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RETINAL HYPERREFLECTIVE FOCI IN TYPE 1 DIABETES MELLITUS

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Purpose: To investigate hyperreflective foci (HF) on spectral-domain optical coherence tomography in patients with Type 1 diabetes mellitus across different stages of diabetic retinopathy (DR) and diabetic macular edema (DME) and to study clinical and morphological characteristics associated with HF.

Methods: Spectral-domain optical coherence tomography scans and color fundus photographs were obtained of 260 patients. Spectral-domain optical coherence tomography scans were graded for the number of HF and other morphological characteristics. The distribution of HF across different stages of DR and DME severity were studied. Linear mixed-model analysis was used to study associations between the number of HF and clinical and morphological parameters.

Results: Higher numbers of HF were found in patients with either stage of DME versus patients without DME ($P < 0.001$). A trend was observed between increasing numbers of HF and DR severity, although significance was only reached for moderate nonproliferative DR ($P = 0.001$) and proliferative DR ($P = 0.019$). Higher numbers of HF were associated with longer diabetes duration ($P = 0.029$), lower high-density lipoprotein cholesterol ($P = 0.005$), and the presence of microalbuminuria ($P = 0.005$). In addition, HF were associated with morphological characteristics on spectral-domain optical coherence tomography, including central retinal thickness ($P = 0.004$), cysts ($P < 0.001$), subretinal fluid ($P = 0.001$), and disruption of the external limiting membrane ($P = 0.018$).

Conclusion: The number of HF was associated with different stages of DR and DME severity. The associations between HF and clinical and morphological characteristics can be of use in further studies evaluating the role of HF as a biomarker for disease progression and treatment response.

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Diabetic retinopathy (DR) occurs as a complication of chronic hyperglycemia, the hallmark of diabetes mellitus (DM), and may lead to vision loss, most frequently as a consequence of diabetic macular edema (DME).[1] Although therapeutic options for DR have improved with the advent of anti–vascular endothelial growth factor, only 3 of 10 people experience a visual acuity gain of 3 or more lines after 1 year.[2,3] Furthermore, the multifactorial origin of DR leads to a heterogeneous clinical manifestation, and disease progression can be highly variable.[4,5] It is, therefore, imperative that we optimize current diagnostic procedures and the therapeutic arsenal. In recent years, precision medicine is emerging, using prognostic biomarkers to establish a tailored therapeutic approach for the individual patient. Prognostic biomarkers may be of clinical or genetic origin but may also be found in imaging characteristics.

Color fundus photography (CFP) and spectral-domain optical coherence tomography (SD-OCT) are

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non-invasive imaging modalities that form the cornerstones of diagnosing and managing diabetic retinal disease. Color fundus photography is a well-established technology that enables visualization of various hallmarks of DR, including microaneurysms, hard exudates, hemorrhages, and neovascularization.[1] Spectral-domain optical coherence tomography can be used to capture cross-sectional imaging of the retina and is widely applied to detect DME and monitor treatment response to intravitreal medication, such as anti–vascular endothelial growth factor and short- and long-acting corticosteroids.[6–9] Furthermore, its high resolution enables evaluation of morphological characteristics of DR that cannot readily be observed by CFP, such as retinal layer integrity or the presence of hyperreflective foci (HF).

Hyperreflective foci are small, well-circumscribed deposits that show high reflectance on SD-OCT and were first described by Bolz et al.[10] The reflectivity of HF is similar to that of hard exudates, but due to their small size, they cannot be detected on CFP as such.[10] This resemblance in reflectivity has led to the hypothesis that HF represent precursors of hard exudates. The notion that HF reflect other DR features such as microaneurysms and hemorrhages is less likely based on reflectance characteristics.[10–12] Conversely, HF can also be detected in various other retinal diseases that are not associated with the presence of hard exudates, such as nonneovascular age-related macular degeneration.[13–15] A common factor between these retinal diseases is its inflammatory-driven nature, and it has therefore been suggested that HF are cells involved in the inflammatory response, such as aggregations of activated microglia.[15,16] Others hypothesized that HF could represent migrating retinal pigment epithelium cells or degenerated photoreceptor cells because of their association with the disruption of the photoreceptor layer.[13,17] They have been observed in patients with DR and other retinal disorders such as nonneovascular age-related macular degeneration.[13–15] However, it is uncertain whether the underlying substrate of HF is identical in these various disorders.

