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Hyperreflective Dots on OCT as a Predictor of Treatment Outcome in Diabetic Macular Edema

A Systematic Review

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Topic: This review aims to evaluate the role of hyperreflective dots (HRDs), detected using OCT, as a predictor of the treatment outcome in patients with diabetic macular edema (DME).

Clinical Relevance: The treatment of DME is possible, but its results are often unsatisfactory. Thus, it is important to develop biomarkers that can help to predict the treatment response to optimize the treatment's effect for individual patients.

Methods: PubMed, Embase, Web of science, and Cochrane library were searched (final search date on May 5, 2021). Participants were patients diagnosed with DME and provided with treatment. The predictor was HRDs, detected using OCT, before treatment. The outcomes were best-corrected visual acuity (BCVA) and central macular thickness (CMT), detected using OCT, after treatment. Two reviewers independently screened the titles and abstracts as well as full text. The refined Quality in Prognosis Studies tool was used to assess the risk of bias for each included study. Because of the clinical heterogeneity of the studies, a meta-analysis was not performed.

Results: Thirty-six studies were included. The Quality in Prognosis Studies assessment showed that most studies had a low or moderate risk of bias in 6 domains. Six studies could not find any correlation between baseline HRDs (either the presence or absence of HRDs [n = 1] or baseline HRD number [n = 5]) and outcome (BCVA or CMT), whereas 12 studies found a significant correlation between these variables. Eight studies reported that baseline HRDs could predict a poor visual outcome (n = 4 on prescence or abscence of HRD and n = 4 on HRD number), and 4 studies (n = 1 on prescence or abscence of HRD and n = 3 on HRD number) found that HRDs were predictive of visual improvement. Fifteen out of 17 studies found that the HRD number decreased after treatment.

Conclusion: Based on the current literature, the HRD numbers decrease with treatment, but it is not clear whether HRDs predict the treatment outcome in patients with DME. Future investigations with more uniform approaches are needed to confirm the nature of this biomarker and its effect on DME treatment outcome. *Ophthalmology Retina 2022;6:814-827 © 2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material is available at www.ophthalmologyretina.org.

Diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus.[1] It is also the leading cause of blindness in the working-age population globally.[2] About 1 in 10 diabetic patients have visual impairment due to DR,[2] and one of the major causes is diabetic macular edema (DME).[3] Diabetic macular edema is characterized by the swelling of the central retina, which can be detected as increased retinal thickness using OCT.[4] The treatment of DME is possible, but its results are often unsatisfactory, and it is difficult to predict which patient benefits from treatment and which does not.

Currently, the first-line treatment of DME is the application of anti-VEGF agents.[5–7] Second-line treatments, such as intravitreal steroid injections or implants, macular laser, and vitrectomy, are available for refractory cases.[6,7] However, their treatment outcomes are unsatisfactory. Only 3 in 10 patients have improvement of visual acuity 1 year after at least 3 consecutive monthly anti-VEGF injections.[5] It is not yet possible to predict whether a given patient will respond to therapy. Thus, it is important to develop biomarkers that can help to predict the treatment response to optimize the treatment's effect for individual patients.

Hyperreflective dots (HRDs), detected using OCT, generate small signals with high reflectivity compared with the background image and are distributed over all retinal layers.[8] Bolz et al[9] first reported the presence of HRDs using OCT in patients with DME more than a decade ago, but the

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origin of this candidate biomarker of DME is still unknown. Several hypotheses of HRDs have been proposed, including activated microglia, a precursor of hard exudates or lipoprotein, and migration of retinal pigment epithelium (RPE) cells.[10,11] Hyperreflective dots have been correlated with treatment response in patients with DME.[10,12] However, previous studies have shown controversial results regarding the correlation between baseline HRDs and final visual outcomes.[10,12,13]

The primary aim of this review was to evaluate whether HRDs assessed before treatment can serve as a biomarker for predicting the treatment outcome in patients with DME. The secondary aim was to evaluate whether the number of baseline HRDs is influenced by treatment. For this purpose, we searched various electronic databases and conducted this systematic review.

Methods

Eligibility Criteria for Considering Studies for this Review

Types of Studies. We included both retrospective and prospective studies that evaluated the role of HRDs, detected using OCT, as a predictor of treatment outcome in patients with DME.

Participants. The participants were patients diagnosed with DME and provided with treatment. All kinds of treatments were included, such as anti-VEGF agents, steroid injections or implants, macular laser, vitrectomy, and tight glucose control.

Predictor. The predictor was the presence or number of HRDs, detected using OCT. Different names were available in the literature, including "hyperreflective dots," "hyperreflective foci," "hyperreflective spots," and "hyperreflective material." All related names were accepted for the review. Both binary (the presence or absence of HRDs) and continuous (the number of HRDs) evaluations of HRDs were included. Hyperreflective dots are defined as non-hard-exudate signals with high reflectivity on OCT images and are spread over all retinal layers. Hard exudates share some OCT features with HRDs. To avoid bias, studies describing the definition of HRDs with the inclusion of hard exudates were excluded. We included studies that did not provide a definition of HRDs, but we performed a separate analysis including only studies with a clear definition, which described at least the characteristics of size and reflectivity (referred to as studies with "clear definitions").

Outcome. The primary outcomes were best-corrected visual acuity (BCVA) and central macular thickness (CMT), detected using OCT, after treatment. Best-corrected visual acuity can be measured using different methods, such as a Snellen chart and ETDRS letters. Based on the current clinical guidelines,[6,7] these 2 parameters are the primary indicators of diagnosis and treatment in patients with DME. All kinds of BCVA and CMT measurements were acceptable. Studies with either BCVA or CMT as an outcome measure were included. As a secondary outcome, we evaluated the change in the number of HRDs related to treatment. No restriction in follow-up time was set for the outcome measures so as to provide as complete an overview as possible of this research question.

Search Methods for Identifying Studies

We searched 4 databases, including PubMed, Embase, Web of Science, and Cochrane library. We did not use any date restrictions. The language was restricted to English at the stage of full-text screen. The keywords we searched were "diabetic macular edema" and "hyperreflective." We first also included "optical coherence tomography" as a major keyword, but we found that adding this keyword would exclude potential studies that did not mention OCT in their title or abstract. The same applied to keywords for visual outcome, i.e., "visual acuity" and "central macular thickness." The details of the search strategy and the search results of each database can be found in Appendix S1 (available at www.ophthalmologyretina.org). The protocol of this review was published on the international prospective register of systematic reviews (PROSPERO, registration number, CRD42021223250).

