Original Article

Behavior of hyperreflective spots noted on optical coherence tomography following intravitreal therapy in diabetic macular edema: A systematic review and meta-analysis

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Purpose: Hyperreflective spots (HRS) are considered as spectral domain optical coherence tomography biomarkers in predicting response to intravitreal therapy (IVT) in diabetic macular edema (DME). We aimed to determine if there was a quantitative reduction in HRS following IVT in DME, if the response to antivascular endothelial growth factor (anti-VEGF) drugs was different from steroids, and if HRS-response was associated with improvement in visual acuity (VA) or reduction in central macular thickness (CMT). **Methods:** PubMed/MEDLINE, Scopus, ProQuest, CINAHL, Wiley online, and Web of Science were searched (between January 1, 2011 and July 1, 2020). Publication bias and heterogeneity were assessed. Meta-analysis was done using the random-effects model. **Results:** Totally, 1168 eyes from 19 studies were eligible for inclusion. IVT was associated with a reduction in quantitative HRS (z = -6.3, $P < 0.0001$). Studies, however, showed heterogeneity (I^2 = 93.2%). There was no difference between anti-VEGF and steroid therapies (P = 0.23). The evidence on predicting VA and CMT outcomes were limited by the number of analyzable studies, owing to the wide variation in individual study designs, and lack of randomized controlled trials. **Conclusion:** We could conclude that there is a definite reduction in quantitative HRS following either form of IVT. We highlight the lacunae in the existing literature on HRS in DME and propose goals for future studies to harness the advantage of this promising biomarker.

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Intravitreal antivascular endothelial growth factor (anti-VEGF) therapy emerged as the first-line treatment for diabetic macular edema (DME) in the last decade after the landmark RISE/RIDE trials and Diabetic Retinopathy Clinical Research Network (DRCR.net) studies demonstrated a significant visual acuity (VA) improvement in ~60% of the eyes treated with IVT.[1,2] However, ~50% of the eyes in protocols I and T of DRCR.net did not respond adequately to these injections.[3,4] Intravitreal steroids are being used in such patients not responding to anti-VEGF injections.[5,6] The rationale behind using steroids is based on the role inflammation has in the pathogenesis of DME.[7,8] However, a subset of patients can show suboptimal response to steroids as well.[9] In a real-life scenario, predicting which patient will or will not respond to intravitreal treatment has become a challenging task.

Various biomarkers are being evaluated on optical coherence tomography (OCT) scans to predict responses like neurosensory detachment,[10,11] ellipsoid zone (EZ) line integrity, cystoid macular edema (CME),[10] hyperreflective spots (HRS),[12] and disorganization of retinal inner layers.[13] HRS are small, dot-like lesions with absent back shadowing on OCT [Fig. 1].[14-17] The pathogenesis of these spots is still unclear. These spots are thought to be extravasated lipoproteins (precursors of hard exudates),[18] inflammatory cells (leucocytes, activated microglia),[19,20] migrated retinal pigment epithelium (RPE) cells,[21] or photoreceptor fragments.[22] Research is underway to estimate the predictive value of this biomarker in determining the final VA, reduction in central macular thickness (CMT), and duration of action of intravitreal implants.[14,15,22-26]

The current literature on HRS in DME consists of small retrospective/prospective cohort studies with a small proportion of studies showing conflicting results. Majority of the studies, however, point that HRS could be a candidate marker in predicting response to therapy in DME. Hence, we tried to synthesize the available information on HRS to (1) investigate if there was a reduction in quantitative HRS following IVT, (2) if the HRS-response to anti-VEGF drugs

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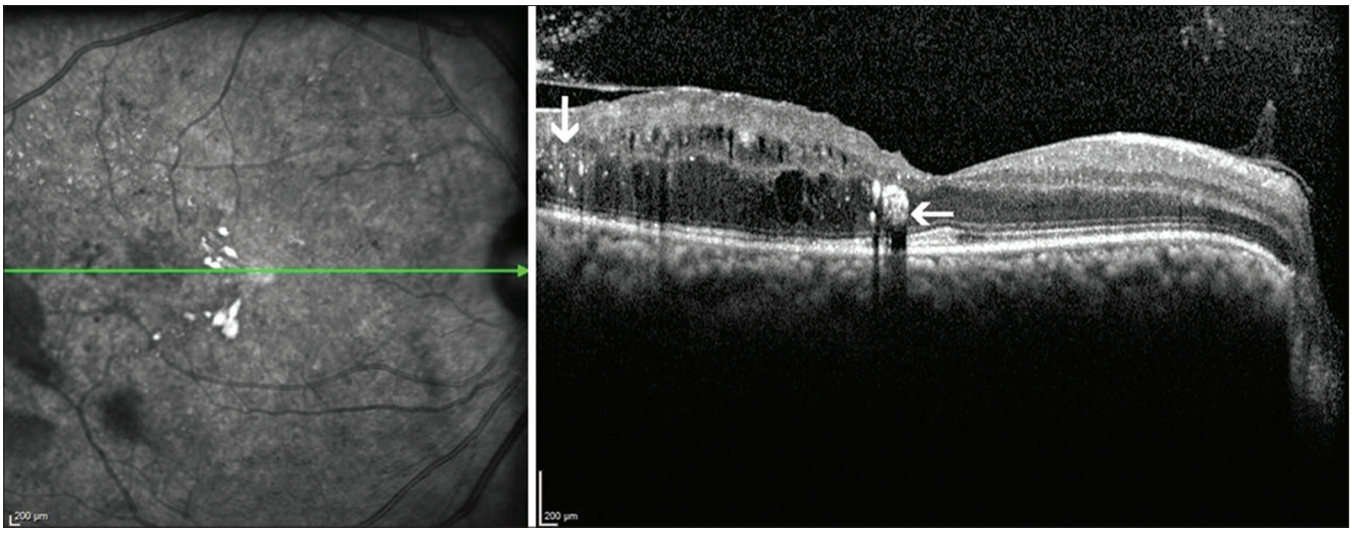


Figure 1: SD-OCT image showing the difference between hard exudates and HRS. Note that HRS are hyperreflective dot-like echoes with absent back shadowing (downward arrow), while hard exudates can be of variable size and cast a back shadow (left arrow)

was different from steroids, and (3) if change in posttreatment quantitative HRS/baseline HRS counts were associated with improvement in VA and/or reduction in CMT. Finally, we highlight the lacunae in the existing literature on HRS in DME and suggest goals for future studies.

Methods

A systematic review was conducted in accordance with Meta-Analyses and Systematic Reviews of Observational Studies guidelines.[27] The protocol was registered in International Prospective Register of Systematic Reviews (CRD42020186820).

This review included all articles that described HRS as an outcome predictor after IVT in DME from peer reviewed journals published in electronic databases (between January 1, 2011 and July 1, 2020).

We excluded studies: (1) not available in English, (2) published in books, conference abstracts, review, comments, letter to editor, case series (<5 subjects), (3) with insufficient quality, (4) where the results of DME were combined with other causes of macular edema like vein occlusion, (5) where additional interventions were done during the study period like laser, vitrectomy, etc., (6) performed in nonhuman subjects, and (7) where time-domain OCT machines were used.