To reliably use HF in the risk assessment of diabetic retinal disease, a thorough understanding of the distribution of HF and their association with clinical and retinal morphological characteristics is needed. The aim of this study was therefore to analyze the distribution of HF in patients with different severity stages of DR and DME and to investigate the association between HF and clinical and morphological characteristics in a population of patients with Type 1 DM. In addition, we aimed to further explore the relationship between HF and visual acuity.

Methods

Study Population

This study was conducted in a population of patients with Type 1 DM visiting the outpatient clinic of the department of Internal Medicine at the Radboud University Medical Center for routine clinical care between September 2011 and August 2016. Patients were included if CFP and SD-OCT of the same date were available. Exclusion criteria were poor image quality and the presence of concurring retinal disease, such as age-related macular degeneration or retinal vein occlusion. This study was approved by the Medical Ethics Committee of the Radboud University Medical Center, Nijmegen, and all participants provided written informed consent. This research was conducted in accordance with the tenets of the Declaration of Helsinki.

Data Collection

All patients underwent a full ophthalmologic examination that included history taking, determination of best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, CFP, and SD-OCT. The BCVA was measured by a certified operator using the Early Treatment of Diabetic Retinopathy Study chart. Standard 7-field 35° CFP was obtained after mydriasis, according to the Early Treatment of Diabetic Retinopathy Study protocol, using the Topcon TRC 50 IX camera (Topcon Corporation, Tokyo, Japan). We acquired high-resolution 20° × 20° SD-OCT scans centered on the fovea with a volume of 25 B-scans using a Spectralis HRA-OCT device (Heidelberg Engineering, Heidelberg, Germany).

Medical charts were assessed to obtain clinical information, including age, sex, DM duration, level of mean glycated hemoglobin (HbA1c, mmol/mol), blood pressure (mmHg), body mass index (calculated as weight/height,^{2} kg/m^2), total cholesterol (mmol/L), high-density lipoprotein (HDL) cholesterol (mmol/L), and albuminuria (mg/L). The presence of microalbuminuria was defined as a urinary albumin excretion of $\geq$30 mg/L in the absence of other renal pathology.[18]

Image Grading

The level of DR was graded on CFP according to the International Clinical DR Severity Scale, distinguishing the levels "no DR," "mild nonproliferative DR (NPDR)," "moderate NPDR," "severe NPDR," and "PDR" by one experienced grader (V.S.).[19] For DME, we distinguished "mild DME," "moderate DME," and "severe DME" on CFP and SD-OCT,

based on the International Clinical DME Severity Scale.[19] The presence of hard exudates was determined on CFP. Spectral-domain optical coherence tomography scans were imported in a custom-built annotation workstation developed using MeVisLab (MeVis Medical Solutions AG, Fraunhover MEVIS, Bremen, Germany). The retinal layers in the central 3 mm of the fovea-centered B-scan were assessed for the presence of HF. We defined HF as small, round or oval-shaped, well-circumscribed dense particles with higher reflectivity than the surrounding background, and a size of <100 μm.[20] The infrared images were checked to exclude HF that corresponded to retinal vessels. The total number of HF between the inner nuclear layer and the external limiting membrane (ELM) was evaluated by means of manual annotations made by two trained graders (V.S. and A.d.B.), masked to all clinical information. The other retinal layers were excluded from analysis because the naturally high reflectivity of these layers impedes the evaluation of HF. The average of both graders was used for analysis. Furthermore, the presence of cysts, subretinal fluid, disruption of the ELM, disruption of the ellipsoid zone (EZ), and disruption of the retinal inner layers (DRIL) was graded in the central 1 mm of the fovea-centered B-scan. Disagreements between graders were solved by open adjudication. Central retinal thickness (CRT) was defined as the average thickness within a 1-mm diameter around the fovea, as derived from Heidelberg retinal mapping software with manual correction of segmentation if the automatic measurement was found to be unreliable.

Statistical Analysis

Values for continuous variables were displayed as mean ± SD in case of a normal distribution or as median with corresponding interquartile range in case of a skewed distribution. Values for categorical variables were displayed as a proportion in percentage.