The first date of the search was December 7, 2020. After we finished data synthesis, we performed the final search on May 5, 2021.

Institutional review board approval was not applicable because this is a review of the literature. This study adhered to the Declaration of Helsinki.

Data Collection and Analysis

All assessments (selection based on the title or abstract, full-text screen, data extraction, and risk-of-bias assessment) were conducted by 2 indenpendent reviewers (H.H. and L.L.), and disagreements were resolved through a discussion between them. Manuscripts were first selected based on their title and abstract. Studies that were selected by $\geq$1 reviewer were subjected to a full-text screen. The reference lists of the included articles were extracted, and the titles and abstracts were screened for eligibility. Figure 1 shows the flowchart of the search and screening process. The following data were extracted:

1. Study design: Prospective or retrospective study.
2. Study population: Definition of DME, treatment history before inclusion (treatment-naïve, refractory, or non-specified DME cases), and sample size (the number of patients and eyes).
3. Treatment: Anti-VEGF agents, steroid implants, steroid injections, macular laser, vitrectomy, or tight glucose control, and the details of treatment, consisting of the number of injections, follow-up time, and treatment protocol.
4. Predictor (HRDs): The type of OCT device, definition of HRDs (the size of dots, reflectivity, and the absence of back shadowing), and HRD calculation methods (the number of OCT B scans used, the diameter of the scan used for calculations, the number of graders, and masked evaluation).
5. Outcome measures: Primary outcomes (final BCVA and CMT after treatment) and secondary outcomes (baseline, follow-up, and final HRD number).
6. Statistical analysis: The correlation or regression analysis of HRDs with treatment outcome; HRD variables included the presence or absence of HRDs at baseline or the baseline HRD number.
7. Conflict of interest statement.

Assessment of Methodological Quality

The risk of bias of the included studies was evaluated using the refined Quality in Prognosis Studies tool.[14] The Quality in Prognosis Studies tool consists of 6 domains that list several assessed items, and each domain is labeled as "high risk," "moderate risk," or "low risk." The domains and specification with regard to our study are described below. More detailed information on each item and examples of assessment can be found in Appendix S2 (available at www.ophthalmologyretina.org).

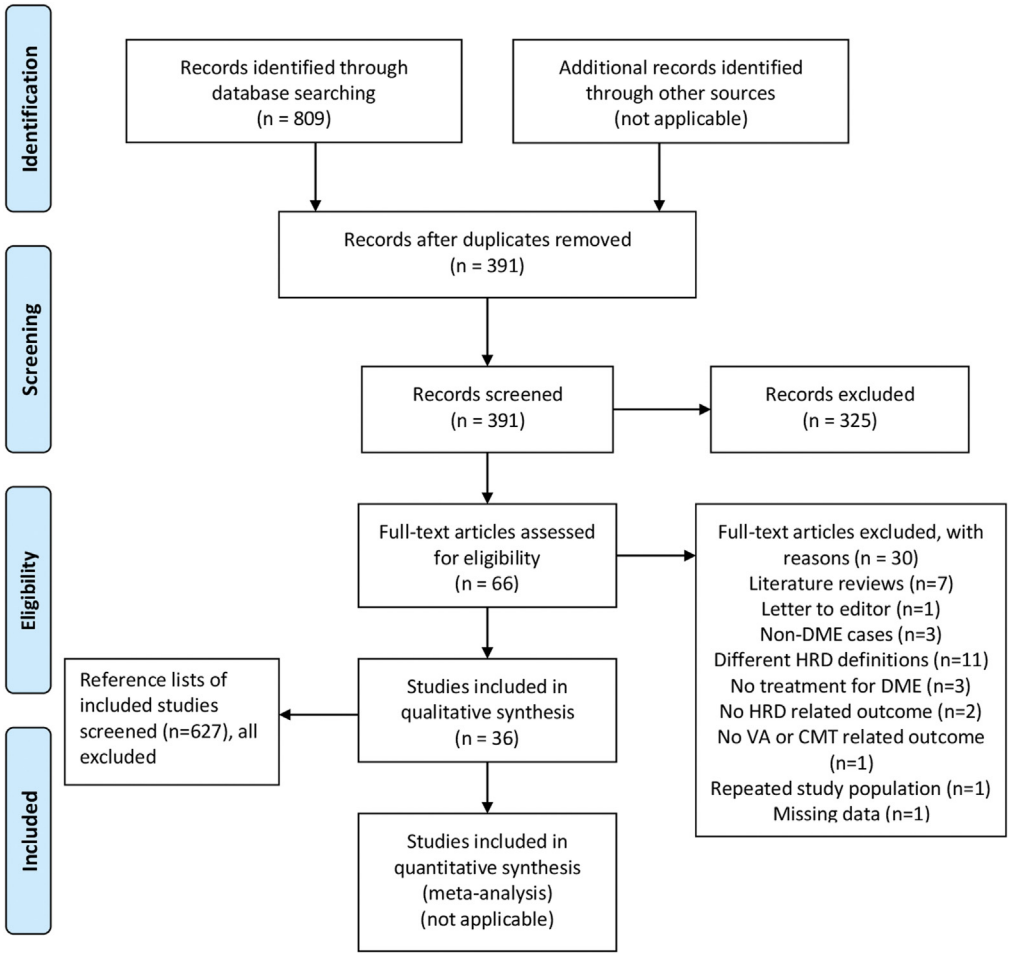

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 flow diagram of the screening process. CMT = central macular thickness; DME = diabetic macular edema; HRD = hyperreflective dot; VA = visual acuity. Included studies are displayed in Appendix S3.

