HYPERREFLECTIVE INTRARETINAL SPOTS IN RADIATION MACULAR EDEMA ON SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY

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Purpose: To better pathophysiologically characterize macular edema secondary to eye irradiation, analyzing the presence of optical coherence tomography (OCT) hyperreflective spots.

Methods: Twenty-five consecutive eyes affected by radiation maculopathy, secondary to irradiation for a primary uveal melanoma, without macular involvement in the irradiation field, were consecutively enrolled. All subjects underwent full ophthalmologic examination, including fluorescein angiography, color fundus photography, and spectral domain OCT, even in en face modality. Optical coherence tomography central subfield thickness was stratified into the following 3 categories: <400 μm, 400 to 600 μm, and >600 μm. Spectral domain OCT images were analyzed to measure and localize hyperreflective spots by two independent masked graders.

Results: Hyperreflective spots were documented in all eyes (100%). Hyperreflective spots significantly increased in number according to OCT central subfield thickness (<400 μm, 400–600 μm, >600 μm, P < 0.05). The intergrader agreement was at least substantial for all measurements (intraclass correlation coefficient: 0.80).

Conclusion: Spectral domain OCT documents discrete intraretinal reflectivity changes (hyperreflective spots) in all (studied) eyes affected by radiation maculopathy. Hyperreflective spots increase in number with increasing central subfield thickness and could be considered as a new clinical biomarker of intraretinal inflammation in patients affected by macular edema secondary to irradiation for uveal melanoma.

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Radiation chorioretinopathy, also known as radiation retinopathy, is one of the major complications of eye irradiation, occurring more frequently after intraocular irradiation of primary intraocular tumors.1–3

Radiation chorioretinopathy is histopathologically characterized by signs of chorioretinal inflammation, progressive vascular occlusion, and vascular remodeling. This pathologic process is clinically characterized by the appearance of microaneurysms, telangiectasia, capillary nonperfusion, macular edema (ME), and hard exudates. Macular microvasculature is more vulnerable to radiation damage because of high capillary density of this part of the retina. Choroidal circulation is also directly compromised.4–8

Macular edema after plaque radiotherapy is associated with significant vision loss.1 Optical coherence tomography (OCT) proved to be useful to identify and grade radiation ME, since its early stages, and correlate it with visual acuity.1

Spectral domain OCT (SD-OCT) has been extensively used for the qualitative and quantitative evaluation of retinal changes in macular diseases.8,9 The presence of retinal hyperreflective spots (HRS) has been documented by OCT in major macular
diseases, such as age-related macular degeneration, diabetic retinopathy with or without ME, and retinal vein occlusion.\textsuperscript{8,12}

The nature of these spots is still under debate, but one of the most relevant hypotheses identifies them as an early marker of inflammation.\textsuperscript{8,11,13}

The aim of this study was to evaluate the presence of OCT HRS in eyes affected by radiation maculopathy, secondary to eye irradiation for intraocular tumors.

Material and Methods

This was an institutional, observational noninterventional study with consecutive enrollment compliant with the tenets of the Declaration of Helsinki. Patients were consecutively recruited from those referred between July 2013 and January 2014. Informed consent was obtained from each patient. Institutional review boards of our institutions approved the study protocol.

Inclusion criteria were patients affected by radiation maculopathy secondary to Iodine 125 brachytherapy for uveal melanoma. Exclusion criteria were direct macular involvement by the irradiation field, diabetes, uncontrolled arterial hypertension, previous ocular surgery (excluding brachytherapy) and previous treatments with any intraocular drug, refractive error >6 diopters (D), glaucoma and ocular hypertension, uveitis, retinal detachment (excluding uveal melanoma associate serous retinal detachment), significant media opacities that precluded fundus imaging, and other degenerative and/or vascular chorioretinal diseases not directly related to brachytherapy.

Twenty-five eyes of 25 consecutive patients were included. Each patient was previously treated with \textsuperscript{125}I brachytherapy according to the COMS guidelines (85 Gy at tumor apex with a dose rate of 0.60–1.05 Gy/hour).\textsuperscript{14}

Radiation-induced ME was defined as the occurrence of macular thickening involving the center of the fovea and secondary to eye irradiation.