Search strategy

The following databases were searched: PubMed/MEDLINE, Scopus, ProQuest, CINAHL, Wiley online, and Web of Science. PICO (participants, intervention, and comparison and outcomes) format search strategy was used to search databases mentioned.

The full search strategy for MEDLINE using keywords is detailed in Appendix 1.

Assessment of methodological quality and risk of bias

The quality and risk of bias of the articles included in the full-text review was assessed by PG and SK using the National Institute of Health Study Quality Assessment Tool.[28] Questions with answer "yes" were scored 1 and those with an answer "no"/"cannot determined"/"not reported" were scored 0. The total score for each study = (the total number of questions answered as "yes"/the total number of questions) × 100. Studies were graded as high quality (80–100%), moderate quality (60–80%), and low quality (<60%).

Statistical analysis

Meta-analysis

We performed a random-effects meta-analysis. All the outcomes of interest (i.e., quantitative HRS reduction, difference in quantitative HRS reduction between steroid and anti-VEGF therapy, and posttreatment change in VA) were set as continuous variables. The variances of combined true effect sizes among the studies were estimated using Hedge's g for all outcomes (with 95% CI). Heterogeneity among studies was estimated using I^2 statistics. Subgroup analysis was performed using analysis of variance of sum of squares.[29] Publication bias was analyzed using Begg and Mazumdar rank correlation test (Δx–y, Kendall Tau a, and CI limits).

Results

Included studies

Fig. 2 shows the flow diagram to summarize inclusion of studies.

Quality of the studies

The quality scores of the 19 studies (13 retrospective cohort studies,[12,15-17,24,26,30-36] 3 prospective cohort studies,[37-39] 2 case series,[14,40] and 1 case-control study[41]) are enumerated in Table 1.

Baseline characteristics

A total of 1168 eyes of 942 patients (mean age: 64.3 ± 4.9 years, males: 59.4%) were analyzed for HRS from the above 19 studies. Eight studies evaluated the response to anti-VEGF injections [intravitreal ranibizumab (IVR), intravitreal bevacizumab (IVB), and conbercept],[16,24,30-32,35,36,41] 11 studies

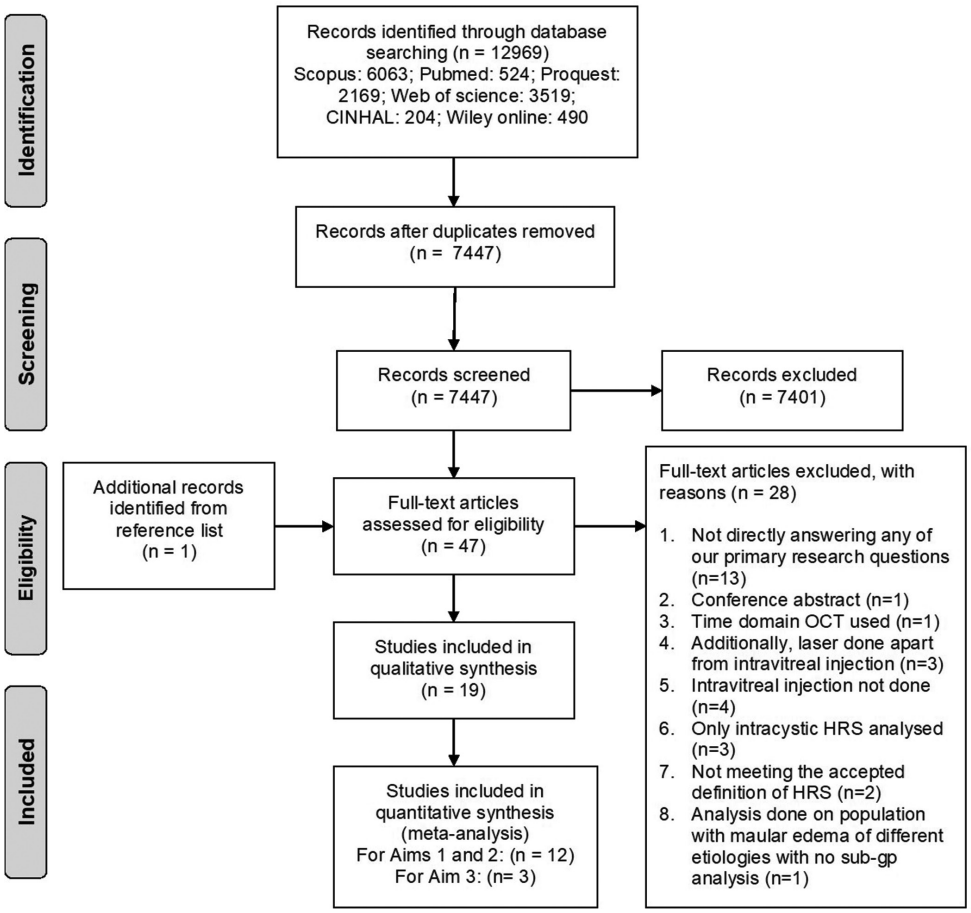


Figure 2: Flow diagram to summarize inclusion of relevant studies

to dexamethasone implant,[12,14,26,32-34,36-40] and 2 studies to sequential use of anti-VEGF and dexamethasone.[15,17] The measurement of HRS was done over different area sizes in the macula (12 studies used 3000 µm area,[12,14-17,26,32-34,36,38,39] 4 studies used 1000 µm area,[24,30,35,40] 2 studies used 1500 µm area,[31,37] and 1 study used area between 500 and 1500 µm from the center of the fovea) [Table 1].[41]

Change in quantitative HRS with IVT

Twelve studies with HRS counts before and after IVT were analyzed. All seven studies where anti-VEGF injections were used[16,17,30-32,35,41] and six out of the seven studies where dexamethasone was used[17,26,32,34,37,40] reported a decrease in quantitative HRS. In the subgroup of patients whose macular edema did not respond to dexamethasone or IVB, there was no significant HRS reduction [Table 2].[17]

Retinal-layer-wise analysis was done in six studies. However, the definition of retinal layers was variable across the studies. Inner retina (IR) was defined as extending from internal limiting membrane (ILM) to outer nuclear layer (ONL) in three studies,[17,31,35] ILM to inner nuclear layer (INL) in one study,[26] and as INL in one study.[16] Similarly, outer retina (OR) was defined as extending from external limiting membrane (ELM) to RPE in two studies,[17,31] ELM to photoreceptors in one study[35] and ELM to outer plexiform layer (OPL) in two studies.[16,26] One study analyzed HRS in three layers, i.e., ILM to inner plexiform layer, INL to OPL and ONL.[41]

HRS change in steroid versus anti-VEGF-treated eyes

Two studies compared the change in HRS counts between these two classes of drugs, in treatment naive eyes.[32,36] Vujosevic *et al*.[32] showed a greater reduction in HRS in dexamethasone-treated eyes (n = 15) versus IVR-treated eyes (n = 18) (24.7% versus 8.0%, P = 0.03) when all baseline parameters were matched. In another study by the same author, the decrease in HRS was not found to be different between the two treatment groups (P = 0.135).[36] However, in this study, the baseline HRS counts were significantly higher in the dexamethasone group compared to the IVR group (P = 0.003). Hwang *et al*.[17] noted that baseline HRS numbers were higher in eyes that did not respond to IVB. When such eyes were treated with dexamethasone implant, the HRS count decreased [Table 2 and Fig. 3a].