1. Study participation: Generally, studies that provided a definition of DME with a CMT range and described the period and place of recruitment were scored as "low risk." If either of them were mentioned, the studies were scored as "moderate risk." If neither of the items were described, the studies were scored as "high risk." Other items were also evaluated during the assessment process, including referral patterns, power calculation, inclusion and exclusion criteria, and baseline characteristics (age, sex, hemoglobin A1C level, and the duration of diabetes).
2. Study attrition: If the response rate was adequate and the reasons for loss to follow-up were well described, the studies were scored as "low risk." Retrospective studies that did not mention any of the listed items were scored as "moderate risk"; prospective studies that did not mention any of the items were scored as "high risk." If some of the items were mentioned, we assessed the risk of bias accordingly.
3. Prognostic factor measurement: Studies that provided clear definitions of HRDs and well-described methods of HRD calculation were scored as "low risk." If 1 aspect was mentioned, the studies were scored as "moderate risk." If none of them were described, the studies were scored as "high risk."
4. Outcome measurement: BCVA and CMT measurements were taken into account in this domain. If the same method was applied to all participants, the study was scored as "low risk." If the methods of either BCVA or CMT measurement differed between the participants, such as the use of different OCT devices for CMT measurements, the study was scored as "moderate risk." Studies that used different methods for both BCVA and CMT were scored as "high risk." Other items, such as a clearly defined duration of follow-up, were also taken into account.
5. Study confounding: We considered prior DME treatment, glycemic control, and phakic status as 3 potentially confounding factors. Studies that considered all of them were scored as "low risk." Studies that did not mention any of these factors were scored as "high risk." Studies in between were scored as "moderate risk."
6. Statistical analysis and reporting: Studies with unilateral eye inclusion or correction for bilateral eye inclusion and those using parametric statistics with normality check were scored as "low risk." Studies that mentioned either of these aspects were scored as "moderate risk." If neither of them were reported, the studies were scored as "high risk." Other items, such as correct statistical model building and

Figure 2. Results of risk-of-bias assessment. TVST = Translational Vision Science and Technology. Included studies are displayed in Appendix S3.

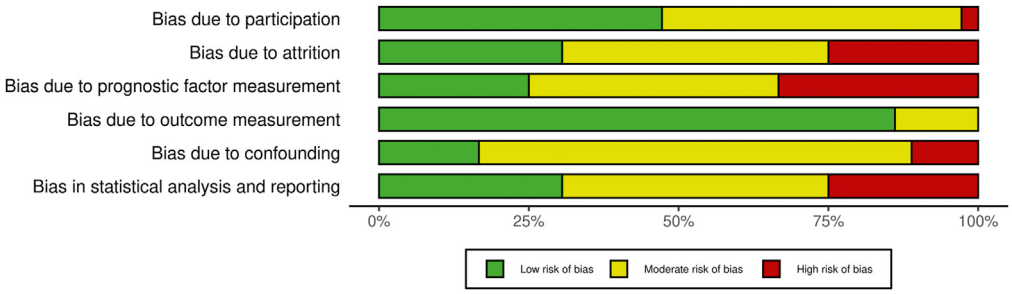

Figure 3. Summary of risk-of-bias assessment.

selective reporting of results, were considered depending on how the results were analyzed and reported.

Statistical Analysis and Data Synthesis

Endnote (version 20; Clarivate) was used to import references from each database and remove duplicates. The screening process (title and abstract screen and full-text screen) was also performed using Endnote. The SPSS tool (version 23; IBM Software) was used to calculate the Cohen kappa of the title and abstract screen and the full-text screen between the 2 reviewers. The robvis R package and Shiny web app[15] was used to produce risk-of-bias visualization results. Because of the clinical and methodological heterogeneity of the studies, we did not conduct a meta-analysis.

Results

Search Results

The electronic search of the 4 databases yielded 809 references. After the removal of duplicates, 391 references remained for the screening of titles and abstracts. Three hundred and twenty-five references were excluded because of the following reasons: 275 studies were not relevant to the current study, 46 were conference abstracts, 1 clinical trial did not have published results, and 3 papers were not written in English. The Cohen kappa of the title and abstract screen between the 2 reviewers was 0.77. Two reviewers independently screened the remaining 66 full-text articles for eligibility. Eleven studies that used arbitrary definitions of HRDs with possible indications of hard exudates were not selected for this review. Three studies that did not provide treatment to patients with DME were also excluded. Sixteen other studies were excluded; the reasons for exclusion are summarized in Figure 1. The Cohen kappa of the full-text screen between the 2 reviewers was 0.76. Finally, 36 articles were included in this review (Appendix S3, available at www.ophthalmologyretina.org). The reference lists of the 36 included articles were also screened. These 627 references did not provide additional studies that met the inclusion criteria.

Methodological Quality of Included Studies

Figure 2 shows the results of risk-of-bias assessment for all included studies. Figure 3 presents a summary of the 6 domains of the Quality in Prognosis Studies tool. Most of the included studies had a low or moderate risk of bias in the 6 domains. The risk of bias due to participation was low in 17 studies (47%), moderate in 18 studies (50%), and high in 1 study (3%). In the study attrition and prognostic factor measurement domains, each grade of assessment applied to about one third of all studies. Detailed information about the prognostic factor, i.e., definitions and calculations of HRDs, is given in the following section. Most studies (31 studies, 86%) had a low risk of bias due to outcome measurement, which indicated clear and reliable methods of BCVA and CMT evaluations. Only 6 articles had a low risk of bias due to confounding. Most of the articles (26 studies, 72%) had a moderate risk of bias, and 4 articles had a high risk of bias. Eleven studies were scored as having a low risk of bias due to their statistical analysis and reporting, 16 had a moderate risk of bias, and 9 had a high risk of bias in this domain.

Description of Studies

Among the 36 studies, 11 were prospective and 25 were retrospective. Fifteen studies had investigated treatment-naïve patients with DME, 6 studies were focused on refractory DME cases that did not respond to anti-VEGF agents, and another 15 articles did not further specify the treatment history of patients with DME. There were 21 articles providing definitions of DME with CMT values, detected using OCT (Table S1, available at www.ophthalmologyretina.org), which varied from a CMT of $>$ 250 μm to a CMT of $>$ 320 μm. The most common definition of DME was a CMT of $>$ 300 μm (13 studies used this definition). Another 15 articles did not provide a clear OCT definition of DME.

Fifteen studies included patients with DME treated with anti-VEGF agents, 12 studies included those treated with steroid implants (in 1 study, fluocinolone acetonide was used, and in all others, dexamethasone was used), and another 5 studies compared patients with DME treated with anti-VEGF agents with those treated with dexamethasone implants. One study investigated patients treated with macular laser, and the remaining 3 studies evaluated patients with DME with various treatments (Table S1).