All eyes underwent full ophthalmic examination including standard digital color fundus photography (Topcon TRC-50IX, 35°, Topcon Corp., Tokyo, Japan) centered onto the macula, fluorescein angiography (HRA2; Heidelberg Engineering, Heidelberg, Germany), and SD-OCT. Spectral domain OCT scans were acquired using Spectralis (Heidelberg Engineering; Spectralis Acquisition Software Version 5.7.4.0.). In each eye, a map centered on the macula using en face OCT by Spectralis was performed. Spectral domain OCT scans in this study were obtained covering a 6.0 × 6.0-mm area centered on the macula and consisting of 97 horizontal B-scan lines with 512 A-scans per line. B-scans were obtained at an interval of 60 μm between lines.

The presence of HRS was analyzed in the central single 180° line OCT scan of the map after segmentation (layering) of retinal layers. The en face complete reconstruction was used to exclude the presence of retinal artifacts, vessels, and other pathologic conditions in the areas involved by HRS. Hyperreflectivity changes from the inner limiting membrane to the inner plexiform layer (ILM–IPL), in the inner nuclear layer and outer plexiform layer (INL–OPL), and in the outer nuclear layer were analyzed. Hyperreflective spots were analyzed and quantified as previously reported.\textsuperscript{12} In brief, the automatic layering of the SD-OCT provided retinal segmentation, further controlled and refined manually. Red vertical lines were traced at 500 μm nasally and temporally to the center of the fovea.\textsuperscript{11} Hyperreflective spots were manually counted in the foveal area (between the 2 lines at 500 μm from the center of the fovea). Macular edema was stratified considering SD-OCT thickness in the central Early Treatment Diabetic Retinopathy Study field (<400 μm, 400–600 μm, and >600 μm), to have a homogeneous population.

Two investigators graded each eye, and intergrader agreement was measured. Graders were masked to radiation ME because they also measured HRS of eyes with ME of different origins.

The difference in the number of HRS was compared among the three groups. \textit{P} values for multiple comparisons were calculated using analysis of variance. The difference in the number of HRS was compared among groups by means of analysis of variance followed by Bonferroni post hoc test.

Results

Twenty-five eyes of 25 consecutive patients were included (mean age, 55.6 ± 10.8 years; range, 36–78). Fourteen patients were male (56%), and 11 were female (44%). The mean age of the different groups, according to OCT subfield thickness, was 56.4 ± 12.2 years in eyes with central subfield thickness (CST) <400 μm, 53 ± 10.4 years in eyes with CST between 400 and 600 μm, and 57.8 ± 10.7 years in eyes with CST >600 μm. There was no statistically significant difference in age among these groups. The right eye was affected in 10 patients (40%), and the left eye was affected in 15 patients (60%). Uveal melanoma location was ciliary body (28%) and purely choroid (72%). Mean tumor largest basal diameter was 13.6 ± 3 mm (range, 7.1–18 mm), and mean tumor thickness was 6 ± 1.9 mm (range, 3.2–9.8 mm). Mean dose rate and mean dose at tumor apex were 0.86 ± 0.14 Gy/hour and 88 ± 4 Gy (Table 1).
Spectral domain OCT scans analyzed with en face modality were compared with fundus photographs. This approach allowed to precisely locate HRS and confirmed that they were not corresponding to microaneurysms, lipids, or other physiological structures (Figure 1).

Hyperreflective spots were found in all studied eyes. Hyperreflective spots count (reported as mean number ± standard deviation) was as follows: in the ILM–IPL, 3.25 ± 1.67 in <400 µm ME eyes, 5.89 ± 2.89 in 400 to 600 µm ME eyes, and 9.25 ± 2.25 in >600 µm ME eyes; in the INL–OPL, 4.13 ± 1.73 in <400 µm ME eyes, 7 ± 4.30 in 400 to 600 µm ME eyes, and 11.38 ± 3.25 in >600 µm ME eyes; in the outer nuclear layer, 2.75 ± 1.28 in <400 µm ME eyes, 5.78 ± 2.68 in 400 to 600 µm ME eyes, and 7.88 ± 1.13 in >600 µm ME eyes (Table 2).

The number of HRS was significantly different among the three groups (analysis of variance, P < 0.01) (Table 2). In particular, the number of HRS was significantly higher in eyes with ME with CST >600 µm compared with the <400 µm CST group.

