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Quantitative Assessment of the Severity of Diabetic Retinopathy

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• PURPOSE: To determine whether a quantitative approach to assessment of the severity of diabetic retinopathy (DR) lesions on ultrawide field (UWF) images can provide new parameters to predict progression to proliferative diabetic retinopathy (PDR).

• METHODS: One hundred forty six eyes from 73 participants with DR and 4 years of follow-up data were included in this post hoc analysis, which was based on a cohort of 100 diabetic patients enrolled in a previously published prospective, comparative study of UWF imaging at the Joslin Diabetes Center. Diabetic Retinopathy Severity Score level was determined at baseline and 4-year follow-up visits using mydriatic 7-standard field Early Treatment Diabetic Retinopathy Study (ETDRS) photographs. All individual DR lesions (hemorrhage [H], microaneurysm [ma], cotton wool spot [CWS], intraretinal microvascular abnormality [IRMA]) were manually segmented on stereographic projected UWF. For each lesion type, the frequency/number, surface area, and distances from the optic nerve head (ONH) were computed. These quantitative parameters were compared between eyes that progressed to PDR in 4 years and eyes that did not progress. Univariable and multivariable logistic regression analyses were performed to identify parameters that were associated with an increased risk for progression to PDR.

• RESULTS: A total of 146 eyes of 73 subjects were included in the final analysis. The mean age of the study cohort was 53.1 years, and 42 (56.8%) subjects were female. The number and surface area of H/ma's and CWSs were significantly ($P \leq .05$) higher in eyes that progressed to PDR compared with eyes that did not progress by 4 years. Similarly, H/ma's and CWSs were located further away from the ONH (ie, more peripheral) in eyes that progressed ($P < .05$). DR lesion parameters that conferred a statistically significant increased risk for proliferative diabetic retinopathy in the multivariate model included hemorrhage area (odds ratio [OR], 2.63; 95% confidence interval [CI], 1.25-5.53), and greater distance of hemorrhages from the ONH (OR, 1.24; 95% CI, 0.97-1.59).

• CONCLUSIONS: Quantitative analysis of DR lesions on UWF images identifies new risk parameters for progression to PDR including the surface area of hemorrhages and the distance of hemorrhages from the ONH. Although these risk factors will need to be confirmed in larger, prospective studies, they highlight the potential for quantitative lesion analysis to inform the design of a more precise and complete staging system for diabetic retinopathy severity in the future.

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INTRODUCTION

DIABETIC RETINOPATHY (DR) IS A LEADING CAUSE of blindness in working-age individuals worldwide,[1] and its prevalence is on the rise with the ongoing epidemic of diabetes, with 360 million diabetics anticipated by 2030.[2] Mechanisms of vision loss in diabetes include macular ischemia, macular edema, and complications of proliferative diabetic retinopathy (PDR). Early detection and appropriate management of DR can prevent blindness in more than 90% of cases. Classification and staging of DR have proven to be critical to defining the pace of disease progression, risk factors for progression, and the appropriate timing of intervention. The Diabetic Retinopathy Study (DRS)[3] and Early Treatment Diabetic Retinopathy Study (ETDRS)[4] established that scatter

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pan retinal photocoagulation (PRP) should be applied without delay for eyes with high-risk PDR, but could be deferred for earlier stages provided that follow-up could be maintained.

The classification and staging systems used in the DRS and ETDRS were based on a modification of the Airlie House classification, which was first devised by a group of experts in 1968.[5] The modified Airlie House classification is based on comparing a patient's findings against reference images from stereo photographs from 7 standard photographic fields that are concentrated in the posterior pole. The reference images provide comparison standards for severity of specific lesions associated with DR, including microaneurysms (ma's), hemorrhages (H's), venous beading (VB), and intraretinal microvascular abnormalities (IRMAs). Using this modified Airlie House system, DR can be classified into 13 levels ranging from level 10 (no retinopathy) to level 85 (severe vitreous hemorrhage or macula-involved retinal detachment). Although this system has proven to be invaluable for clinical trials and clinical research, it is generally considered to be too complex for use in routine clinical practice. As a result, simplifications of the classification system have been proposed, the most common of which is the International Clinical Diabetic Retinopathy Severity Scale.[6]