Twenty-one studies provided definitions of HRDs (Table 1), whereas the other 15 did not. Among studies with a definition of HRDs, 17 provided size limits, 15 mentioned the reflectivity of HRDs, and 7 mentioned the absence of back shadowing. The most commonly defined sizes of HRDs were a diameter of $<$30 μm (7 studies) and a diameter between 20 and 40 μm (5 studies). Eight studies specified that the reflectivity was similar to that of RPE, 5 studies specified that it was similar to that of the nerve fiber layer, and 2 studies mentioned higher reflectivity than that of the background. Only 6 studies provided a definition of HRDs based on all 3 characteristics, including the size, reflectivity, and absence of back shadowing. For studies that did not provide an HRD definition in their methods section, some

Table 1. Summary of Definitions of HRDs

Study	Size (μm)	Reflectivity	Absence of Back Shadow
Bonfiglio 2019	≤30	Similar to the retinal nerve fiber layer	Absence of back shadowing and absence of vessels or any other lesion on en face image
Busch 2020	<30	Similar to the retinal nerve fiber layer	Presenting without back shadowing
Cavalleri 2020	<30	Similar to the retinal nerve fiber layer	Absence of back shadowing
Ceravolo 2020	<30	Similar to the retinal nerve fiber layer	Absence of back shadowing
Chen 2020	<50	Similar or higher than the RPE band	Absence or minimal back shadowing
Choovuthayakorn 2021	<30	-	No back shadowing
Fonollosa 2019	≤40	Higher than the background	-
Huang 2021	20–40	-	-
Hwang 2017	20–40	Equal or higher than the RPE band	-
Kang 2016	20–40	Equal or higher than the RPE band	-
Kim 2019	20–40	Equal or higher than the RPE band	
Lee 2018	20–50	Similar to the RPE band	
Liu 2019	-	Equal or higher than the RPE band	-
Narnaware 2020	20–40	Equireflective to RPE	-
Park 2019	20–50	-	-
Pessoa 2021	<30	Similar to the retinal nerve fiber layer	No back shadowing
Rübsam et al,[13] 2021	≤30	Identical to the RPE band	-
Schreur 2018	≤100	Higher than the background	-
Chatziralli 2016	The presence of HFs was defined as the presence of small focal, hyperreflective material scattered in all retinal layers, observed in ≥1 scan.		
Framme 2012	The number of HFs—which are HFs of round or oval shape and of different sizes—within the parafoveal area was subjectively determined in all patients by grading them into 3 stages (A = few, representing 2–10 HFs; B = moderate, representing 11–20 HFs; C = many, representing ≥21 HFs).		
Vujosevic 2016	A manual count of hyperreflective spots—defined as small, punctiform discrete white lesions—was performed between the 2 markers.		

HF = hyperreflective focus; HRDs = hyperreflective dots; RPE = retinal pigment epithelium. Included studies are displayed in Appendix S3.

descriptions of HRD were available in other parts of the manuscript. These are summarized in Appendix S4 (available at www.ophthalmologyretina.org).

Most studies (32 studies) mentioned HRD calculation methods but in different ways (Table S2, available at www.ophthalmologyretina.org). Nonautomated manual calculations were used in all the studies. Twelve studies used 1 B scan per eye, 3 studies used 3 B scans per eye, 3 studies used 6 B scans per eye, and another 4 studies used 5, 7, 25, and 49 B scans each. Ten studies did not mention the number of B scans used for calculation. The diameter of the scan ranged from 1 to 6 mm and was reported in 25 studies. The most common diameter was 3 mm centered on the fovea (12 studies used this area), which corresponds to the diameter of the middle circle of the ETDRS grid. Eighteen articles had 2 graders to calculate HRDs, and 11 of these mentioned masked evaluation. Another 3 articles had 1 masked grader to calculate HRDs. Two articles had 3 or 4 masked graders, but each person evaluated a separate proportion of OCT scans. Other articles (13 studies) did not provide any grader or masking information. Most articles (33 studies) used 1 type of OCT device to evaluate their patients with DME, whereas 3 articles used different OCT devices within their study (Table S2).

Twelve papers calculated HRDs in different OCT layers. Most of them separated the retina into outer and inner retinal layers. The cutoff points differed in these studies. Five studies took the external limiting membrane–RPE as the outer retinal layer and the internal limiting membrane–outer nuclear layer as the inner retinal layer. Three articles took the outer plexiform layer–external limiting membrane as the outer retinal layer and the internal limiting membrane–inner nuclear layer as the inner retinal layer. Two articles used an extra layer of subretinal fluid for HRD calculation, 1 article used an extra layer under the RPE, and 1 article separated the retina into 3 layers that were different from the abovementioned definitions.

Most studies disclosed conflicts of interest in their article, but 2 studies did not give relevant information on this aspect (Appendix S5, available at www.ophthalmologyretina.org). Eleven articles reported competing interests, and 23 papers reported no conflict of interest.

Findings

Six studies investigated whether the presence of HRDs at baseline can predict the visual outcome (BCVA or CMT) in patients with DME after treatment (Table 2). In total, 1030 eyes were included, and the follow-up duration ranged from 4 months to 2 years. The results were inconsistent among those studies. One study found no statistically significant correlation between the presence of HRDs at baseline and final visual outcome, whereas 5 studies found a statistically significant correlation, for either BCVA or CMT. Of these 5 studies, 4 found that the presence of HRDs could predict a poor visual outcome, whereas 1 study found that the presence of HRDs was predictive of BCVA improvement.

Twelve other studies evaluated the correlation between the baseline HRD number and final BCVA or CMT (Table 3). Five studies found no significant correlation between the baseline HRD number and final visual outcome, whereas 7 studies found significant correlations. Three out of the 7 studies showed that the baseline HRD number could predict visual improvement; the

Table 2. Prediction Model of Presence vs. Absence of HRDs and Final Visual Outcome*

Studies	No. of Patients/Eyes	DME Type	Treatment	Follow-up Time (mo)	Prediction Outcome	Statistical Analysis
Zur 2018	284/299	Unspecified	DEX × 1	4	VA	Generalized estimating equation
Chatziralli 2017	54/54	Refractory	DEX × 1 + PRN	12	VA	GLS linear regression
Choovuthayakorn 2021	173/226	Unspecified	Anti-VEGF × 3 + PRN	12	VA	Multivariable linear regression
Venkatesh 2021	101/123	Treatment-naïve	Variable	≥ 12	VA†	Univariate and multivariate linear regression
Chatzirallis 2020	112/112	Treatment-naïve	Anti-VEGF × 3 + PRN	18	VA	GLS linear regression
Chen 2020‡	142/216	Treatment-naïve	Anti-VEGF × 1 + PRN	24	CMT	Generalized estimating equation

CMT = central macular thickness; DEX = dexamethasone implant; DME = diabetic macular edema; GLS = generalized least squares; HRDs = hyperreflective dots; PRN = pro re nata; VA = visual acuity.
*The gray arrow represents insignificant correlation between the presence of HRDs and final visual outcome. The red arrows indicate that the presence of HRDs predicts poorer visual outcome, and the green arrows indicate that the presence of HRDs predicts visual improvement. The length of the arrow represents the follow-up time.
†The prediction outcome of the study by Venkatesh 2021 showed that the presence of HRDs was significantly correlated with final VA in univariate but not in multivariate linear regression analysis.
‡Represents articles with clear definitions of HRDs. Included studies are displayed in Appendix S3.

remaining 4 studies found that the baseline HRD number was predictive of a poor visual outcome.

