material scattered mainly in outer retinal layers but also spreading to all retinal layers, in 4 different clinical cases of active exudative AMD at the initial visit, before anti-VEGF treatment. **a** Pre-epithelial CNV. **b** Occult subepithelial CNV. **c** PCV. **d** CRAs.

Material and Methods

In this prospective study, 100 consecutive patients presenting with exudative AMD who presented at the Centre d'Ophtalmologie de l'Odéon in Paris were enrolled. Inclusion criteria were: 50 years or older in age, the presence of symptomatic subfoveal AMD, the presence of active exudative AMD and a minimum follow-up of 12 months after the initial visit. Exclusion criteria were: previous anti-VEGF treatment, high myopia, bad-quality images because of strong eye movements or extensive media opacities, signs of any other active retinal disease in the study eye, such as retinal vasculopathy (i.e. diabetic retinopathy and retinal vein occlusion) or vitreoretinal diseases (i.e. vitreomacular traction syndrome and epiretinal membrane).

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity (VA; determined in all subjects with logMAR and Early Treatment Diabetic Retinopathy Study charts), determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, SD-OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) and fluorescein and indocyanine green angiography (HRA2; Heidelberg Engineering), and in some cases, autofluorescence fundus photography. At each scheduled monthly follow-up visit, each patient underwent a complete ophthalmologic examination, including VA measurement, slit-lamp biomicroscopy, indirect fundus

ophthalmoscopy and OCT examination. Fluorescein and indocyanine green angiography were performed, as deemed necessary.

For a more accurate statistical analysis, patients were classified into 3 groups according to the initial logMAR VA: Group 1 good VA (if logMAR VA was better than 0.3), Group 2 moderate VA (if logMAR VA was from 0.4 to 0.6) and Group 3 poor VA (if logMAR VA was worse than 0.7). All eyes were divided into 4 groups based on the type of lesion: occult CNV (type 1), classic or pre-epithelial CNV (type 2), chorioretinal anastomosis (CRA, type 3) and polypoidal choroidal vasculopathy (PCV).

Each eye was treated with ranibizumab intravitreal injections. After the loading phase of 3 injections, additional injections were administered on an as-needed basis (i.e. a decrease in vision of 5 letters or more, new intraretinal hemorrhage, increased angiographic leakage and/or an increased central foveal thickness of more than 100 μm).

The characteristics of the HRD were evaluated in all SD-OCT scans obtained. The presence of HRD was defined as the presence of small focal hyperreflective material scattered mainly in outer retinal layers but also spreading to all retinal layers, observed in at least one available scan (fig. 1).

Considering the horizontal B-scan passing through the fovea, HRD were classified on the basis of quantity (i.e. absent, few if less than 10, moderate if between 10 and 20, or numerous if more than 20) and localization (i.e. near the retinal pigment epithelium, at the border of the serous detachment or disseminated in all retinal layers).

Statistical calculations were performed using the χ^2 test and McNamara's test to analyze morphological changes (i.e. fluid accumulation, central foveal thickness and HRD) during the treatment and to correlate them with functional changes, i.e. VA.

All the research and measurements adhered to the tenets of the Declaration of Helsinki; the study was approved by the local ethics' committee, and informed consent was obtained from all individuals after a detailed discussion of the nature and possible consequences of the study procedures.

Results

One hundred eyes from 100 patients (70 men and 30 women) with exudative AMD (mean age 79.2 ± 6.7 years) were examined. Of these 100 eyes, 62 had occult CNV, 13 had classic CNV, 12 had chorioretinal anastomosis (CRA) and 13 had PCV.

At baseline, the mean initial VA was 0.5 logMAR (20/63). According to our classification, 27% of patients had good VA, 48% had moderate VA and 25% had poor VA. The mean foveal thickness was 397 μm (range 264–559 μm).

Active CNV lesions in all eyes were treated with ranibizumab intravitreal injections. During the 12 months of follow-up, these varied from 4 to 9 injections (mean 6.2 injections). At the 12-month follow-up visit, the VA had significantly increased to 0.3 logMAR (20/40) ($p < 0.001$).