Both the modified Airlie House system and the International Clinical Diabetic Retinopathy Severity Scale are based on a relatively small sample of the posterior retina (approximately 30% of the total retinal surface area). The use of this limited 7-standard field sample was in part due to the limitations of the existing camera technologies of that era. DR lesions however, can be present throughout the retina, and the lesions may not be uniformly distributed. For example, using ultrawidefield (UWF) imaging, Wessel[7] and associates demonstrated that some patents with DR could have extensive peripheral nonperfusion with relatively little evidence of DR in the posterior pole. Similarly, Silva and associates[8,9] showed that in the cohort of diabetic patients, more than 30% of the H/ma's, IRMA, and neovascularization elsewhere were distributed outside the ETDRS fields. Notably, 10% of the time, the extent of the peripheral lesions suggested a more severe assessment than what would be determined based on the EDTRS 7 fields alone. Importantly, eyes with predominantly peripheral DR (defined as more DR lesions in at least 1 peripheral field compared to its corresponding ETDRS field) had a more than 4-fold increased risk of progression to PDR at 4 years compared to eyes with predominantly central disease. DRCR.net Protocol AA is currently in progress with the aim of confirming the importance of these peripheral lesions with the results expected in the middle of 2020.

The optimal definition of predominantly peripheral disease, however, is uncertain.[10] For example, should it be defined based on the surface area or numbers of lesions? Should it be based on a single peripheral field or a combination of all fields? In addition, subjective assessment of predominantly peripheral disease can be challenging and may differ compared with assessments based on precise quantification of individual DR lesions.

Considerable progress has been made over the past decade in the objective and automated assessment of DR. A number of artificial intelligence and deep learning–based systems have demonstrated excellent performance for detecting referral-warranted DR,[11] and some health care systems have deemed such technology to be suitable for use in national screening programs.[12,13] Most initial studies applied these software tools to conventional posterior pole flash photographs such as those used for ETDRS 7-field imaging. However, we demonstrated that these automated algorithms could also be applied for the automated identification of referral warranted retinopathy on ultrawidefield pseudocolor images.[14] Furthermore, data from prospective multicenter clinical trials analyzed using deep-learning models has highlighted the importance of the predictive signal of the peripheral retinal fields, which are not routinely collected for DR assessments.[15]

Although automated algorithms for screening applications in DR appear to be well established, accurate detection and quantification of individual DR lesions represents a more significant challenge. Silva and associates used an automated tool to count H/ma's in UWF images and demonstrated a good correlation between these counts and H/ma severity within the ETDRS photo fields. They did not, however, consider other features of DR such as IRMA and cotton wool spots, and they did not assess the distribution of these lesions across the retina.[16]

In this study, we precisely quantify a range of DR lesions on UWF photos and evaluate which quantitative metrics predict progression to PDR.

METHODS

• **SUBJECTS AND IMAGING DATA:** The subjects and imaging data for this study were derived from a collection of consecutive UWF pseudocolor images of patients with DR who were recruited at the Beetham Eye Institute of the Joslin Diabetes Center as they arrived for regularly scheduled eye appointments. In the post hoc analysis described in the present report, we included 200 eyes of 100 patients who had been enrolled in a previously published prospective, comparative instrument validation study.[8,9,17] This post hoc analysis was approved by the institutional review board of the University of California, Los Angeles, and study was performed in accordance with the tenets set forth in the Declaration of Helsinki. The original data collection was approved by the institutional review board of the Harvard Medical School and patients signed written informed consent.

Patients were eligible for the initial Joslin study if they met all of the following inclusion criteria: age 18 years or

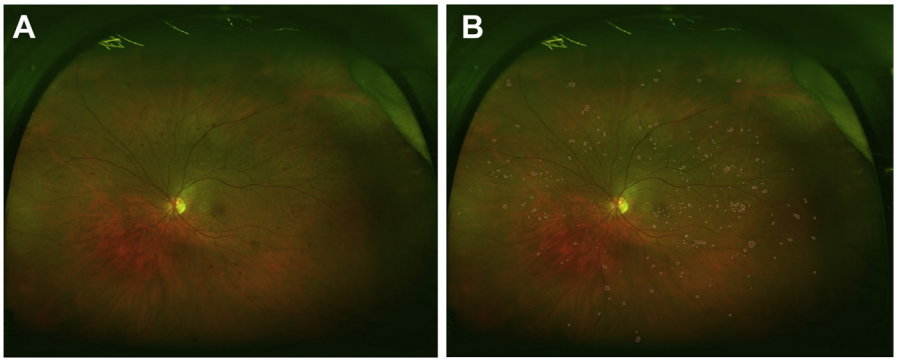

FIGURE 1. Stereographic projected ultra widefield fundus images showing diabetic retinopathy lesions (A) in an eye with severe nonproliferative diabetic retinopathy; manual segmentation of the diabetic retinopathy lesions (shaded) using GRADOR software (B).