For statistical analyses, BCVA was converted to the logarithm of the minimum angle of resolution. In addition, to study the distribution of HF according to BCVA, groups were created, comprising normal vision (BCVA $\geq$ 20/25), mild to moderate visual impairment (BCVA $\geq$ 20/60 and $<$20/25), and low vision (BCVA $<$ 20/60).[21] Intergrader agreement for the grading of HF was determined using an intraclass correlation coefficient. The intereye symmetry for the number of HF was assessed with a Pearson correlation coefficient (r_p). Patient characteristics were compared across the groups "no DR," "mild–moderate NPDR," and "severe NPDR–PDR" using a mixed-effects multinomial logistic regression model. We applied univariable and multivariable linear mixed-model analysis to study associations with the total number of HF and logarithm of the minimum angle of resolution BCVA as outcome measures. For the multivariable analyses, we used backward selection, eliminating variables with a P value of $\geq$0.1. Multicollinearity was checked using Pearson correlation coefficients (r_p) for parametric distributions and Spearman rank correlation coefficients (r_s) for nonparametric distributions or categorical variables. P values $<$0.05 were considered statistically significant. Analyses were conducted using SPSS version 25 (SPSS, Chicago, IL).

Results

A total of 260 patients were recruited for this study. In four patients, one eye had become blind as a consequence of diabetic retinal disease, and therefore only one eye could be evaluated. We excluded five eyes of three patients because of either age-related macular degeneration or retinal vein occlusion and eight eyes of six patients because of poor image quality. Subsequently, a total of 503 eyes of 256 patients were eligible for analysis.

We detected no signs of DR in 156 eyes (31%), mild NPDR in 177 eyes (35%), moderate NPDR in 127 eyes (25%), severe NPDR in 26 eyes (5%), and PDR in 17 eyes (3%). Regarding DME, we found no DME in 417 eyes (83%) and classified 34 eyes (7%) as having mild DME, 25 eyes (5%) as moderate DME, and 26 eyes (5%) as severe DME. Patient characteristics of these patients are shown in Table 1. Patients with mild or moderate NPDR were significantly older, had a longer duration of diabetes, higher levels of HbA1c and HDL cholesterol, and more often a history of PRP, than patients with no DR. Patients with severe NPDR or PDR had significantly higher levels of HbA1c, lower levels of HDL cholesterol and BCVA, and a more frequent history of PRP compared with patients without DR.

There was good agreement for the number of HF between the two graders, with an interrater coefficient of 0.86, 95% CI (0.81–0.90). There was a weak but significant correlation for the number of HF between both eyes of one patient (r_p 0.310, $P < 0.001$). Hyperreflective foci were detected in 414 eyes (82% of total), throughout all stages of DR and scattered through all studied retinal layers (Figure 1). Hyperreflective foci were detected in 96 of 156 eyes (62%) without DR, in 125 of 177 eyes (71%) with mild NPDR, in 108 of 127 eyes (85%) with moderate NPDR, in 21 of 26 eyes (81%) with severe NPDR, and in 14 of 17 eyes (82%) with PDR. Compared with

Table 1. Patient Characteristics According to the Groups "No DR," "Mild–Moderate NPDR," and "Severe NPDR and PDR"