Baseline HRS and change in VA

A total of 14 studies were analyzed. Five studies made a qualitative reporting of HRS as present or absent at baseline;[12,14,24,38,39] three studies had categorized the patients into those with HRS <10–15 and those with HRS >10–15 on baseline scans.[15,33,40] In the remaining six studies, baseline HRS counts were correlated with final VA using regression/correlation statistics.[16,30,31,35,37,41] Three studies showed that higher HRS counts at baseline were associated with worse final VA.[31,35,38] Five studies showed no correlation between baseline HRS counts and final VA.[15,16,33,40,41] In a study by Cavalleri *et al*.,[15] dexamethasone therapy resulted in a greater gain in VA in eyes with high baseline

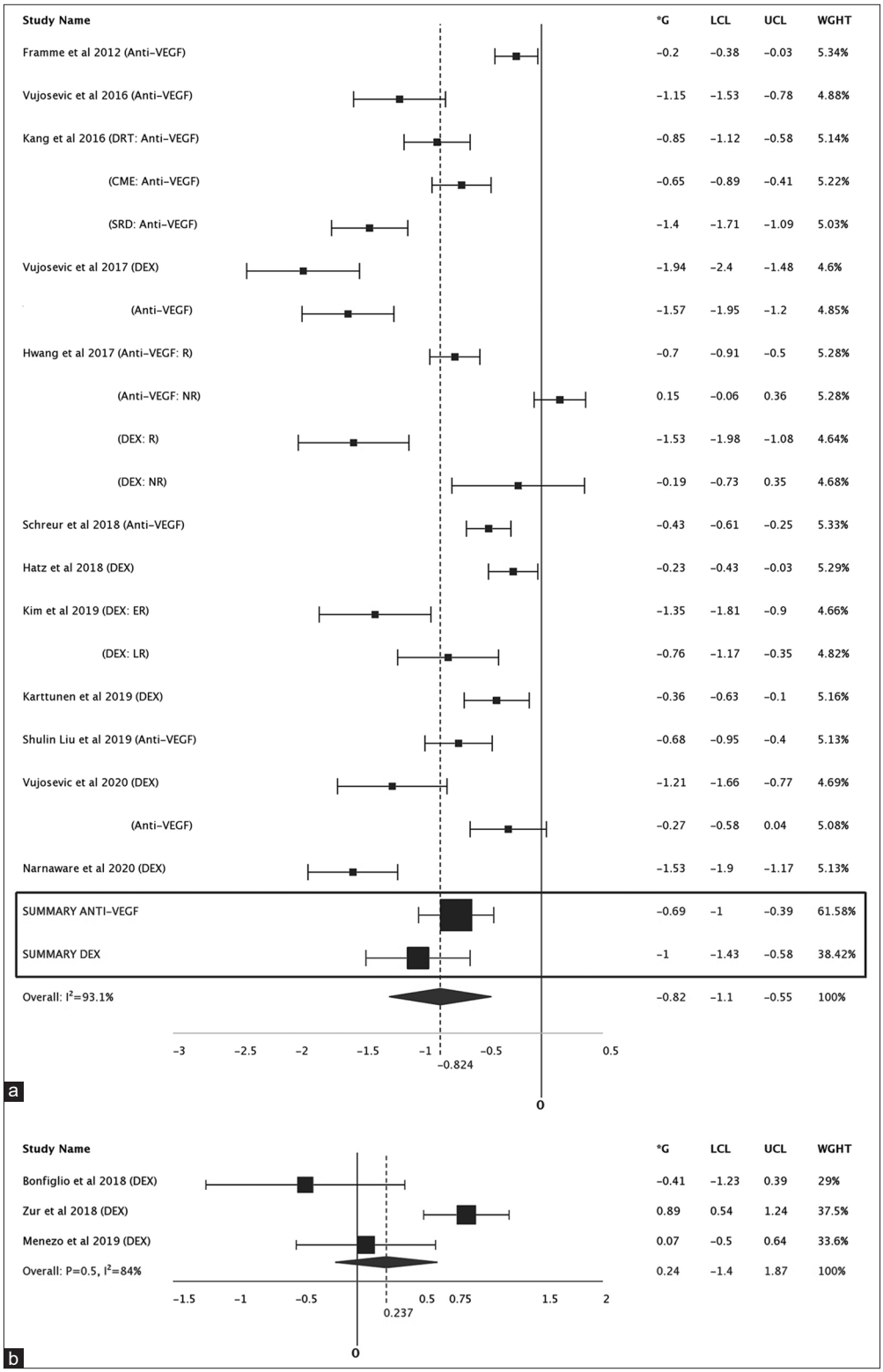


Figure 3: (a) Forest plot showing the change in quantitative HRS following intravitreal injection. There were a total of 12 studies among which there were 20 effect sizes to be analyzed. The box and whisker plot for individual studies represent the effect size (Hedges' g) and 95% confidence intervals ($CI_{95\%}$). Subgroup analyses for dexamethasone and anti-VEGF groups are summarized within the plot. The overall effect size is represented by the polygon. (b) Forest plot showing the association between HRS at baseline and change in VA. [*G = Hedges' g; LCL = lower confidence limit; UCL = upper confidence limit; WGHT = weight of the study; dotted vertical line = overall effect size; I^2 = heterogeneity of the studies; within parenthesis = therapeutic group; VEGF = vascular endothelial growth factor; DEX = dexamethasone; DRT = diffuse retinal thickening; CME = cystoid macular edema; SRD = serous retinal detachment; R = responder; NR = nonresponder; ER = early recurrence; LR = late recurrence]

Table 1: Baseline characteristics of the studies and participants included in the systematic review