older, diagnosis of type 1 or type 2 diabetes mellitus as defined by the American Diabetes Association, willingness to comply with the study imaging procedures, and willingness to sign the institutionally approved informed consent form for this study. Patients were excluded if they had no history of diabetes, had a history of a condition in either eye that might preclude pupil dilation, or were using eye drops (mydriatic/miotic) that would alter pupil size or reactivity. Patient enrollment was stratified to ensure the inclusion of a wide distribution of various levels of DR, ranging from no DR (ETDRS level 10) to high-risk PDR (ETDRS level 75).

Patients were dilated by topical administration of 2.5% phenylephrine hydrochloride and 1.0% tropicamide. Certified photographers obtained mydriatic nonsimultaneous stereoscopic 200° UWF images using the Optos P200MA (Optos plc, Dunfermline, Scotland, UK) in all 200 eyes of 100 patients. Nonsimultaneous stereoscopic ultrawide field images were acquired by capturing sequential images approximately 2 to 5° apart. ETDRS photographs were also obtained at baseline and again 4.2 ± 0.3 years later. DR severity was determined from these ETDRS photographs at baseline and follow-up by readers at the Joslin center as previously described, to determine which subjects progressed to PDR.

• **ULTRAWIDE FIELD IMAGE LESION SEGMENTATION:** UWF images were transferred to the Doheny Image Reading Center (DIRC) for quantitative analysis by experienced, masked graders (WT, AV, SV, CS). Images were first imported into custom-designed GRADOR software that has been described in many previous reports.[10,18–22] The boundaries of individual DR lesions relevant to DR severity assessment were manually segmented throughout the UWF image. Segmented lesions included microaneurysms (Ma's), hemorrhages, cotton wool spots (CWSs), IRMAs, neovascularization elsewhere, and neovascularization of the disc (Figure 1). All visible lesions were segmented, resulting in several hundreds of segmented lesions in some cases. Manual segmentation was used for this analysis as opposed to automated segmentation (used in prior reports),[16] as we wanted to be able to precisely compute area as well as numbers of lesions. Regions of venous beading were not manually segmented as it was uncertain to graders as to how to delineate the borders of such lesions or the area of involvement precisely. In addition, lipid exudates were not segmented as they have not been included as a feature in DR severity scoring.

Before computation of individual DR lesion statistics, manually segmented images were stereographically projected using proprietary manufacturer's software to a format that unifies the projection of the curved retina onto the imaging plane, accounting for differences in gaze direction, and pixel-to-micron ratios throughout the retina.[23,24] It should be noted that this transformation was determined from optical models of the UWF systems and Navarro model eye. As a result, some small discrepancies in measurement may be expected with nonemmetropic eyes or eyes with long or short axial lengths. However, performing this transformation allows for lesion sizes and distances between lesions to be expressed in real physical dimensions (mm^2 and mm).

• **QUANTITATIVE LESION PARAMETERS AND STATISTICAL ANALYSIS:** With progression to PDR in 17 (11.6%) of 146 eyes, this study was 76% powered to detect significance associations ($P < .05$). DR lesion frequencies for each lesion type, total surface areas of individual lesions, and average distance from the optic nerve head (ONH) center to each individual lesion were computed for each eye.

The list of quantitative parameters generated in this study are shown in Table 1. For the purpose of analysis and to align with previous DR severity assessment approaches, we also combined microaneurysm + hemorrhages into a single lesion type termed "H/ma's."

TABLE 1. Comparison of Demographic/Systemic Factors and Quantitative Diabetic Retinopathy Lesion Parameters in Eyes That Did and Did Not Progress to Proliferative Diabetic Retinopathy in 4 Years