Table 1. Characteristics of the study population

Number of eyes	100
Mean age, years (mean ± SD)	79.2 ± 6.7
Gender (M/F)	70/30
Pre-treatment data	
VA (mean, logMAR/Snellen)	0.5 (20/63)
Central foveal thickness (mean, range)	397 (264–559)
Post-treatment data	
VA (mean, logMAR/Snellen)	0.3 (20/40)
Central foveal thickness (mean, range)	311 (240–416)
Intravitreal anti-VEGF injections (mean, range)	6.2 (4–9)

Moreover, on SD-OCT, a statistically significant ($p < 0.001$) reduction of subretinal fluid and reduction of cystoid macular edema ($p < 0.01$) were observed (mean foveal thickness: 311 μm and range 240–416 μm). While this was associated with some improvement in VA and was clinically evident in all cases, it did not always correlate with final VA ($p = 0.12$).

Intraretinal HRD were present in all cases. There were few HRD in 21% of eyes, moderate HRD in 36% and numerous HRD in 43% (table 2). In particular, there was a similar distribution of HRD in all active CNV, in both occult and classic lesions (respectively, few in 17.7 and 15.4% of eyes, moderate in 37.1 and 38.4% and numerous in 45.2 and 46.2%). A different distribution was found in CRA (few in 25% of eyes and numerous in 75% of eyes) and in PCV (few in 30.8% of eyes, moderate in 61.5% and numerous in 7.7%) (table 2).

The HRD were primarily detected in the outer retinal layers and near the retinal pigment epithelium layer. They were not only at the border of serous retinal detachment and cystoid spaces but also disseminated into all retinal layers (but less numerous), respectively, in 21.5 and 17.5% of eyes (fig. 1). After treatment, a significant reduction of subretinal fluid and cystoid spaces was evident in all cases, but this was not directly correlated with final VA ($p = 0.12$).

The HRD rapidly regressed in a few days. One month after the first injection, there was a statistically significant regression ($p < 0.04$) and an even more significant regression at the 3-month follow-up examination ($p < 0.01$) (fig. 2).

Considering the VA sub-groups, after treatment there was a statistically significant reduction of HRD in the good and moderate VA subgroups (respectively, $p < 0.01$ and $p < 0.02$). The persistence of HRD correlated significantly with the poor VA group ($p < 0.02$).

Table 2. Hyperreflective dots

	Few (<10)		Moderate		Numerous (>20)	
	Patients	%	Patients	%	Patients	%
Occult CNV	11	17.7	23	37.1	28	45.2
Pre-epithelial CNV	2	15.4	5	38.4	6	46.2
CRA	4	25	0	0	8	75
PCV	4	30.8	8	61.5	1	7.7
Total	21	21	36	36	43	43

Post-treatment regression of HRD was evident in all VA subsets and was statistically significant for occult CNV lesions. HRD resolution was associated with better final VA ($p < 0.001$).

Discussion

Using SD-OCT to examine patients with active exudative AMD, previously undetected intraretinal changes, termed HRD, can be visualized [1]. The HRD may also be present in different retinal diseases, such as retinal vein occlusion, diabetic macular edema or uveitis [11–17]. The HRD are scattered, punctiform, small in size, mainly located in the outer retinal layers and/or around pockets of fluid accumulation and are typically not confluent (fig. 1).

Using SD-OCT, we investigated the presence of HRD and its post-treatment evolution in 100 patients affected by exudative AMD. The presence of HRD was detected in all patients affected by active exudative AMD, primarily in the outer retinal layers, but could be scattered in all retinal layers, even in cases where the continuous external limiting membrane was still detectable and continuous.

Considering the number of HRD, classic and occult active CNV had a similar distribution. Less than the 55% of patients had few and/or moderate numbers of HRD. However, active PCV lesions had a different distribution: few and/or moderate numbers of HRD were detected in more than 92% of the patients.

After treatment, we demonstrated rapid and statistically significant resolution of HRD in a few days and at the 1-month and even more at the 3-month follow-up visit after anti-VEGF treatment. Considering the VA subgroups, there was a statistically significant reduction of HRD only in the good and moderate VA subgroups, but less regression in the poor VA group.

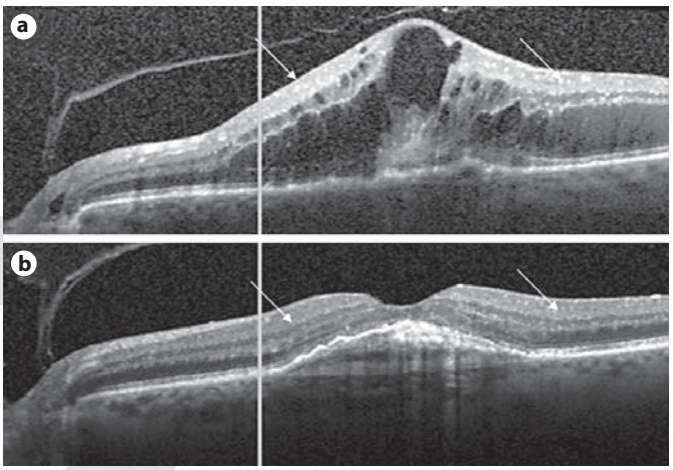

Fig. 2. SD-OCT examination showing the reduction of the HRD (arrows) numbers before (**a**) and after (**b**) 3 anti-VEGF injections in a patient (with a good response) with exudative AMD.