Author (year)	Study design	Study population	*Eyes	Mean age (years)	*Follow up (months)	Macular area analyzed (μm)	Intervention	Study quality
Framme *et al.* (2012)[21]	Retrospective cohort	DME (previously no anti-VEGF)	51	67	1	1000	IVR=30, IVB=21	Moderate
Vujosevic *et al.* (2016)[41]	Prospective case control	Treatment naive DME	40	63.0	6	500-1500	IVR	High
Kang *et al.* (2016)[31]	Retrospective cohort	Treatment naive DME	97	60.11	6.71±3.7	1500	IVB	Moderate
Vujosevic *et al.* (2017)[32]	Retrospective cohort	Treatment naive DME	49	66.0	Unclear	3000	DEX (23)/ IVR (26)	Moderate
Chatziralli *et al.* (2017)[38]	Prospective cohort	Refractory DME	54	69.2	12	3000	DEX	Moderate
Hwang *et al.* (2017)[17]	Retrospective cohort	Treatment naive DME	82	55.13	3 m post IVB/1 m post DEX	3000	3 IVB; if no response add DEX	Moderate
Zur *et al.* (2018)[12]	Retrospective cohort	Treatment naive and refractory DME	299	64	4	3000	DEX	High
Schreur *et al.* (2018)[16]	Retrospective cohort	Treatment naive DME	54	67	3	3000	IVR	High
Hatz *et al.* (2018)[40]	Case series	Refractory DME	40	68.3	2	1000	DEX	Moderate
Bonfiglio *et al.* (2019)[14]	Case series	Refractory DME	44	69.7	6	3000	DEX	High
Fonollosa *et al.* (2019)[33]	Retrospective cohort	Naive or previously treated DME patients	64	67.5	6	3000	DEX	High
Karttunen *et al.* (2019)[34]	Retrospective cohort	Refractory DME	24	65.6	2	3000	DEX	Moderate
Menezo *et al.* (2019)[39]	Prospective cohort	Treatment naive DME	50	66.4	12	3000	DEX	Moderate
Liu *et al.* (2019)[35]	Retrospective cohort	DME (previously no anti-VEGF)	26	53.9	3	1000	Conbercept	High
Kim *et al.* (2019)[26]	Retrospective cohort	Refractory DME	29	58.3	12	3000	DEX	Moderate
Vujosevic *et al.* (2020)[36]	Retrospective cohort	Treatment naive DME	33	63.3	3 m post IVR/2 m post DEX	3000	DEX (15 eyes)/IVR (18)	Moderate
Cavalleri *et al.* (2020)[15]	Retrospective cohort	Treatment naive DME	28	72.1	12	3000	Loading dose of IVR followed by DEX	Moderate
Yoshitake *et al.* (2020)[24]	Retrospective cohort	DME (unspecified)	77	69	12	1000	IVR	High
Narnaware *et al.* (2020)[37]	Prospective cohort	Treatment naive and refractory DME	27	61.11	4	1500	DEX	Low

IVR: intravitreal ranibizumab; IVB: intravitreal bevacizumab; DEX: dexamethasone implant; *: number of eyes with respect to HRS analysis; refractory DME: diabetic macular edema unresponsive to previous anti-VEGF injections, μm: micrometers; m: months

HRS counts compared to IVB. Bonfiglio *et al.*[14] and Yoshitake *et al.*[24] compared eyes with and without HRS at baseline and showed a greater gain in VA following dexamethasone and anti-VEGF injections, respectively, in eyes with HRS. Zur *et al.*[12] reported a greater gain in eyes without HRS at baseline[12] and Menezo *et al.*[39] showed no association between gain in VA and the presence of HRS at baseline [Appendix 2 and Fig. 3b].

Baseline HRS and CMT change

A total of 10 studies were included for this analysis. Bonfiglio *et al.*[14] and Yoshitake *et al.*[24] reported greater reduction in CMT in eyes with HRS compared to those without. Menezo *et al.*[39] found no association between the two parameters. Two studies which evaluated the association between a decrease in HRS and change in CMT showed contrasting results, with Liu *et al.*[35] reporting a significant correlation between the

Table 2: Quantitative HRS change following intravitreal therapy

Author (year)	Drug (Number of eyes)	HRS (Mean±SD)				
			Baseline		After treatment	*P* value
Framme *et al.* (2012)[21]	IVR (30); IVB (21)		16.02±8.0		14.32±8.46	0.000*
Vujosevic *et al.* (2016)[41]	IVR	ILM-IPL		20.9±6.1	18.4±8.7	0.2993
		INL-OPL		16.2±5.8	13.8±5.8	0.1986
		ONL		4.8±2.8	2.5±3.2	0.02*
Kang *et al.* (2016)[31]	IVB	DRT	TR	16.97±5.68	12.23±4.93	<0.001*
			IR	12.33±4.34	8.97±3.77	<0.001*
			OR	4.63±2.94	3.27±3.03	0.01*
		CME	TR	14.62±4.45	11.15±5.47	0.002*
			IR	10.21±4.69	7.56±3.81	0.004*
			OR	4.56±2.08	3.71±2.78	0.047*
		SRD	TR	20.97±5.93	12.82±5.23	<0.001*
			IR	11.76±3.66	8.93±3.09	0.002*
			OR	7.33±2.78	3.15±2.83	<0.001*
			SRF	1.88±2.04	0.73±1.70	0.003*
Vujosevic *et al.* (2017)[32]	DEX (23); IVR (26)	DEX		101.3±16.4	68.8±10.4	0.0001*
		IVR		80.6±18.2	52.5±14.1	0.0001*
Hwang *et al.* (2017)[17]	3 IVB; if no response add DEX	IVB Responder	TR	11.26±3.64	8.72±3.44	<0.001*
			IR	7.46±2.74	6.22±2.56	0.002*
			OR	3.59±1.67	2.50±1.52	<0.001*
		IVB non-responder	TR	16.06±6.60	17.00±5.39	0.377
			IR	11.50±4.95	12.36±4.18	0.262
			OR	4.42±2.13	4.58±1.96	0.678
		DEX responder	TR	20.78±3.34	14.78±3.92	<0.001*
			IR	15.33±2.47	10.72±3.34	<0.001*
			OR	5.38±1.97	4.06±1.92	<0.001*
		DEX nonresponder	TR	14.00±3.85	13.00±4.38	0.53
			IR	10±3.52	8.67±4.18	0.41
			OR	4±1.55	4.17±2.14	0.74
Schreur *et al.* (2018)[16]	IVR	TR		14.8±9.7	10.7±6.5	0.002*
		IR		10.4±7.3	7.9±5.0	0.01*
		OR		4.5±4.9	4.5±4.9	0.013*
Hatz *et al.* (2018)[40]	DEX	10.9±7.9			9.1±7.6	0.077
Kim *et al.* (2019)[26]	DEX	Early recurrence group	TR	11.38±3.07	7.19±2.29	NA
			IR	5.44±1.50	3.69±1.14	<0.001*
			OR	5.94±2.74	3.31±2.15	<0.001*
		Late recurrence group	TR	7.54±3.60	4.69±3.30	NA
			IR	4.08±1.70	3.15±1.57	0.027*
			OR	3.46±2.30	1.31±1.44	0.001*
Karttunen *et al.* (2019)[34]	DEX	67±20			59±22	0.04*
Shulin Liu *et al.* (2019)[35]	Conbercept	IR		5.39±4.24	2.19±2.00 (2 m)	0.002*
		OR		5.15±5.17	3.35±4.40 (1 m)	<0.0001*
		SRF		0.88±1.9	0.08±0.27 (1 m)	0.004*
Vujosevic *et al.* (2020)[36]†	DEX (15); IVR (18)	DEX		85.5±16.9	64.4±11.5	0.0004*
		IVR		72.7±15.7	66.9±21.4	0.36
Narnaware *et al.* (2020)[37]	DEX	23.88±10.31			7.04±5.58	<0.0001*