Parameter	Did Not Progress to PDR, Mean ± SD (Median; Range) (n=129 Eyes)	Progressed to PDR, Mean ± SD (Median; Range) (n=17 Eyes)	*P*
Age (y)	54.08 ± 13.94 (56; 25.20-88.33)	58.54 ± 12.98 (60.76; 27.76-74.55)	.31
HbA_{1c} (average of 1-year data)	7.70 ± 1.06 (7.55; 6-10)	8.11 ± 0.70 (8.165; 7-9)	.36
HbA_{1C} (average of 2-year data)	7.91 ± 1.56 (7.78; 5.17-15.20)	9.14 ± 2.77 (8.49; 7.16-15.20)	.09
DM duration (years)	24.64 ± 11.83 (23; 2-49)	20.83 ± 8.80 (19.50; 9-40)	.35
Hemorrhage count	31.05 ± 38.07 (18.5; 0-205)	71.25 ± 41.31 (63; 19-147)	**<.001**
Microaneurysm count	2.58 ± 7.23 (0; 0-54)	7.5 ± 21.19 (0; 0-80)	.07
H/ma count	33.63 ± 41.44 (21.5; 0-216)	78.75 ± 49.23 (64; 19-170)	**<.001**
Cotton wool spot count	0.24 ± 0.87 (0; 0-7.87)	0.7 ± 1.12 (0; 0-3.04)	**.05**
IRMA count	0.09 ± 0.46 (0; 0-3)	0.13 ± 0.35 (0; 0-1)	.78
Hemorrhage total surface area (mm^2)	0.7 ± 1.19 (0.31; 0-9.18)	2.02 ± 1.36 (1.67; 0.43-4.3)	**<.001**
Microaneurysm total surface area (mm^2)	0.03 ± 0.06 (0; 0-0.46)	0.06 ± 0.17 (0; 0-0.61)	.06
H/ma total surface area (mm^2)	0.72 ± 1.2 (0.32; 0-9.22)	2.07 ± 1.42 (1.67; 0.43-4.45)	**<.001**
Cotton wool spots total surface area (mm^2)	0.27 ± 0.94 (0; 0-7.88)	1 ± 3.16 (0.02; 0-12.74)	**.03**
IRMA total surface area (mm^2)	0.04 ± 0.24 (0; 0-2.41)	0.05 ± 0.17 (0; 0-0.68)	.88
Hemorrhage average distance from the ONH center (mm)	9.48 ± 6.59 (11.19; 0-20.88)	13.64 ± 2.93 (13.41; 8.27-19.04)	**.02**
Microaneurysm average distance from the ONH center (mm)	1.44 ± 3.85 (0; 0-17.84)	1.59 ± 4.33 (0; 0-12.86)	.85
H/ma average distance from the ONH center (mm)	10.91 ± 8.28 (12.15; 0-33.74)	15.22 ± 5.14 (14.01; 8.27-27.4)	**.04**
Cotton wool spot average distance from the ONH center (mm)	0.82 ± 2.87 (0; 0-18.14)	2.92 ± 5.16 (0; 0-17.03)	**.01**
IRMA average distance from the ONH center (mm)	0.23 ± 1.65 (0; 0-18.1)	0.69 ± 2.76 (0; 0-11.04)	.31

DM = diabetes mellitus, HbA_{1c} = glycated hemoglobin A_{1c}, H/ma = hemorrhage and microaneurysm, IRMA = intraretinal microvascular abnormality, ONH = optic nerve head, PDR = proliferative diabetic retinopathy. Significant *P*-values are denoted in bold.

Imaging descriptive statistics (count, area, and distance to the ONH) were computed for each lesion type. The frequency distribution of each DR lesion with respect to baseline Diabetic Retinopathy Severity Score (DRSS) level are presented in box plots with error bars. Univariate and multivariate logistic regression analysis was performed to assess the relationship between these various quantitative DR lesion risk factors, demographic factors (age, gender), average glycated hemoglobin A_{1c} for 1 year and 2 years, duration of diabetes mellitus, and baseline DRSS (considered as independent variables) and progression to PDR over the follow-up period (considered as the dependent variable). From the univariate analysis, variables with $P \leq .05$ were included in the multivariate logistic regression analysis to derive the final model. Generalized estimating equations were used to adjust for correlation between the 2 eyes of the same subject. All statistical analyses were performed using SPSS for Windows version 18.0 (SPSS Science, Chicago, IL). A *P* value ≤.05 was considered significant.

RESULTS

OF THE 200 EYES INITIALLY SELECTED FOR THIS POST HOC analysis, 50 of the eyes (25%) did not have data from the 4-year follow-up visit and were excluded. An additional 4 of the eyes (2%) were excluded because the quality of the baseline UWF images was not sufficient to allow the graders to reliably segment all DR lesions. Thus, the final analysis cohort included 146 eyes from 74 subjects. At the 4-year follow-up visit, 17 (11.6%) of these 146 eyes were graded to have progressed to PDR. The mean age of the study cohort was 54.47 years (SD, 14.74; range 18-88) and 61 subjects (41.7%) were female. A total of 7,432 DR lesions were segmented on 146 UWF images. The frequency of baseline DR lesions according to the baseline DRSS level are shown in Figure 2. The number of hemorrhages generally increased with increasing DRSS level, but the relationship with microaneurysms was not as clear. Few eyes had IRMA lesions identified during segmentation of individual lesions on UWF images in this cohort.