	No DR (n = 156)	Mild–Moderate NPDR (n = 304)	Severe NPDR–PDR (n = 43)
Demographics			
Sex, male, n (%)	69 (44)	147 (49)	16 (37)
Age, years (mean ± SD)	50 ± 15	53 ± 13*	47 ± 15
Diabetes duration, years (mean ± SD)	28 ± 15	32 ± 12*	30 ± 11
Clinical characteristics			
HbA1c, mmol/mol (mean ± SD)	61 ± 11	66 ± 13**	64 ± 14*
Systolic blood pressure, mmHg (mean ± SD)	130 ± 14	132 ± 15	133 ± 17
Diastolic blood pressure, mmHg (mean ± SD)	73 ± 10	72 ± 11	72 ± 9
Body mass index, kg/m^2 (mean ± SD)	26 ± 4	27 ± 5	27 ± 5
Total cholesterol, mmol/L (mean ± SD)	4.7 ± 0.8	4.7 ± 0.9	4.8 ± 0.8
HDL cholesterol, mmol/L (mean ± SD)	1.56 ± 0.40	1.44 ± 0.39*	1.35 ± 0.35*
Microalbuminuria, n (%)	27 (17)	60 (20	8 (19)
Ophthalmological characteristics			
BCVA, logMAR (median, IQR)	0.00 (−0.08 to 0.10)	0.00 (−0.08 to 0.10)	0.10 (0.00 to 0.40)*
BCVA, Snellen equivalent (median, IQR)	20/20 (20/25–20/17)	20/20 (20/25–20/17)	20/25 (20/50–20/20)
History of panretinal photocoagulation, n (%)	35 (22)	108 (36)*	22 (52)**
CFP features			
Presence of hard exudates, n (%)	0 (0)	48 (16)**	15 (35)**
Optical coherence tomography features			
No. of HF, (mean ± SD)	1.6 ± 1.8	2.4 ± 2.6*	3.5 ± 4.4**
CRT, μm (mean ± SD)	280 (261–295)	280 (260–296)	284 (260–345)**
Cysts, n (%)	3 (2)	26 (9)*	11 (26)**
Subretinal fluid, n (%)	0 (0)	2 (1)	5 (12)**
Disorganization of retinal inner layers, n (%)	15 (10)	27 (9)	5 (12)
ELM disruption, n (%)	5 (3)	6 (2)	1 (2)
EZ disruption, n (%)	5 (3)	6 (2)	1 (2)

*$P < 0.05$; ** $P < 0.001$.
IQR, interquartile range; logMAR, logarithm of the minimum angle of resolution; n, number; PDR, proliferative DR.

no DR (1.6 ± 1.8), the number of HF was significantly higher in moderate NPDR (3.0 ± 3.1, $P = 0.001$) and PDR (3.0 ± 2.1, $P = 0.019$) after correction of the P value for the interaction with the presence of central DME. No significant difference was found between the number of HF in patients with mild NPDR (2.0 ± 2.1, $P = 0.058$) or severe NPDR (3.9 ± 5.4, $P = 0.801$) versus no DR (Figure 2A). Higher numbers of HF were found for mild, moderate, or severe DME when compared with no DME (3.9 ± 2.6, $P < 0.001$; 4.0 ± 3.3, $P < 0.001$ and 7.4 ± 5.7, $P < 0.001$ vs. 1.7 ± 1.7, respectively, Figure 2B). In addition, we found higher numbers of HF in patients with previous PRP than in those without previous PRP (3.3 ± 3.0 vs. 1.7 ± 2.2, $P < 0.001$). When patients presented with hard exudates, the number of detected HF was higher than when no hard exudates were present (4.6 ± 4.7 vs. 1.9 ± 2.0, $P < 0.001$, Figure 2C).

We then assessed the relationship between clinical characteristics and the number of HF. In univariable analysis, we found a longer diabetes duration ($P = 0.002$), a lower HDL cholesterol ($P = 0.005$), and the presence of microalbuminuria ($P = 0.001$) to be significantly associated with higher levels of HF (Table 2). In multivariable analysis, longer diabetes duration ($P = 0.029$), lower HDL cholesterol ($P = 0.005$), and the presence of microalbuminuria ($P = 0.005$) remained significantly associated with a higher number of HF (Table 2).

To gain insight in the relationship between HF and other morphological characteristics that can be distinguished on SD-OCT, we used mixed-model analysis