IVR: intravitreal ranibizumab; IVB: intravitreal bevacizumab; DEX: dexamethasone implant; IR: inner retina; OR: outer retina; TR: total retina; SRF: subretinal fluid; ILM: internal limiting membrane; INL: inner nuclear layer; IPL: inner plexiform layer; OPL: outer plexiform layer; ONL: outer nuclear layer; DRT: diffuse retinal thickening; CME: cystoid macular edema; SRD: subretinal detachment; *P<0.05, NA=not available; †data obtained after contacting author

reduction in inner and total retinal HRS and the decrease in CMT at 3 months (r = 0.422, P = 0.032 and r = 0.429, P = 0.029, respectively) and Framme *et al*.[30] reporting no significant association between the two variables at the end of 1 month. Vujosevic *et al*.[32] showed greater CMT reduction in eyes with more HRS (>87) at baseline than those with less HRS (<87) (ρ = -0.28, P = not reported). Schreur *et al*.[16] reported that the number of HRS at baseline was independently associated with a decrease in CMT ($\beta_{standardized}$ = -2.61, P = 0.006). On the contrary, Fonollosa *et al*.[33] found that the CMT reduction was not significantly different between groups with scare (<10) or abundant (>21) HRS. Finally, Kang *et al*.[31] and Vujosevic *et al*.[41] found no significant correlation between the baseline HRS counts and the final retinal thickness [Appendix 2].

Meta-analysis

From the systematic review, we found (i) that the qualities of the studies were moderate, (ii) result reporting was inconsistent across studies, and (iii) conflicting results across various studies. Hence, results summarized using a random effects meta-analysis on 12 studies testing the quantitative HRS change following IVT [Fig. 2a] showed high heterogeneity in the studies (I^2 = 93.16%) and significant publication bias (Δ = -100; Kendell's Tau = -0.526, $CI_{95\%}$ = -0.47 to -0.36, P = 0.001). There was no significant difference between dexamethasone (Hedges' g = -1.0, $CI_{95\%}$ = -1.42 to -0.57) and anti-VEGF groups (Hedges' g = -0.69, $CI_{95\%}$ = -0.99 to -0.38) in terms of HRS reduction (Q* = 1.4, df = 1, P = 0.23).

To analyze the association between HRS and VA, we performed a meta-analysis on three studies [Fig. 3b].[12,14,39] The presence/absence of HRS at baseline was not associated with improved VA at the end of treatment (Hedges' g = 0.237, $CI_{95\%}$ = -1.39 to 1.87, I^2 = 84%, P = 0.5) [Fig. 3b].

We could not perform a meta-analysis to see the effect of HRS on CMT reduction due to heterogeneity in reporting results.

Discussion

In this review, we found that there is a definite reduction in HRS counts following IVT and no significant difference between anti-VEGF and steroid groups. The role of HRS in predicting VA outcome and CMT change was limited by the number of analyzable studies owing to the wide variation in the study designs and reporting.

Various theories have been proposed regarding the exact nature of HRS.[18-22,42] Of these, the hard exudate and inflammatory theories are most popular in DME. Cusick *et al*.[43] using immunochemistry found apolipoprotein-B deposits corresponding to the HRS. An inflammatory basis for HRS was postulated by Lee *et al*.[44] The authors found that soluble CD14 (sCD14) levels in the aqueous humor and HRS counts in inner retina on OCT were raised in patients with DME compared to controls. Hence, they concluded that since sCD14 is released by retinal microglia, HRS might represent aggregates of activated microglial cells in DME eyes. Intravitreal dexamethasone is a potent antiinflammatory agent. Anti-VEGF injections, although not as potent as steroids in their antiinflammatory action, have been shown to have antiactivated microglial activity.[45] The reduction in HRS within 3 months of starting IVT as seen in most studies of this review strongly points toward their inflammatory origin. If HRS were to be hard exudates, we do not expect such rapid regression.

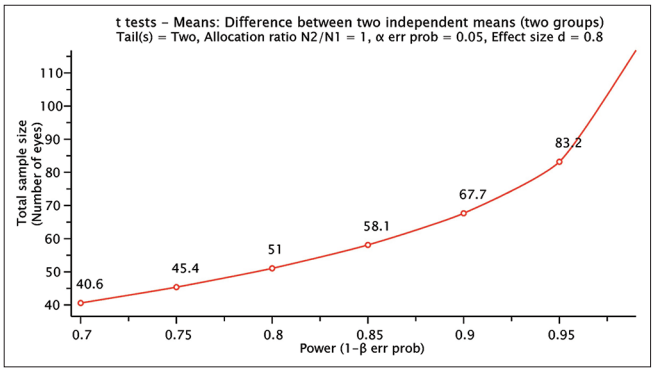
Although HRS are mainly located in the inner retina, with progressing retinopathy, HRS reach the outer retinal layers. Studies have shown that OR-HRS were associated with ELM and EZ disruption[46] and that there was a positive correlation between OR-HRS counts and final EZ and ELM disruption length.[31] Further studies have shown that HRS in OR had greater shortening of EZ line disruption following intravitreal anti-VEGF therapy than those without HRS at baseline.[24] Nishijima *et al*.[47] showed that HRS in OR were predictive of photoreceptor damage and poor vision after vitrectomy for DME. Kang *et al*.[31] found that in the DRT and CME groups, the final VA was worse in those with greater number of OR-HRS. Yoshitake *et al*.[24] reported that eyes with HRS in OR had greater VA improvement and greater CSF thickness reduction. HRS in the inner retinal layers were not associated with VA improvement in this study. In an observational study on treatment naïve DME patients, Arthi *et al*.[48] found that there no differences in CMT, BCVA, ELM, and EZ continuity between those with and without IR-HRS or OR-HRS.

A recent study showed that greater proportion of diabetics with HRS had coexistent hypertension compared to those who did not have HRS and those with higher number of HRS had significantly lower levels of serum triglycerides.[48] However, Davoudi *et al*.[49] showed that the presence of HRS was associated with higher total cholesterol and higher low-density lipoprotein levels. Framme *et al*.,[30] Wong *et al*.,[50] and De Benedetto *et al*.[18] have shown that poor glycometabolic control is associated with more HRS. They postulate that hyperglycemia could activate retina microglial cells in diabetic patients, which are seen as HRS on OCT. On the contrary, Arthi *et al*.[48] showed no association of HRS with glycemic control.

HRS noted in the inner wall of cystoid spaces have been called the "pearl necklace sign."[51] This sign indicates the presence of lipoproteins or lipid-laden macrophages in patients with chronic CME. In a study by Ajay *et al*.,[52] this sign was seen in 13.1% of the eyes with DME. In 75% of such eyes, clinically visible hard exudates developed in exactly the same location as the pearl necklace sign after the resolution of DME. This could cause irreversible damage to photoreceptors if present subfoveally. Terada *et al*.[53] noted that HRS were accompanied by hyperreflective walls in foveal cystoid spaces. Eyes with hyperreflective walls in foveal cystoid spaces had poorer VA, more severe photoreceptor disruption, and poorer DME remissions than did those without such findings.