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Table 1. Clinical and Demographic Characteristics of Enrolled Patients

|                     | <400 µm Thickness Group | 400–600 µm Thickness Group | >600 µm Thickness Group | All Groups |
|---------------------|-------------------------|----------------------------|-------------------------|------------|
| Number of patients, N | 8                       | 9                          | 8                       | 25         |
| Age, mean (range), years | 56.4 ± 12.2 (36–78)     | 53 ± 10.4 (37–67)          | 57.8 ± 10.7 (56–72)     | 55.6 ± 10.8 (36–78) |
| Sex: male/female     | 4/4 (50%/50%)           | 3/6 (33.3%/66.7%)          | 7/1 (87.5%/12.5%)       | 14/11 (56%/44%)   |
| Affected eye: right/left | 5/3 (62.5%/37.5%)      | 4/5 (44.4%/55.6%)          | 3/5 (37.5%/62.5%)       | 10/15 (40%/60%)   |
| Tumor location: choroid/ciliary body | 5/3 (62.5%/37.5%) | 7/2 (77.8%/22.2%) | 6/2 (75%/25%) | 18/7 (72%/28%) |
| Tumor thickness (range), mm | 4.2 ± 1.8 (3.2–7)    | 5.9 ± 1.1 (4–7)            | 8 ± 1.1 (6–9.8)         | 6 ± 1.9 (3.2–9.8) |
| Tumor largest basal diameter (range), mm | 11 ± 2.8 (7.1–15) | 13.8 ± 2.4 (8.1–16) | 16.1 ± 1.5 (13.6–18) | 13.6 ± 3 (7.1–18) |

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Fig. 1. Radiation maculopathy: OCT and OCT en face images of the same patient focusing on two HRS at different retinal layers (OPL and ONL). The OCT en face images (A and C) document that the HRS (B and D) are not corresponding to vessels, microaneurysms, and lipids. ONL, outer nuclear layer.
both in the ILM–IPL (P = 0.0001) and INL–OPL (P = 0.0007), and with the 400 to 600 µm group (P = 0.0228) in the ILM–IPL (P = 0.0228) and INL–OPL (P = 0.0383). The number of HRS in the outer nuclear layer was significantly lower in eyes with CST <400 µm compared with other groups (P = 0.0095 and P < 0.0001, respectively) (Figure 2). The intergrader agreement was at least substantial for all measurements (intraclass correlation coefficient: 0.80); therefore, data from 1 examiner are reported.

The type of ME (cystoid, noncystoid, foveolar, and extrafoveolar),¹ the presence of exudation, macular ischemia by angiography, and optic neuropathy did not show statistical significant correlation with HRS.

### Discussion

Radiotherapy is the current standard of treatment for the management of uveal melanoma, the most common primary malignancy of the eye.¹⁴ This conservative approach is characterized by high local tumor control (>95%) and comparable overall survival compared with enucleation.¹⁴,¹⁵ One of the most important

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| Retinal Layer on OCT | Number of HRS per 1000 µm Foveal Area in CST Groups, number (SD) | P (ANOVA) |
|----------------------|-------------------------------------------------------------|------------|
|                      | <400 µm | 400–600 µm | >600 µm |
| ILM–IPL              | 3.25 (1.67) | 5.89 (2.89) | 9.25 (2.25) | 0.0002 |
| INL–OPL              | 4.13 (1.73) | 7 (4.30) | 11.38 (3.25) | 0.0010 |
| ONL                  | 2.75 (1.28) | 5.78 (2.68) | 7.88 (1.13) | <0.0001 |

ONL, outer nuclear layer; SD, standard deviation; ANOVA, analysis of variance.

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Fig. 2. Hyperreflective spots in different CST macular edema: the arrows are placed in correspondence of each HRS individualized by the grader. Hyperreflective spots appear to increase with increasing CST.
and visually disabling complications is radiation (chorio)retinopathy.\textsuperscript{3,5,16} The ischemic and edematous components of radiation chorioretinopathy have already been described in detail.\textsuperscript{4,5} Radiation maculopathy is characterized by ME and ischemia. Macular ischemia is commonly present when the macula is included or borders the irradiation field. Most of the current treatments for radiation maculopathy are substantially similar to those used in the management of other ischemic and/or edematous maculopathies, that is, retinal photocoagulation, intravitreal anti-vascular endothelial growth factor, and intravitreal corticosteroids. No treatment led to satisfactory long-term visual results.\textsuperscript{3,17–19} Recently, an inflammatory component in the pathophysiology of radiation chorioretinopathy has been suggested.\textsuperscript{20} Extracellular and intracellular stress due to a high dose irradiation induces persistent DNA damage leading to cell “senescence,” a condition of permanent cell-cycle arrest.\textsuperscript{20–23} Cellular senescence is the main mechanism of solid tumor regression, but most of the senescent cells remain active for several months and years, acquiring a “senescence-associated secretory phenotype,” activating a complex mechanism of autocrine/paracrine signaling, which leads to increased secretion of inflammatory cytokines.\textsuperscript{24,25} This process increases the communication between senescent cells and their microenvironment, promoting a local inflammatory response in which healthy cells are also involved.\textsuperscript{20,21,23}