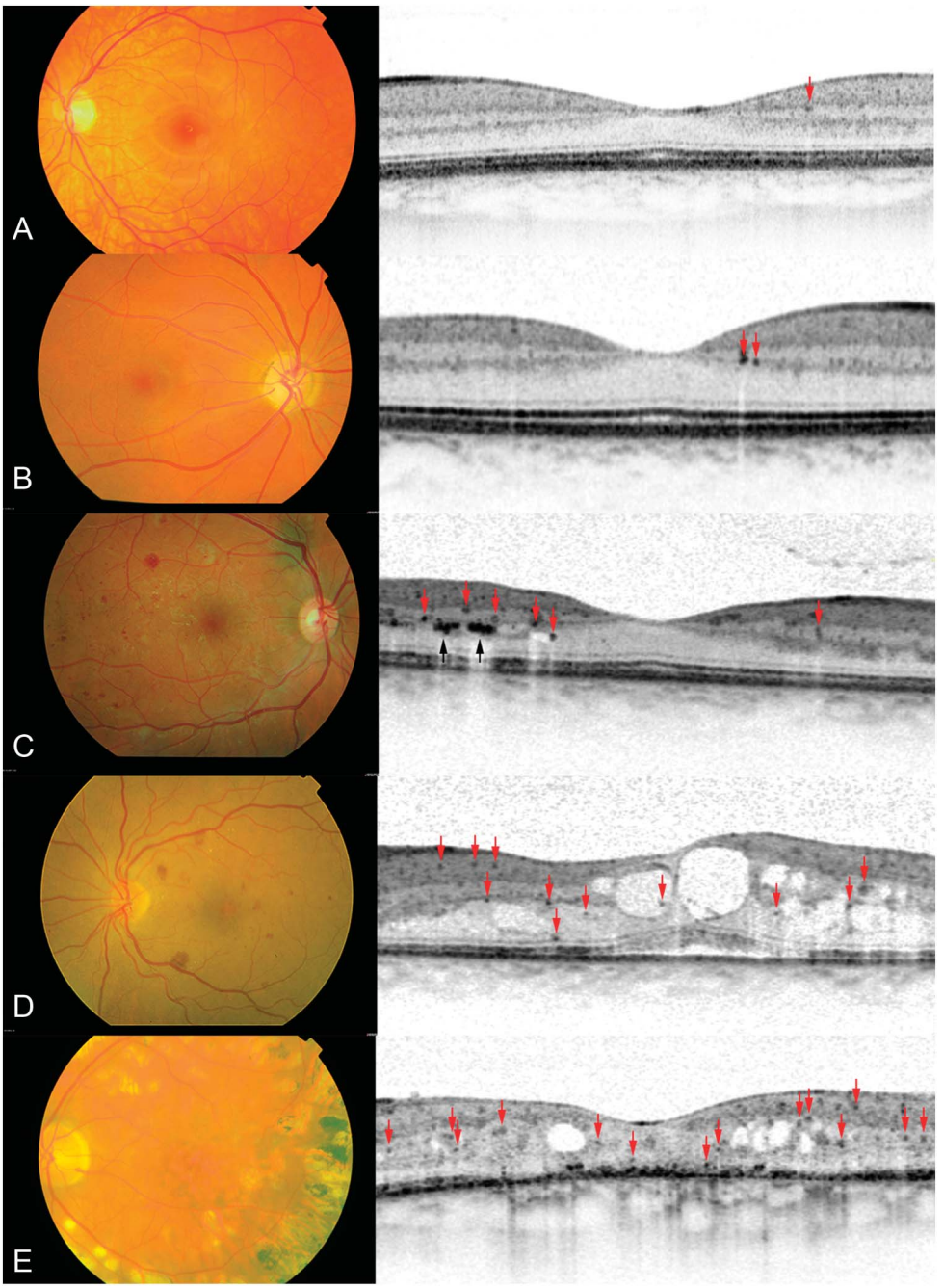

Fig. 1. Color fundus photographs with corresponding 3-mm fovea-centered optical coherence tomography scan, showing representative examples of (**A**) no DR; (**B**) mild DR; (**C**) moderate DR, showing hard exudates on both SD-OCT and CFP, as indicated by the black arrows; (**D**) severe DR with severe macular edema; (**E**) longstanding macular edema with disruption of the ELM and inner segment/outer segment layer. Red arrows indicate HF.

with HF as a dependent outcome measure. We found significant associations for CRT ($P < 0.001$), the presence of cysts ($P < 0.001$), subretinal fluid ($P < 0.001$), DRIL ($P < 0.001$), ELM disruption ($P = 0.003$), and EZ disruption ($P = 0.004$) in univariable analysis. In multivariable analysis, CRT ($P = 0.004$), the presence of cysts ($P < 0.001$), subretinal fluid ($P = 0.001$), and ELM disruption ($P = 0.018$) were significantly associated with HF (Table 3).