In DME, SD-OCT often shows HRS at the outer border of the detached neurosensory retina and/or within the subretinal space. Arthi *et al*.[48] showed that a greater proportion of eyes with HRS also had SRF. Ota *et al*.[54] compared eyes with no/few subretinal HRS and eyes with many subretinal HRS. While there was no difference in the baseline foveal thickness between the groups, foveal thickness of the group with few dots was significantly thicker than that of the group with many dots at 6 months, and this difference was abolished at 12 months. However, the VA at

Table 3: Lacunae in existing literature and goals for future studies

Lacunae in existing literature	Goals for future studies
Uncertainty regarding the biological composition of HRS, its origin and natural history	Histopathological studies (immunohistochemistry/electron microscopy) on animal models of diabetic macular edema wherein OCT features are correlated with histological findings Studies on the natural history of these lesions on OCT in human eyes
Lack of standardization in the protocols used to evaluate these lesions on imaging	Standardization of image acquisition protocols to study HRS: OCT images to be taken on spectral domain or swept source OCT devices with real-time eye tracking and automated follow-up scanning Images to have a minimum signal strength of 80% and an axial resolution of 7 μm or less Standardization of the definition of HRS on OCT in terms of its size, site, reflectivity and backshadowing
Lack of large-scale prospective clinical trials on the effect of intravitreal drugs on HRS	We recommend studies with the following characteristics: Sample size: Studies will have to recruit at least~68 eyes to test any two comparisons such as (1) treatment responsive vs unresponsive or (2) Dex vs anti-VEGF, at an expected power of 0.9, alpha of 0.05 and a large effect size of 0.8  Patient population: DME patients with Well controlled systemic parameters (HbA1c<7%, normal lipid profile and renal parameters) No past history of panretinal photocoagulation or intravitreal therapy or intraocular surgery in the last 6 months Macular ischemic index on FFA of ETDRS grade 2 or better No vitreo-retinal interface abnormality Standard methods of OCT acquisition to study HRS as discussed above HRS counting done on standard software which can scale lesion size to magnification; HRS counted in the entire area of 3000 μm from the fovea; blinding of investigators involved in data acquisition Random allocation of interventional drug (steroids versus anti-VEGF drugs) to the patients Follow-up of at least 1 year to understand the temporal changes in these lesions

12 months was significantly poorer in the groups with many HRS owing to the subfoveal deposition of hard exudates.

This meta-analysis could not bring out a significant effect of baseline HRS on the change in VA. However, on closer look, eyes with treatment naive DME[15,16,30-32,35,37,41] as against those with refractory DME[14,38,40] showed a positive correlation between HRS and VA gain (5/8 studies versus 0/3 studies) and CMT reduction (7/8 studies versus 1/3 studies) implying a significant role of inflammation in treatment naïve DME as against a multifactorial pathogenesis in refractory-DME eyes.

The limitations of the studies included in this review include the following: retrospective designs, inadequate sample sizes, varied HRS measurements (i.e., HRS measured in different macular areas, manual versus automated counting, inconsistent definition of retinal layers), short follow-up duration, lack of adjustment for confounders (blood lipid/sugar levels[18,30,49,50]), and varying statistical reporting methods and significant publication bias. Also, there has been a lack of uniform definition of HRS in these studies.

There is a need to standardize such variability in quantitative research when evaluating a biomarker to ensure reproducibility and test–retest reliability. Hence, we recommend a stage-wise approach to understand the exact nature and the role of this biomarker in DME [Table 3].

Conclusion

In conclusion, there is a definitive quantitative reduction in HRS after intravitreal anti-VEGF or intravitreal steroid therapy for DME. However, its correlation with reduction in CMT and VA change is inconclusive. HRS appear to be a promising biomarker in predicting therapeutic response to intravitreal treatment in DME.

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Conflicts of interest

There are no conflicts of interest.

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Appendix 1: Pubmed search strategy (searched on July 4, 2020)