Among studies with clear definitions of HRD, 6 studies investigated the correlation between HRDs at baseline and treatment outcome (Tables 2 and 3). One study showed that the presence of HRDs can predict a poor visual outcome. Five studies evaluated whether the baseline HRD number can predict the final visual outcome (Table 3). Two studies found insignificant correlations between these 2 parameters, 2 studies found that the baseline HRD number can predict a poor visual outcome, and 1 study found that it can predict CMT improvement but not BCVA improvement.

There were 17 studies, including 24 groups of patients, comparing BCVA, CMT, and the HRD number before and after treatment (Fig 4A, Table 4). Eight studies (11 groups) included treatment-naïve patients with DME, 4 studies (5 groups) included patients with refractory DME, and the other 5 studies (8 groups) did not specify prior treatment history. Of the 24 groups, 22 showed a statistically significant decrease in the HRD number after treatment, and 2 groups found an insignificant change after treatment. Seventeen groups showed improved BCVA after treatment, 3 groups showed a statistically insignificant change in BCVA after treatment, and 4 groups did not report statistical results in terms of BCVA. Twenty groups showed decreased CMT after treatment, 2 groups had an insignificant change, and 2 groups had no statistical report regarding CMT change. The follow-up time ranged from 1 to 12 months. The treatment included anti-VEGF injections (13 studies), steroid implants (10 studies), and macular laser (1 study). Most studies showed a consistent change in terms of reduction in the HRD number and the improvement of visual acuity (15 out of 18 studies) and CMT (17 out of 21 studies, Table 4).

Similar results were found in studies with clear definitions of HRDs. Nine studies, including 15 groups of patients, evaluated the change in HRDs before and after treatment (Fig 4B, Table 4). Thirteen groups found a significant decrease in the HRD number after treatment, whereas 2 groups found an insignificant change.

Table 3. Prediction Model of HRD Number and Final Visual Outcome*

Studies	No. of Patients/Eyes	DME Type	Treatment	Follow-up Times (mo)	Prediction Outcome	Statistical Analysis
Framme 2012	51/51	Treatment-naïve	Anti-VEGF × 1	1	VA/CMT change	Spearman correlation
Rübsam et al,[13] 2021†	51/59	Unspecified	Anti-VEGF or DEX × 1	1	VA	Pearson correlation
Vujosevic 2017	49/49	Treatment-naïve	DEX × 1	1	CMT	Pearson correlation
Ceravolo 2020†	156/156	Treatment-naïve	Anti-VEGF ×3 or DEX × 1	3/2	VA >10 letters	Multiple regression
Liu 2019	13/26	Unspecified	Anti-VEGF × 3	3	VA	Pearson correlation
Schreur 2018†	41/54	Treatment-naïve	Anti-VEGF × 3	3	CMT/VA‡	Multivariable linear mixed model
Narnaware 2020†	24/27	Unspecified	DEX × 1	4	VA/CMT	Pearson correlation
Kang 2016†	80/97	Unspecified	Anti-VEGF × 1 + PRN	6	VA	Multivariate linear regression
Vujosevic 2016	20/20	Treatment-naïve	Anti-VEGF × 3 + PRN	6	VA/CMT	Spearman correlation
Meduri 2021	39/39	Treatment-naïve	DEX × 1	12	VA >10 letters	Logistic regression
Huang 2021	106/106	Unspecified	Anti-VEGF × 2–3 + PRN	12	VA/CMT	Linear regression
Yoshitake 2020	71/77	Unspecified	Anti-VEGF × 3 + PRN	12	VA	Multivariate analysis

CMT = central macular thickness; DEX = dexamethasone implant; DME = diabetic macular edema; HRD = hyperreflective dot; PRN = pro re nata; VA = visual acuity.

*The gray arrows represent insignificant correlation between the HRD number and final visual outcome. The red arrows indicate that HRD number predicts poorer visual outcome, and the green arrows indicate that HRD number predicts visual improvement. The length of the arrow represents the follow-up time.

†Represents articles with clear definitions of HRDs.

‡The regression outcome of Schreur 2018 showed that the baseline HRD number is significantly correlated with CMT improvement but insignificantly correlated with VA. Included studies are displayed in Appendix S3.