The number of HF was significantly higher in patients with mild–moderate visual impairment (3.5 ± 1.9) or low vision (4.5 ± 2.9) when compared to patients with normal vision (1.9 ± 2.3, $P < 0.001$ for both comparisons, Figure 2D). There was no significant difference in the number of HF between patients with mild–moderate visual impairment and low vision patients ($P = 0.148$). To further investigate the relationship between HF and phenotypic characteristics on

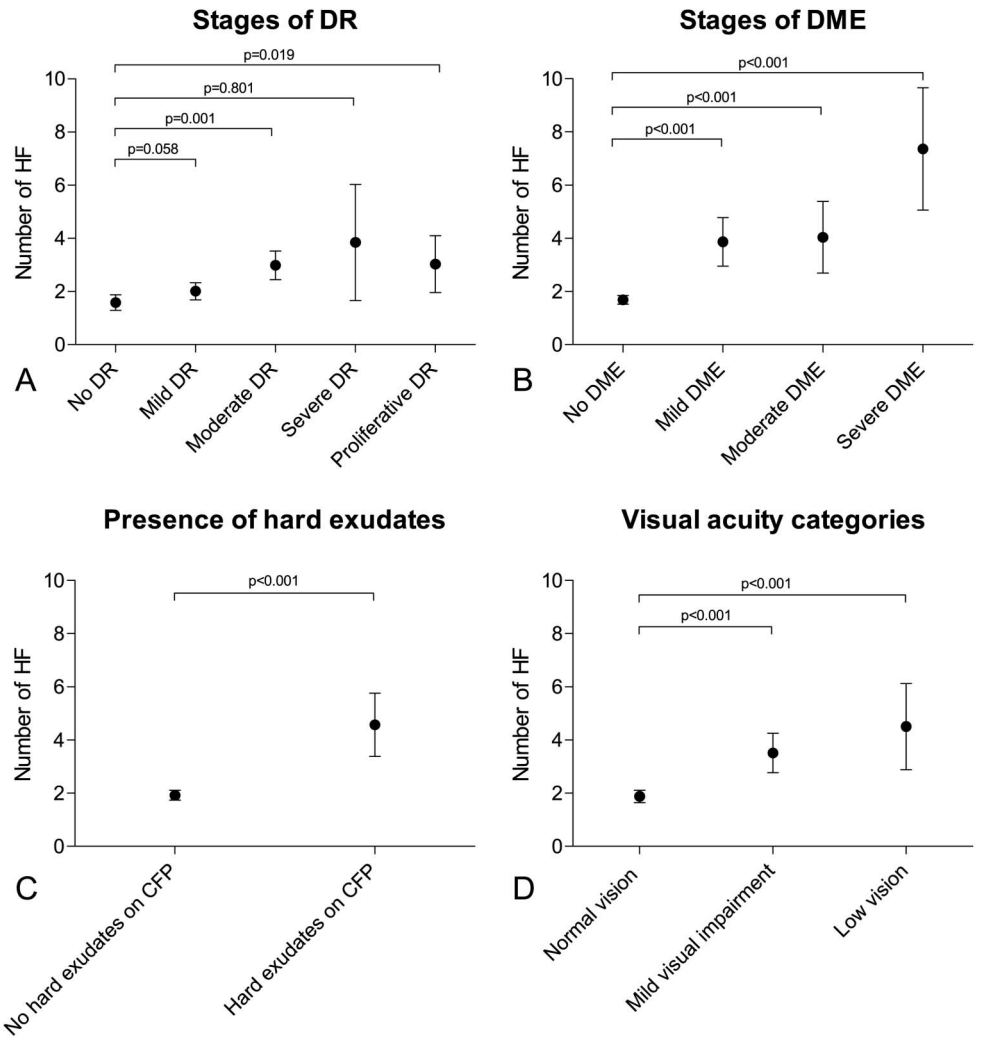

Fig. 2. Distribution of HF, according to (**A**) different stages of DR; (**B**) different stages of DME; (**C**) the presence of hard exudates; (**D**) visual acuity categories.

SD-OCT with BCVA, we performed mixed-model analysis. In univariable analysis, we found a higher number of HF, the presence of cysts, DRIL, ELM disruption, and EZ disruption to be significantly associated with worse BCVA (Table 4). In multivariable analysis, we found independent associations between higher numbers of HF and the presence of DRIL and ELM disruption with worse BCVA (Table 4).