No.	Search no	Query	Results
1.	#S1	Search ((((“DMO” OR “DME” OR “macular oedema” OR “Macular edema” OR “Center-involving” OR “Maculopathy”))) OR ((“DMO”[Title/Abstract] OR “DME”[Title/Abstract] OR “macular oedema”[Title/Abstract] OR “Macular edema”[Title/Abstract] OR “Center-involving”[Title/Abstract] OR “Maculopathy”)[Title/Abstract])) OR ((“DMO” OR “DME” OR “macular oedema” OR “Macular edema” OR “Center-involving” OR “Maculopathy”)[MeSH Terms]) Filters: Humans	14561
2.	#S2	Search ((((“Bevacizumab” OR “Optical coherence tomography” OR “Anti VEGF” OR “ranibizumab” OR “SD-OCT” OR “Intravitreal” OR “antivascular endothelial growth factor” OR “OCT” OR “BVZ” OR “dexamethasone” OR “steroid” OR “Intra vitreal” OR “Intra-vitreal” OR “avastin” OR “Lucentis” OR “accentrix” OR “Aflibercept” OR “Eyelea” OR “ozurdex” OR “Triamcinolone acetonide” OR “IVTA” OR “Conbercept” OR “Anti-VEGF” OR “AntiVEGF”))) OR ((“Bevacizumab” OR “Optical coherence tomography” OR “Anti VEGF” OR “ranibizumab” OR “SD-OCT” OR “Intravitreal” OR “antivascular endothelial growth factor” OR “OCT” OR “BVZ” OR “dexamethasone” OR “steroid” OR “Intra vitreal” OR “Intra-vitreal” OR “avastin” OR “Lucentis” OR “accentrix” OR “Aflibercept” OR “Eyelea” OR “ozurdex” OR “Triamcinolone acetonide” OR “IVTA” OR “Conbercept” OR “Anti-VEGF” OR “AntiVEGF”)[MeSH Terms])) OR ((“Bevacizumab”[Title/Abstract] OR “Optical coherence tomography”[Title/Abstract] OR “Anti VEGF”[Title/Abstract] OR “ranibizumab”[Title/Abstract] OR “SD-OCT”[Title/Abstract] OR “Intravitreal”[Title/Abstract] OR “antivascular endothelial growth factor”[Title/Abstract] OR “OCT”[Title/Abstract] OR “BVZ”[Title/Abstract] OR “dexamethasone”[Title/Abstract] OR “steroid”[Title/Abstract] OR “Intra vitreal”[Title/Abstract] OR “Intra-vitreal”[Title/Abstract] OR “avastin”[Title/Abstract] OR “Lucentis”[Title/Abstract] OR “accentrix”[Title/Abstract] OR “Aflibercept”[Title/Abstract] OR “Eyelea”[Title/Abstract] OR “ozurdex”[Title/Abstract] OR “Triamcinolone acetonide”[Title/Abstract] OR “IVTA”[Title/Abstract] OR “Conbercept”[Title/Abstract] OR “Anti-VEGF”[Title/Abstract] OR “AntiVEGF”)[Title/Abstract]) Filters: Humans	1738178
3.	#S3	Search ((((“Hyper” OR “reflective” OR “foci” OR “central macular thickness” OR “macular volume” OR “CST” OR “CMT” OR “FT” OR “hyperreflective” OR “foveal thickness” OR “spots” OR “HRS” OR “HF” OR “Small” OR “Dense” OR “Best” OR “Corrected” OR “Visual” OR “acuity” OR “BCVA” OR “outcomes” OR “Hyper-reflective” OR “dots” OR “material” OR “points” OR “aggregates” OR “particles” OR “clumps” OR “retinal” OR “HRF” OR “HS” OR “HRD” OR “inflammatory” OR “biomarkers*” OR “Prognostic” OR “markers”))) OR ((“Hyper” [Title/Abstract] OR “reflective”[Title/Abstract] OR “foci”[Title/Abstract] OR “central macular thickness”[Title/Abstract] OR “macular volume”[Title/Abstract] OR “CST”[Title/Abstract] OR “CMT”[Title/Abstract] OR “FT”[Title/Abstract] OR “hyperreflective”[Title/Abstract] OR “foveal thickness”[Title/Abstract] OR “spots”[Title/Abstract] OR “HRS” [Title/Abstract] OR “HF”[Title/Abstract] OR “Small”[Title/Abstract] OR “Dense”[Title/Abstract] OR “Best”[Title/Abstract] OR “Corrected”[Title/Abstract] OR “Visual”[Title/Abstract] OR “acuity”[Title/Abstract] OR “BCVA”[Title/Abstract] OR “outcomes”[Title/Abstract] OR “Hyper-reflective”[Title/Abstract] OR “dots”[Title/Abstract] OR “material”[Title/Abstract] OR “points”[Title/Abstract] OR “aggregates”[Title/Abstract] OR “particles”[Title/Abstract] OR “clumps”[Title/Abstract] OR “retinal”[Title/Abstract] OR “HRF”[Title/Abstract] OR “HS”[Title/Abstract] OR “HRD”[Title/Abstract] OR “inflammatory”[Title/Abstract] OR “biomarkers*”[Title/Abstract] OR “Prognostic”[Title/Abstract] OR “markers”)[Title/Abstract])) OR ((“Hyper” OR “reflective” OR “foci” OR “central macular thickness” OR “macular volume” OR “CST” OR “CMT” OR “FT” OR “hyperreflective” OR “foveal thickness” OR “spots” OR “HRS” OR “HF” OR “Small” OR “Dense” OR “Best” OR “Corrected” OR “Visual” OR “acuity” OR “BCVA” OR “outcomes” OR “Hyper-reflective” OR “dots” OR “material” OR “points” OR “aggregates” OR “particles” OR “clumps” OR “retinal” OR “HRF” OR “HS” OR “HRD” OR “inflammatory” OR “biomarkers*” OR “Prognostic” OR “markers”)[MeSH Terms]) Filters: Humans	3886156
4	#S1 AND S2 AND S3	Search (((((((“DMO” OR “DME” OR “macular oedema” OR “Macular edema” OR “Center-involving” OR “Maculopathy”))) OR ((“DMO”[Title/Abstract] OR “DME”[Title/Abstract] OR “macular oedema”[Title/Abstract] OR “Macular edema”[Title/Abstract] OR “Center-involving”[Title/Abstract] OR “Maculopathy”)[Title/Abstract])) OR ((“DMO” OR “DME” OR “macular oedema” OR “Macular edema” OR “Center-involving” OR “Maculopathy”)[MeSH Terms])) AND Humans[Mesh])) AND ((((((“Bevacizumab” OR “Optical coherence tomography” OR “Anti VEGF” OR “ranibizumab” OR “SD-OCT” OR “Intravitreal” OR “antivascular endothelial growth factor” OR “OCT” OR “BVZ” OR “dexamethasone” OR “steroid” OR “Intra vitreal” OR “Intra-vitreal” OR “avastin” OR “Lucentis” OR “accentrix” OR “Aflibercept” OR “Eyelea” OR “ozurdex” OR “Triamcinolone acetonide” OR “IVTA” OR “Conbercept” OR “Anti-VEGF” OR “AntiVEGF”))) OR ((“Bevacizumab” OR “Optical coherence tomography” OR “Anti VEGF” OR “ranibizumab” OR “SD-OCT” OR “Intravitreal” OR “antivascular endothelial growth factor” OR “OCT” OR “BVZ” OR “dexamethasone” OR “steroid” OR “Intra vitreal” OR “Intra-vitreal” OR “avastin” OR “Lucentis” OR “accentrix” OR “Aflibercept” OR “Eyelea” OR “ozurdex” OR “Triamcinolone acetonide” OR “IVTA” OR “Conbercept” OR “Anti-VEGF” OR “AntiVEGF”)[MeSH Terms])) OR ((“Bevacizumab”[Title/Abstract] OR “Optical coherence tomography”[Title/Abstract] OR “Anti VEGF”[Title/Abstract] OR	

Contd...

Appendix 1: Contd...