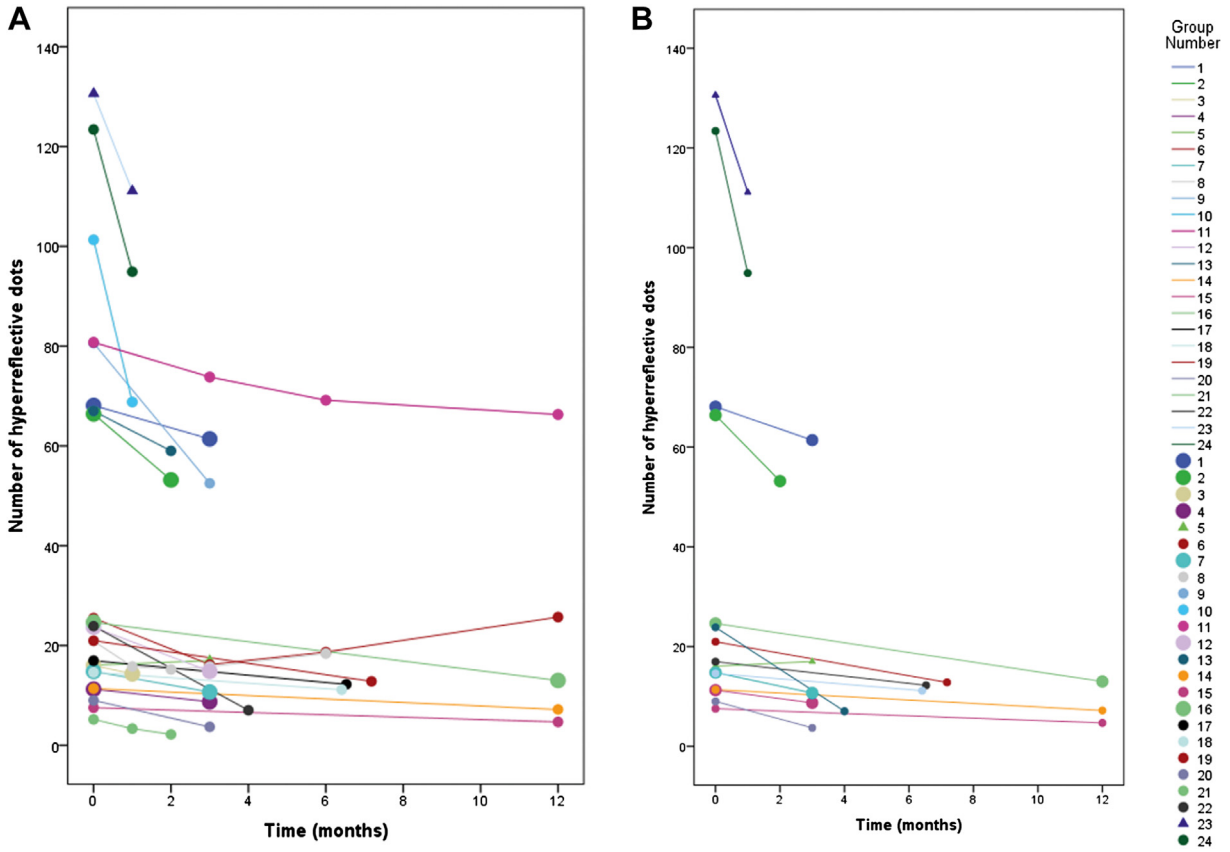

Figure 4. Decrease in the number of hyperreflective dots (HRDs) after treatment. Baseline (time 0), follow-up, and final HRD number from (**A**) 24 groups (17 studies) and (**B**) 15 groups (9 studies) with clear definitions of HRDs. Each color represents one group. The dots and triangles represent time points during follow-up. The triangles indicate an insignificant change in the HRD number after treatment (2 groups), and the dots represent a significant reduction thereof (22 groups). The larger dots refer to groups including at least ≥40 eyes, and the smaller dots refer to groups including <40 eyes. See Table 4. Included studies are displayed in Appendix S3.

Eight studies evaluated the change in the HRD number in the outer and inner retinal layers before and after treatment, and most studies found that the HRD number decreased in both layers. Only 1 study found an insignificant change in the outer retina and a significant change in the inner retina, but the authors did not clarify where the separation between the inner and outer retina was located.

Five studies compared the change in the HRD number in patients with DME treated with anti-VEGF agents with that in patients with DME treated with dexamethasone implants (Table 5). Three studies found a greater reduction in the HRD number in the dexamethasone group than in the anti-VEGF group, whereas the other 2 studies did not find a significant difference in HRD reduction between the 2 treatment groups.

We also evaluated whether a change in the HRD number preceded a change in BCVA or CMT. Of the 5 articles that provided 1-month follow-up data, including HRD number, only 2 articles also provided data on 3- or 6-month follow-up (Table S3, available at www.ophthalmologyretina.org). In this limited amount of data, none of the parameters consistently changed before any of the others. Similar findings were found in terms of changes in the HRD number, BCVA, and CMT 3 months after treatment (Table S4, available at www.ophthalmologyretina.org).

Discussion

Inconsistent results are found when the presence of HRDs or the number of HRDs at baseline is used as a biomarker for treatment response and final outcome (BCVA or CMT) in patients with DME. Therefore, the potential role of HRDs as a biomarker predicting treatment response in patients with DME is unclear. A consistent result is that the HRD number significantly decreases after treatment.

The predictive role of HRDs was controversial among the included studies even after we set stricter criteria of HRD definitions. The 3 studies that found that HRD presence or number can predict BCVA improvement did not specify prior treatment history, other than stating that the patients did not receive any DME treatment in the 3 to 6 months before inclusion. This aspect might have been a major source of bias because a consistent finding among the studies was that treatment reduces HRD numbers and is mostly accompanied by improved vision and CMT (Table 4). Other methodological aspects that varied between the studies could have contributed to the inconsistent and insignificant findings, such as different and incomplete definitions of HRDs (Table 1) as well as different HRD

Table 4. Comparison of Baseline and Final BCVA, CMT, and HRD

Group Number	Study	Number of Eyes (Number of Patients)	Treatment	Follow-up Duration (mo)	Baseline /Final BCVA	Baseline /Final CMT (μm)	Baseline /Final HRD No.
Treatment-naïve DME (8 studies, 11 groups)							
1†	Ceravolo 2020	75 (75)	Lucentis × 3	3	*51.6 ± 17.1* /*56.9 ± 17.3*	511 ± 113 /342 ± 86	68.1 ± 19.8 /61.4 ± 17.9
2†	(Ceravolo 2020)	81 (81)	DEX × 1	2	*47.8 ± 16.8* /*55.4 ± 16.8*	588 ± 178 /339 ± 117	66.4 ± 18.6 /53.2 ± 18.7
3	Framme 2012[16]	51 (51)	Anti-VEGF × 1	1	*62.0 ± 11.8* /*66.0 ± 12.0*	446 ± 106 /374 ± 94	16.0±8.1 /14.3±8.5
4†	Hwang 2017	46 (total 82 eyes of 63 patients)	Avastin × 3	3	0.56 ± 0.33 /0.40 ± 0.26	484 ± 131 /322 ± 63	11.3±3.6 /8.7±3.4
5†	(Hwang 2017)	36 (total 82 eyes of 63 patients)	Avastin × 3 + DEX × 1	4	**0.57 ± 0.30** **/0.63 ± 0.42**	507 ± 136 /545 ± 131	**16.1 ± 6.6** **/17.0 ± 5.4**
6	Meduri 2021	39 (39)	DEX × 1–2	12	*51.6 ± 17.1* /*56.9 ± 17.5*	434 ± 156 /337 ± 151	25.5 ± 13.8 /25.7 ± 12.6
7†	Schreur 2018	54 (41)	Avastin × 3	3	**0.54 ± 0.36** **/0.48 ± 0.35**	482 ± 128 /419 ± 127	14.8 ± 9.7 /10.7 ± 6.5
8	Vujosevic 2016	20 (20)	Lucentis × 3 + PRN	6	*64.9 ± 9.1* /*70.4 ± 10.2*	**496 ± 119** **/458 ± 138**	20.9 ± 6.1 /18.4 ± 8.7 (ILM/IPL retinal layer)
9	Vujosevic 2017	26 (26)	Lucentis × 3	3	*57.6 ± 10.2* /*64.0 ± 8.7*	518 ± 113 /355 ± 136	80.6 ± 18.2 /52.5 ± 14.1
10	(Vujosevic 2017)	23 (23)	DEX × 1	1	*50.8 ± 14.9* /*57.4 ± 15.7*	595 ± 183 /301 ± 87	101.3 ± 16.4 /68.8 ± 10.4
11	Vujosevic 2020 (TVST)	37 (37)	Subthreshold micropulse laser	12	*69.4 ± 12.0* /*76.0 ± 9.1*	**305 ± 51** **/294 ± 40**	80.8 ± 20.4 /66.3 ± 18.5
Refractory DME (4 studies, 5 groups)							
12	Al-Latayfeh 2021	72 (72)	DEX × 1	3	0.68/0.44	539 ± 132 /3791 ± 99	23.7 ± 16 /14.8 ± 13
13	Karttunen 2019	24 (22)	DEX × 1–3	4 ± 1	0.54 ± 2.7 /0.4 ± 3.2 (138 ± 35 days)	Not available	67 ± 20 /59 ± 22 (61 days)
14†	Kim 2019	16 (total 29 eyes of 26 patients)	DEX × 1 (early recurrence group)	12	0.68 ± 0.39 /0.61 ± 0.51 (no statistical report)	605 ± 191 /352 ± 115 (no statistical report)	11.4 ± 3.1 /7.2 ± 2.3
15†	(Kim 2019)	13 (total 29 eyes of 26 patients)	DEX × 1 (late recurrence group)	12	0.79 ± 0.45 /0.47 ± 0.22 (no statistical report)	577 ± 173 /309 ± 64 (no statistical report)	7.5 ± 3.6 /4.7 ± 3.3
16†	Pessoa 2021	41 (30)	Fluocinolone acetonide implant×1	12	*42.54 ± 18.41* /*54.85 ± 19.84*	535 ± 192 /337 ± 152	24.6 ± 16.2 /13.0 ± 13.2
Other/unspecified DME (5 studies, 8 groups)							
17†	Kang 2016	30 (26)	Avastin × 1 + PRN (diffuse retinal thickening group)	6.53 ± 2.96	0.45 ± 0.43 /0.26 ± 0.26	441 ± 142 /315 ± 51	17.0 ± 5.7 /12.2 ± 4.9
18†	(Kang 2016)	34 (28)	Avastin × 1 + PRN (cystoid macular edema group)	6.41 ± 4.02	0.40 ± 0.23 /0.27 ± 0.19	384 ± 71 /323 ± 42	14.6 ± 4.5 /11.2 ± 5.5