In this study, we clinically looked for HRS in retinal layers in patients affected by radiation ME to support (or not) the theory of the role of inflammation in the pathogenesis of radiation ME and eventually visualize early intraretinal signs of radiation-related inflammation. Spectral domain OCT retinal layers analysis enabled us to document specific reflectivity changes that have already been found to have a relevant role in other macular diseases, such as age-related macular degeneration, edema secondary to branch vein occlusion, and diabetic retinopathy, namely the hyperreflective (intra)retinal spots.

Hyperreflective spots are punctiform, hyperreflective, small, and discrete elements typically not confluent. Hyperreflective spots may be considered as microaggregates of inflammatory activated and swollen microglia cells that spread from the inner retinal layers to the outer ones, as recently confirmed by Karlstetter et al.\textsuperscript{8,11,13,20,26}

We documented the presence of HRS in the inner retinal layers (from ILM to IPL), in the INL to OPL, and in the outer nuclear layer of all patients with radiation ME. Moreover, we documented that the number of HRS increases with increasing CST up to double in eyes with CST >600 μm compared with eyes with CST <400 μm. Hyperreflective spots proved to be located preferentially in the central retinal layers (INL–OPL), where cystoid spaces usually develop. Moreover, using the en face OCT modality, the exact location of the HRS was documented to exclude artifacts, hard exudates, microaneurysms, or other physiological structures such as vessels.

We have not considered the photoreceptor layer in our analysis because of the specific nature of this layer and the morphologic abnormalities progressively observed with increasing radiation maculopathy. In fact, even if the whole retina is involved by important morphologic alteration in the radiation damage, degeneration and disorganization of photoreceptors and fragmentation of the external limiting membrane as well as loss of junction integrity could prevent clear detection and differentiation of HRS creating a confounding factor.\textsuperscript{27,28} In diabetic ME, a reduction of HRS has been observed after treatment, with a stronger decrease in eyes showing a complete edema resolution.\textsuperscript{29} In long-lasting exudative age-related macular degeneration, it has been confirmed that HRS were the first detectable sign of ME at recurrence, and the first to disappear after treatment.\textsuperscript{30}

In diabetes, HRS occurred in all retinal layers as observed in radiation maculopathy, and they do not tend to become confluent.\textsuperscript{8,29} Coscas et al\textsuperscript{8} have already correlated these features in exudative age-related macular degeneration with retinal microglia cells. Retinal microglia cells are the primary resident retinal immune cells, which rapidly respond to metabolic stress with migration, accumulation, and activation.\textsuperscript{13} Microglia activated cells, spreading from inner retinal layers to the outer ones, can ultimately induce alterations of the retinal pigment epithelium resulting in further inflammation, increasing both vascular permeability and neuronal damage.\textsuperscript{26,30,31} This hypothesis on HRS nature seems to be confirmed by data obtained in diabetic retinopathy and could explain the behavior of HRS in the present population.\textsuperscript{11} A limitation of this study is the impossibility of a histologic analysis, but the en face OCT may be an effective method to exclude possible confounding lesions.

Further confirmation is derived from previous data, which document that corticosteroids are probably better than anti-vascular endothelial growth factor to control side effects of intraocular irradiation, because radiation damage seems to be mainly driven by chronic tissue inflammation.\textsuperscript{32} We also observed a significant decrease of both radiation-induced ME and HRS number after intravitreal dexamethasone implant (unpublished data).

The clinical phenotype of radiation maculopathy may differ substantially among patients, both in its clinical and angiographic aspect.\textsuperscript{33} The limited number of patients included in this study did not allow us to identify
different subgroups of patients by specific clinical or angiographic aspects (type of ME, presence of exudation, presence of macular ischemia by angiography, presence of optic neuropathy) showing statistically significant correlation with HRS. Nevertheless, larger studies may further address the correlation between HRS and these clinical and angiographic features.

In conclusion, considering the growing importance of inflammation in the pathophysiology of radiation ME, HRS, documented by SD-OCT, might be considered a novel, in vivo noninvasive biomarker to better qualify and monitor retinal inflammation in radiation-induced choriotinal damages.

Key words: brachytherapy, hyperreflective intraretinal spots, melanoma, OCT, radiation macular edema, senescence.

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