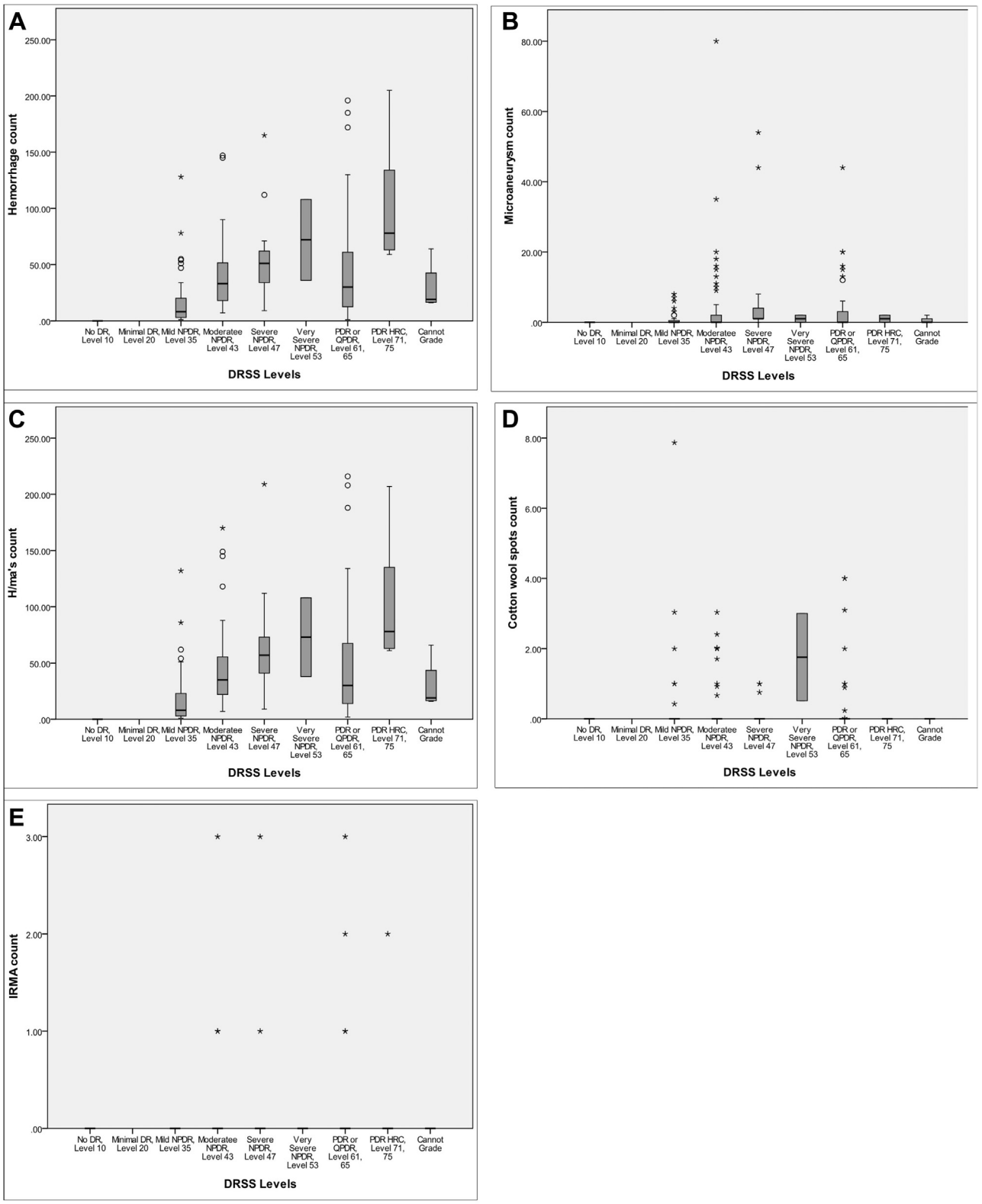

FIGURE 2. Frequency of diabetic retinopathy lesions on ultrawidefield images according to the level of diabetic retinopathy classified using the Diabetic Retinopathy Severity Scale (DRSS) on 7 standard Early Treatment Diabetic Retinopathy Study fields. Hemorrhages (A), microaneurysm (B), hemorrhages/microaneurysms (H/ma's) (C), cotton wool spots (D), and intraretinal microvascular abnormalities (IRMAs) (E).