(*Continued*)

Table 4. (Continued.)

Group Number	Study	Number of Eyes (Number of Patients)	Treatment	Follow-up Duration (mo)	Baseline /Final BCVA	Baseline /Final CMT (μm)	Baseline /Final HRD No.
19†	(Kang 2016)	33 (26)	Avastin × 1 + PRN (serous retinal detachment group)	7.18 ± 4.01	0.37 ± 0.26 /0.24 ± 0.17	471 ± 160 /334 ± 86	21.0 ± 6.0 /12.8 ± 5.2
20†	Lee 2018	30 (unspecified)	Avastin × 1 + PRN (SRD group)	3	**0.32 ± 0.18 /0.39 ± 0.31 (*P* = 0.07)**	387 ± 69 /359 ± 85	9.0 ± 4.8 /3.7 ± 2.3
21	Liu 2019	26 (13)	Conbercept × 3	3	0.75 ± 0.48 /0.39 ± 0.22	576 ± 192 /259 ± 105	5.4 ± 4.2 /2.2 ± 2.0 (2 mo, inner retinal layer)
22†	Narnaware 2020	27 (24)	DEX × 1	4	0.70 ± 0.46 /0.48 ± 0.33	600 ± 170 /378 ± 174	23.9 ± 10.3 /7.0 ± 5.6
23†	Rübsam et al,[13] 2021	32 (27)	Anti-VEGF × 1	1	Not available (overall BCVA did not change: 0.41 ± 0.31 to 0.41 ± 0.31; *P* = 0.929)	381 ± 123 /456 ± 139	**130.6 ± 100 /111.1 ± 88 (*P* = 0.062)**
24†	Rübsam et al,[13] 2021	19 (16)	DEX × 1	1	Not available	472 ± 112 /382 ± 99	123.4 ± 94 /94.9 ± 89 (*P* = 0.020)

BCVA = best-corrected visual acuity; CMT = central macular thickness; DEX = dexamethasone implant; DME = diabetic macular edema; HRD = hyperreflective dots; ILM = internal limiting membrane; IPL = inner plexiform layer; PRN = pro re nata; TVST = Translational Vision Science and Technology.

*BCVA in italic font represents ETDRS letters. Increased values of ETDRS letters mean increase of vision. BCVA in default (black) color represents logarithm of the minimum angle of resolution (logMAR). Decreased values of logMAR mean increase of visual acuity. Groups that had references in brackets mean that they are the same studies as the one above. Bold text represents an insignificant difference between baseline and final values.

†Represents study that provides clear definitions of HRD. Vadala 2020 was excluded in this table because the baseline number of eyes was 24, at 12 and 24 months the number of eyes were 12. Included studies are displayed in Appendix S3.

Table 5. Comparison of HDR Number Reduction between Anti-VEGF and Dexamethasone Implant Groups

Study	Study Population	Anti-VEGF Group (No. of Patients/Eyes)/Follow-up Time (mo)	Dexamethasone Implant Group (No. of Patients/Eyes)/Follow-up Time	Conclusion
Chatziralli 2016[17]	Unspecified DME	Lucentis*3 + PRN (44/44)/9	DEX*3 + PRN (48/48)/9	Similar HRD reduction between the 2 groups
Vujosevic 2017	Treatment-naïve DME	Lucentis*3 (26/26)/3	DEX*1 (23/23)/1	Similar HRD reduction between the 2 groups
Vujosevic 2020 (Acta Diab)	Treatment-naïve DME	Lucentis*3 (18/18)/3	DEX*1 (15/15)/2	Greater HRD number reduction in Ozurdex group
Ceravolo 2020	Treatment-naïve DME	Lucentis*3 (75/75)/3	DEX*1 (81/81)/2	Greater HRD number reduction in Ozurdex group
Rübsam 2021[13]	Unspecified DME	Anti-VEGF*1 (27/32)/1	DEX*1 (16/19)/1	Greater HRD number reduction in Ozurdex group

DEX = dexamethasone implant; DME = diabetic macular edema; HRD = hyperreflective dots; PRN = pro re nata.
Included studies are displayed in Appendix S3.

calculation methods. Different numbers of B scans per eye and differently sized diameters (range, 1−6 mm) were evaluated, potentially resulting in sampling errors. Manual calculation and nonmasked evaluation might have produced a subjective bias. Some articles used different types of OCT devices in their studies, which also might have produced a bias for both CMT measurement and HRD calculation. Different OCT devices can produce different measurements regarding CMT and HRDs.[18,19] The unilateral or bilateral selection of eyes with DME may have influenced the outcome. Lastly, differences in the treatment protocol, such as the switching of medication, and different follow-up times may have influenced the results as well.