Discussion

With this study, we provide insight in the distribution of HF across different stages of DR and DME in patients with Type 1 DM. We assessed the associations between HF and clinical characteristics, including BCVA, and other OCT parameters.

The potential clinical relevance of HF was demonstrated in previous studies showing that HF may be a predictor of treatment response to intravitreal anti–vascular endothelial growth factor or corticosteroid therapy in DME.[20,22] In the current study, we observed significantly higher numbers of HF in all stages of DME versus patients without DME. The number of HF was especially high in severe DME, suggesting the presence of a strong link between HF and exudative retinal disease. We deem it unlikely that HF will replace other commonly used morphological biomarkers that predict or define treatment success, such as CRT, but they may be used in combination with other markers, thereby optimizing monitoring and potentially predicting treatment strategies. An advantage of HF with their high reflectance is that they may be more suitable for automated detection and inclusion in prediction models than other, less quantifiable, biomarkers that have shown associations with treatment response, such as DRIL.[23,24] Moreover, the evaluation of en face OCT images instead of single B-scan could facilitate faster manual quantification of HF.[25]

Table 2. Univariable and Multivariable Linear Mixed-Model Analysis for the Association of Clinical Variables With the Number of HF

	Estimate	95% CI	*P*
Univariable			
Sex	0.40	−0.13 to 0.94	0.139
Age	0.01	−0.01 to 0.03	0.210
Diabetes duration	0.03	0.01 to 0.05	0.002*
HbA1c	0.00	−0.03 to 0.02	0.738
Systolic blood pressure	0.02	0.00 to 0.04	0.062
Diastolic blood pressure	0.00	−0.03 to 0.02	0.968
Body mass index	0.01	−0.06 to 0.07	0.864
Total cholesterol	−0.09	−0.40 to 0.23	0.593
HDL cholesterol	−1.04	−1.72 to −0.37	0.003*
Microalbuminuria	1.00	0.42 to 1.59	0.001*
Multivariable			
Diabetes duration	0.021	0.002 to 0.040	0.029*
HDL cholesterol	−0.867	−1.468 to −0.267	0.005*
Microalbuminuria	0.839	0.257 to 1.422	0.005*

*$P < 0.05$.
CI, confidence interval.

In this study, we furthermore aimed to relate the amount of HF to the severity of DR. We observed a trend for the association between higher numbers of HF and DR severity; however, these differences did not all reach statistical significance. For the early stages, our findings on the distribution of HF are in accordance with Vujosevic et al,[12] who reported a higher number of HF in patients with NPDR than in diabetic patients without DR. In patients with severe DR, there was a large variation in the number of HF, potentially due to the high prevalence of DME in this group. After correction for the presence of DME, the size of the remaining subgroup may have been too small to detect significant differences. Another reason why the relationship between HF and DR severity in our study was not strong may be that the observed area was limited to the central 3 mm surrounding the fovea. This area was selected because of its importance for visual function and because the labor-intensive nature of HF grading impedes the evaluation of multiple B-scans per person. This is logical for diseases affecting the macula, such as DME; however, diabetic retinal disease activity is not restricted to the macula, and peripheral disease activity may not correspond to macular disease activity. As for now, we may conclude that macular HF are not a clinically relevant biomarker for DR severity.

We further substantiated the relationship between HF and disease activity by the correlation we found between HF and visual acuity, in consistence with previous reports.[17,20,26] The occurrence of HF in diabetic patients may act as an early warning sign for oncoming vision loss. The exact nature of the relation of HF and visual acuity is yet unclear. It is possible that DME is the reason for visual loss in patients with high numbers of HF, but this cannot fully account for the relationship we found: HF were independently associated with BCVA loss irrespective of the hallmarks of DME (CRT and the presence of cysts). In previous studies, a direct link between HF and visual impairment through degenerated photoreceptor cells

Table 3. Correlation Between the Number of Hyperreflective Foci and Other Optical Coherence Tomography Characteristics