The HRD were the first change detectable at each clinical recurrence and the first features to disappear after treatment. This observation suggests that HRD could represent a clinical marker of an early inflammatory reaction.

Many different etiologies could be suggested about the possible nature of HRD. As there has been no human histopathological data until now, we can only refer to the different imaging modalities used during post-treatment follow-up. Blue-light fundus photography and autofluorescence images eliminate the possibility that HRD could be the focal accumulation of pigment or lipofuscin granules. These imaging techniques do not reveal any alteration supporting this origin, and this has also been agreed by other investigators [2–13]. Punctuate hemorrhages are usually larger in size and are located mainly in the inner retinal layers. Moreover, color- and green-light fundus

photography does not show any hemorrhage in the area corresponding to HRD.

We do not support the hypothesis that these HRD could be lipid exudates, even if this has been suggested in diabetic macular edema and retinal vein occlusion [13–16], since they are not visible in any monochromatic and angiographic photos.

Some authors have suggested that HRD may represent small intraretinal proteins or lipid deposits acting as a precursor of hard exudates diffused into the retinal layers after the breakdown of the blood-retinal barrier that is usually present mainly in patients affected by diabetic macula edema and retinal vein occlusion. These authors have suggested that the hyperreflective foci, initially isolated, may become confluent and similar to hard exudates [13].

In fact, hard exudates are confluent, more voluminous, deeper in the retina, near the retinal pigment epithelium layer and easily visible. They could regress after treatment, but very slowly, usually in a few months or at least many weeks after fluid reabsorption. This is different from the rapid and significant resolution of HRD seen a few days after anti-VEGF or anti-inflammatory treatment [11].

Moreover, the external limiting membrane, which corresponds to the adherent junctions between the Müller cells and photoreceptor cells, will restrict the migration of extravasated material and macromolecules into the outer retinal layers [18]. This concept is contrary to the evidence that these alterations are present in all retinal layers.

Although multiple mechanisms are likely to contribute to the onset and progression of AMD, its pathogenesis remains unclear [19]. The role of inflammation has been given much consideration [20], even if the nature of the immune cell interactions that drive cellular and tissue changes in AMD is still not fully understood [21].

It has been demonstrated that retinal microglia cells are the primary immune cell type in the retina [22] that can respond rapidly to tissue injury, which they do by altering their activation-state-acquiring capabilities of migration and proliferation and by secreting inflammatory mediators and neurotrophic agents [14, 21].

In the young healthy retina, microglia cells are found distributed only in the inner retinal layers [23] and not in the subretinal space and outer retina. It has been demonstrated that advanced age and disease can alter this normal distribution. In the older retina, microglia cells are found to be displaced into the outer retina and subretinal space, acquiring morphological and immunohistochemical features of activation [22]. The presence of displaced and activated microglia in the outer retina results in altered cellular interactions that help drive AMD pathogenesis and progression [21].

Therefore, we believe that HRD found in a high percentage of patients affected by exudative AMD may be inflammatory activated and swelled cells that spread in all retinal layers and not lipids or pre-exudates. The high-resolution images currently available with SD-OCT instruments may visualize these inflammatory cells and their rapid disappearance after anti-VEGF treatment.

In conclusion, these HRD very likely represent active and in vivo alteration of damaged retinal tissue. The limitation of the current studies is that it is not possible to have an exact correspondence between HRD and the histopathological examination of human retinal tissue. Otherwise, we believe that the location of HRD, the rapid response to the treatment and the growing role that early biological inflammatory reaction plays in the development of AMD suggest HRD are most likely activated microglia cells. Therefore, using SD-OCT to follow-up HRD (which are ophthalmoscopically invisible), will become an extremely useful tool in detecting early macular changes in exudative AMD.

Consequently, the presence or the recurrence of these HRD could be considered as a simple clinical marker to facilitate early decision-making for treatment and retreatment with anti-VEGF (and/or anti-inflammatory) drugs in exudative AMD.

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