The number of HRDs decreased after treatment in 15 out of 17 studies. The results remained the same when we selected studies with clear definitions of HRDs. Of the 2 studies that had insignificant results, 1 (group 23, Table 4) found an insignificant reduction in the number of HRDs 1 month after only 1 anti-VEGF injection, which is considered to be an incomplete treatment of DME.[5] The other (group 5, Table 4) found no change in the number of HRDs in a subgroup of patients unresponsive to 3 anti-VEGF injections. Both studies had another comparing group that showed a significant reduction in the HRD number after treatment. Thus, most of the evidence supports the assumption that the number of HRDs decreases with treatment. Therefore, the number of HRDs can be used as a biomarker of change induced by treatment. Whether this biomarker has an additional value compared with visual acuity and CMT measured using OCT remains to be established. The included studies did not specifically evaluate this aspect and provided insufficient information to clarify this.

We considered prior treatment, glycemic control, and phakic status as the 3 potentially confounding factors in the risk-of-bias assessments. As explained earlier, prior DME treatment would have influenced the baseline HRD number and treatment outcome, making it a potential confounding factor. Intensive diabetes therapy may delay the development and progression of DR; thus, the glycemic level and changes herein during the study period may have affected the treatment outcome.[20] Some studies have shown that the HRD number is correlated with hemoglobin A1C levels[16] and the duration of dexamethasone action,[21] which supports the idea that glycemic control may be a confounding factor. As an OCT biomarker, the evaluation of HRDs is obviously affected by image quality, which is 1 reason why we took phakic status into consideration. Patients with cataract have poorer OCT images than those with clear lenses, thus causing inaccurate calculation of HRD number. The progression of cataract during follow-up most likely occurs in studies using steroid-based treatments, and this obviously affects visual outcomes. Therefore, future studies should provide information on these aspects, and especially, studies using steroid-based treatments should provide detailed information on cataract progression.

Currently, the origin of HRDs is still unknown. There are several hypotheses of HRDs, including precursors of hard exudates, the migration of RPE cells, and activated microglia. Rübsam et al[13] found that the number of hard exudates remained unchanged and that the number of HRDs significantly decreased 30 days after treatment in the same group of patients with DME. Because of this different response to treatment, it is unlikely that they share the same origin.

The absence of back shadowing can help distinguish HRDs from hard exudates, which absorb penetrating light and block tissue signal below. Moreover, HRDs are distributed over all retinal layers, whereas hard exudates are mostly deposited around the outer plexiform layer, as observed using OCT. Therefore, it is not likely that HRDs are the precursors of hard exudates. In addition, HRDs can be present in diabetic patients without clinically detectable retinopathy.[22] These eyes have a relatively intact blood−retinal barrier; so, RPE cells are unlikely to penetrate into the neurosensory retina. Therefore, the most plausible hypothesis of HRDs is that they represent activated microglia. Inflammation is involved in the pathogenesis of DR, and microglia play an important role in this process.[23] Other retinal diseases, such as retinal venous

occlusion and age-related macular degeneration, also have similar pathological changes, with the involvement of activated microglia,[24] and HRDs are present in these diseases.[17,25,26]

Microglia are activated in a hyperglycemic or ischemic environment and change from a resting state, with a small cell body, to an activated state, with an ameboid cell body.[27] Such activation results in an inflammatory response, including the upregulation of VEGF and the migration and proliferation of microglia in the retina.[27] This phenomenon is consistent with the change in HRDs during the development of DR and their increasing numbers with the progression of retinopathy.[22] A positive correlation was found between the number of HRDs and CD14 levels, an inflammatory biomarker only expressed in macrophages and microglia,[28] which supports this opinion. The cell body size of resting microglia in the human retina is about 10 to 20 μm.[29,30] Resting microglia have a small cell body and lengthy ramifications. On activation, the size of the cell body increases and the length of the ramifications decreases. In an animal model, the latter was associated with a tendency of the cells to cluster.[31] The typical resolution of OCT is 20 μm in the axial direction and 5 μm in the lateral direction.[32] Thus, only enlarged microglia can be visualized using OCT. The generally used size limits of 20 to 40 μm of HRDs in OCT would, thus, correspond to a doubling of the cell size or the clustering of cells. Because activated microglia spread over all retinal layers, the separation of the inner and outer layers would be unnecessary. Most studies also showed that the HRD number decreased after treatment in both the layers.

A definition of HRDs could, therefore, be as follows: lesions with a size between 20 and 40 μm, lesions with high reflectivity similar to the RPE band or retinal nerve fiber layer, lesions with no back shadowing, and lesions with the involvement of all retinal layers. Reliable and reproducible calculations should be based on a high number of B scans and a large diameter (6 mm rather than 1 mm). Automated calculation[33] might be advantageous in terms of repeatability and may be time saving compared with manual calculation.

The strength of this review is that we conducted it in a systematic way and analyzed possible factors related to the predictive role of HRDs. In this way, we tried to provide a better approach for future investigations. A major limitation of this review is that a meta-analysis could not be conducted because of the methodological heterogeneity of the evaluated studies, mostly because of the different definitions and calculation methods of HRDs.

In conclusion, the predictive role of HRDs in treatment effect (increase in visual acuity or reduction in macular thickness, detected using OCT) is not yet confirmed based on the current literature, but it is clear that they themselves can be a biomarker of treatment response. Recruiting subjects with a clear treatment history, defined treatment protocol, and considerably longer follow-up time will produce more convincing results. It may also be valuable to further look into the time course of changes in the HRD number, BCVA, and CMT to establish which parameter is the most sensitive indicator of treatment response. Future investigations with the above-described approaches may help to uncover the nature of this biomarker and its effect on treatment outcome in patients with DME.

Footnotes and Disclosures

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Analysis and interpretation: Huang, Jansonius, Los

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Abbreviations and Acronyms:
BCVA = best-corrected visual acuity; **CMT** = central macular thickness; **DME** = diabetic macular edema; **DR** = diabetic retinopathy; **HRDs** = hyperreflective dots; **RPE** = retinal pigment epithelium.

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