	Estimate	95% CI	*P*
Univariable			
CRT	0.02	0.01 to 0.02	<0.001*
Cysts	4.82	4.08 to 5.57	<0.001*
Subretinal fluid	6.84	4.95 to 8.73	<0.001*
DRIL	1.70	0.89 to 2.51	<0.001*
ELM disruption	2.31	0.81 to 3.80	0.003*
EZ disruption	2.22	0.73 to 3.72	0.004*
Multivariable			
CRT	0.01	0.00 to 0.01	0.004*
Cysts	3.89	3.11 to 4.68	<0.001*
Subretinal fluid	3.20	1.33 to 5.07	0.001*
DRIL	0.72	−0.01 to 1.45	0.054
ELM disruption	1.76	0.31 to 3.22	0.018*

Central retinal thickness was evaluated as a continuous variable, explaining why the estimate is much lower in comparison with the other variables. In multivariable analysis, EZ disruption was excluded because of the high correlation with ELM disruption and its relatively lower estimate in univariable analysis.
*$P < 0.05$.

Table 4. Univariable and Multivariable Linear Mixed-Model for the Association of Phenotypic Characteristics on SD-OCT and logMAR BCVA

	Estimate	95% CI	*P*
Univariable			
No. of HF	0.018	0.010 to 0.025	<0.001*
Cysts	0.107	0.031 to 0.182	0.006*
CRT	0.000	−0.001 to 0.001	0.916
Subretinal fluid	0.087	−0.089 to 0.263	0.331
DRIL	0.301	0.229 to 0.372	<0.001*
ELM disruption	0.762	0.645 to 0.878	<0.001*
EZ disruption	0.739	0.622 to 0.857	<0.001*
Multivariable			
No. of HF	0.010	0.003 to 0.016	0.005*
DRIL	0.152	0.844 to 0.220	<0.001*
ELM disruption	0.655	0.537 to 0.774	<0.001*

In multivariable analysis, EZ disruption was excluded because of the high correlation with ELM disruption and its relatively lower estimate in univariable analysis.
*$P < 0.05$.
CI, confidence interval; logMAR, logarithm of the minimum angle of resolution.

was suggested.[17] However, HF were associated with visual acuity, independent of EZ disruption, and we therefore hypothesize that other processes pathognomonic for DR may affect retinal tissue integrity, such as activated microglial cell aggregations and precursors of hard exudates.[10,12]

Regarding clinical patient characteristics, we found that a longer diabetes duration, lower levels of HDL cholesterol, and the presence of microalbuminuria were associated with higher numbers of HF. The HF in these Type 1 DM patients seemed to be related to several other retinal morphological abnormalities on SD-OCT, including CRT, cysts, subretinal fluid, and ELM and EZ disruption, as is in line with previous reports.[17,26–28] The variables that associated with HF are also known risk factors for DR or DME severity. To a certain extent, HF, DR severity, and the investigated clinical variables may all be related to each other, and further research should be conducted to distinguish what is the cause and what is the consequence in this case.

A strength of this study is the large sample size with patients representing all stages of DR and DME. In addition, we used a homogeneous cohort with carefully phenotyped patients with Type 1 diabetes. Our study also has several limitations. Because we only studied patients with Type 1 diabetes, our findings should be replicated in other diabetic cohorts. However, due to the shared pathophysiological mechanisms, we hypothesize that it is likely that similar results could be found in Type 2 diabetes. Another limitation is that the grading of HF is challenging and currently not standardized, which makes comparison with other studies difficult. Nevertheless, we found good interrater agreement implicating that with this grading protocol, replicable results can be obtained. In addition, the use of one fovea-centered B-scan for HF evaluation should be considered a limitation because the number of HF may vary between B-scans. Automated detection of HF will make the detection of HF more objective, easier to apply in multiple B-scans or more peripheral areas, and more feasible for application in clinical practice.

In conclusion, our results confirm that HF are associated with diabetic retinal disease. Future research should be directed toward the relationship between HF and progression of DR, and histopathological studies should be conducted to unravel the origin of HF. Although HF were directly correlated with retinal pigment epithelium cells and lipid-filled nonretinal pigment epithelium in age-related macular degeneration, their exact entity in diabetic retinal disease is still unknown.[29] Ultimately, this knowledge could contribute to further development of personalized medicine through the implementation of HF as a prognostic biomarker for oncoming vision loss and evaluation of treatment response to current and future treatments.

Key words: hyperreflective foci, diabetic macular edema, diabetic retinopathy, optical coherence tomography, image analysis.

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