No.	Search no	Query	Results
		"ranibizumab"[Title/Abstract] OR "SD-OCT"[Title/Abstract] OR "Intravitreal"[Title/Abstract] OR "antivascular endothelial growth factor"[Title/Abstract] OR "OCT"[Title/Abstract] OR "BVZ"[Title/Abstract] OR "dexamethasone"[Title/Abstract] OR "steroid"[Title/Abstract] OR "Intra vitreal"[Title/Abstract] OR "Intra-vitreal"[Title/Abstract] OR "avastin"[Title/Abstract] OR "Lucentis"[Title/Abstract] OR "accentrix"[Title/Abstract] OR "Aflibercept"[Title/Abstract] OR "Eyelea"[Title/Abstract] OR "ozurdex"[Title/Abstract] OR "Triamcinolone acetonide"[Title/Abstract] OR "IVTA"[Title/Abstract] OR "Conbercept"[Title/Abstract] OR "Anti-VEGF"[Title/Abstract] OR "AntiVEGF")[Title/Abstract])) AND Humans[Mesh])) AND ((((((("Hyper" OR "reflective" OR "foci" OR "central macular thickness" OR "macular volume" OR "CST" OR "CMT" OR "FT" OR "hyperreflective" OR "foveal thickness" OR "spots" OR "HRS" OR "HF" OR "Small" OR "Dense" OR "Best" OR "Corrected" OR "Visual" OR "acuity" OR "BCVA" OR "outcomes" OR "Hyper-reflective" OR "dots" OR "material" OR "points" OR "aggregates" OR "particles" OR "clumps" OR "retinal" OR "HRF" OR "HS" OR "HRD" OR "inflammatory" OR "biomarkers*" OR "Prognostic" OR "markers"))) OR (("Hyper" [Title/Abstract] OR "reflective"[Title/Abstract] OR "foci"[Title/Abstract] OR "central macular thickness"[Title/Abstract] OR "macular volume"[Title/Abstract] OR "CST"[Title/Abstract] OR "CMT"[Title/Abstract] OR "FT"[Title/Abstract] OR "hyperreflective"[Title/Abstract] OR "foveal thickness"[Title/Abstract] OR "spots"[Title/Abstract] OR "HRS" [Title/Abstract] OR "HF"[Title/Abstract] OR "Small"[Title/Abstract] OR "Dense"[Title/Abstract] OR "Best"[Title/Abstract] OR "Corrected"[Title/Abstract] OR "Visual"[Title/Abstract] OR "acuity"[Title/Abstract] OR "BCVA"[Title/Abstract] OR "outcomes"[Title/Abstract] OR "Hyper-reflective"[Title/Abstract] OR "dots"[Title/Abstract] OR "material"[Title/Abstract] OR "points"[Title/Abstract] OR "aggregates"[Title/Abstract] OR "particles"[Title/Abstract] OR "clumps"[Title/Abstract] OR "retinal"[Title/Abstract] OR "HRF"[Title/Abstract] OR "HS"[Title/Abstract] OR "HRD"[Title/Abstract] OR "inflammatory"[Title/Abstract] OR "biomarkers*"[Title/Abstract] OR "Prognostic"[Title/Abstract] OR "markers")[Title/Abstract])) OR (("Hyper" OR "reflective" OR "foci" OR "central macular thickness" OR "macular volume" OR "CST" OR "CMT" OR "FT" OR "hyperreflective" OR "foveal thickness" OR "spots" OR "HRS" OR "HF" OR "Small" OR "Dense" OR "Best" OR "Corrected" OR "Visual" OR "acuity" OR "BCVA" OR "outcomes" OR "Hyper-reflective" OR "dots" OR "material" OR "points" OR "aggregates" OR "particles" OR "clumps" OR "retinal" OR "HRF" OR "HS" OR "HRD" OR "inflammatory" OR "biomarkers*" OR "Prognostic" OR "markers")[MeSH Terms])) AND Humans[Mesh]) Filters: Journal Article; Publication date from 2011/01/01 to 2020/06/01; Humans; English	524

Appendix 2: Summary of studies reporting association between HRS and VA/CMT

Association between HRS and VA/CMT					
Author (Year)	**Results**				
Framme *et al.* (2012)[21]	No correlation between the HRS reduction and the course of VA/decrease in CMT				
Vujosevic *et al.* (2016)[41]	Weak correlation between the number of HRS and BCVA (r = -0.37)/CMT (data not shown)				
Kang *et al.* (2016)[31]	Positive association between baseline number of HRS in OR and final VA (LogMAR) in DRT ($\beta_{standardized}$=0.037; P=0.004) and CME groups ($\beta_{standardized}$=0.048; P=0.002) and between baseline number of HRS in IR and OR and final VA (LogMAR) in the SRD group ($\beta_{standardized}$=0.014, 0.024, respectively; P<0.04) The final foveal thickness showed no association with the baseline HRS counts (P>0.2 in all three groups)				
Chatziralli *et al.* (2017)[38]	Presence of HRS at baseline was associated with poorer visual outcomes (coefficient = - 6.02; CI $_{95\%}$ = - 10.12 to - 2.21; P<0.001)				
Zur *et al.* (2018)[12]	Absence of HRS at baseline predicted increased odds to gain >10 letters after 4 months (OR=5.33; $CI_{95\%}$ = 1.81-15.72; P=0.002) and good clinical response at 4 months (absent vs. present HRS: OR=3.66; CI $_{95\%}$ = 1.40-9.62; P=0.01)				
Schreur *et al.* (2018)[16]	No effect of baseline number of HRS on change in VA (3 m) ($\beta_{standardized}$ = -0.002; CI $_{95\%}$ = -0.009 to -0.004; P=0.473) The number of HRS at baseline was independently associated with a decrease in CMT (3 m) (P=0.006) Adequate responders had higher numbers of HRS at baseline than insufficient responders (21.6±9.5 versus 12.7±8.8; OR=1.106; $CI_{95\%}$ = 1.012-1.210; P=0.030)				
Hatz *et al.* (2018)[40]		HRS <15		HRS >15	*P*
	Change in VA	8.0±7.7		3.1±12.0	0.163
Fonollosa *et al.* (2019)[33]		HRS<10		HRS>21	
	Change in VA	4.1 (0.3-7.9)		4.4 (1.3-7.5)	0.336
	Change in CMT	-106.3 (59.8-152.7)		- 94.2 (34.7-153.7)	NA
Cavalleri *et al.* (2020)[15]		HRS<13		HRS>13	
	IVR (VA)	Baseline	Final	Baseline	Final
		63.3±24.2	76.3±17.1	63.9±16.7	63.1±21.3 NA
	DEX (VA)	79±15.4	84.1±15	59.6±22.2	70.1±15.6 NA
Bonfiglio *et al.* (2019)[14]		HRS present at baseline		HRS absent at baseline	
	VA	52.3±6.4	55.2±8.4	51.4±8.9	51.8±8.0 NA
	CMT	607±69	493±123	569±94	510±125 NA
Menezo *et al.* (2019)[39]		HRS present at baseline		HRS absent at baseline	
	Change in VA	7.6±11.3		8.6±15.9	0.85
	Change in CMT	-130.4 (142.6)		-102.5 (143.9)	0.49
Yoshitake *et al.* (2020)[24]		HRS present at baseline		HRS absent at baseline	
	Change in VA (LogMAR) (6 m)	0.140±0138		0.074±0.110	0.022*
	Change in VA (LogMAR) (12 m)	0.179±0.150		0.048±0.124	<0.001*
	Change in CMT	171±138		110±86	0.028*
Shulin Liu *et al.* (2019)[35]	Positive correlation between the baseline number of HRS in OR and baseline VA (r=0.42; P=0.034) Positive correlation between the baseline number of HRS in the IR, OR, and SRD and final VA (r=0.571, P=0.002; r=0.464, P=0.017; r=0.405, P=0.04, respectively) No correlation between the HRS reduction in OR and TR and increase in VA (r=0.40, P=0.043 and r=0.393, P=0.04, respectively) Positive correlation between the HRS reduction in IR and TR and decrease in CMT (r=0.422, P=0.032 and r=0.429, P=0.029, respectively)				
Narnaware *et al.* (2020)[37]	Positive but not significant correlation between the change in HRS and change in VA (LogMAR) (r=0.3343; P>0.05)				
Vujosevic *et al.* (2017)[32]	Inverse correlation between the HRS number at baseline and CMT change (q = -0.28, P=NA)				

All CMT values are measured in micrometers, VA measured in ETDRS letters unless specified; m: months; IVR: intravitreal ranibizumab; DEX: dexamethasone implant; IR: inner retina; OR: outer retina; TR: total retina; DRT: diffuse retinal thickening; CME: cystoid macular edema; SRD: subretinal detachment; NA: not available