TABLE 2. Regression Analysis to Evaluate the Effect of Demographic or Systemic Factors and Various Quantitative Diabetic Retinopathy Lesion Parameters on Progression to PDR

	Univariate Analysis			Multivariate Analysis		
Parameter	Odds Ratio	95% CI	*P*	Odds Ratio	95% CI	*P*
Age	1.04	1.00-1.08	.06	1.03	0.85-1.25	.76
Gender						
Female	1					
Male	2.36	0.80-6.94	.12	—	—-	—
HbA_{1C} (average of 1-year data)	1.42	0.63-3.20	.39	—	—-	—
HbA_{1C} (average of 2-year data)	1.42	0.99-2.04	.06	1.85	0.30-11.60	.52
DM duration (years)	0.97	0.93-1.02	.26	—	—-	—
Hypertension						
No	1					
Yes	2.78	0.60-12.92	.19	—	—-	—
Baseline DR status						
Baseline Diabetic Retinopathy Severity Score						
No/minimal/mild NPDR	1			1		
Moderate NPDR	4.64	0.93-23.16	.06	3.12	0.36-27.47	.31
Severe NPDR	11.79	1.89-73.58	**.008**	12.26	1.08-138.70	**.04**
Hemorrhage count	1.02	1.01-1.03	**.001**	0.99	0.96-1.03	.67
Microaneurysm count	1.03	1.00-1.07	.07	0.98	0.92-1.05	.62
H/ma count	1.02	1.01-1.03	**< .001**	0.99	0.96-1.03	.72
Cotton wool spot count	1.4	0.96-2.05	.08	0.94	0.44-2.05	.89
IRMA count	1.12	0.43-3.17	.78	—	—	—
Hemorrhage total surface area	1.63	1.21-2.20	**.001**	7.45	1.75-31.75	**.007**
Microaneurysm total surface area	NA	NA	NA	—-	—	—
H/ma total surface area	1.63	1.21-2.18	**.001**	7.44	1.73-32.01	**.007**
Cotton wool spot total surface area	1.26	0.98-1.62	.07	0.8	0.41-1.53	.49
IRMA total surface area	1.18	0.17-8.25	.86	—	—	—
Hemorrhage average distance from the ONH center	1.14	1.02-1.27	**.02**	1.1	1.02-1.26	**.049**
Microaneurysm average distance from the ONH center	1.01	0.89-1.15	.89	—	—	—
H/ma average distance from the ONH center	1.07	1.00-1.14	**.046**	1.06	1.04-1.17	**.049**
Cotton wool spot average distance from the ONH center	1.14	1.02-1.27	**.02**	1.16	0.87-1.54	.32
IRMA average distance from the ONH center	1.1	0.91-1.33	.34	—	—	—

DM = diabetes mellitus, HbA_{1C} = glycated hemoglobin A_{1c}, H/ma = hemorrhage and microaneurysm, IRMA-Intraretinal Microvascular Abnormality, NPDR = nonproliferative diabetic retinopathy, ONH = optic nerve head, PDR = proliferative diabetic retinopathy.

Factors with significance levels ≤.010 in the univariate model were adjusted for the multivariate model. NA indicates variables were not included in the model because of the small sample size. Significant *P*-values are denoted in bold.

• **COMPARISON OF LESION PARAMETERS IN PROGRESSED AND NONPROGRESSED EYES:** *Lesion number*. The mean number of baseline H/ma's in eyes that did not progress to PDR in 4 years was 33.63 ± 41.44, which was significantly lower compared with eyes that did progress to PDR, 78.75 ± 49.23 ($P < .0001$). Of note when H/ma's were further subdivided into hemorrhages and ma's individually, only hemorrhage count showed a significant difference between progressed and nonprogressed eyes (though ma's showed a trend; $P = .07$). CWSs also appeared to be more frequent in eyes that progressed to PDR though there was no significant ($P > .05$) difference in number of IRMA between nonprogressed and progressed eyes (Table 1).

Lesion surface area. The mean lesion surface area of H/ma's was significantly (<.001) greater in progressed eyes (2.07 ± 1.42 mm^2) when compared to eyes that did not progress (0.72 ± 1.2 mm^2) to PDR by 4 years, which again appeared to be driven primarily by the hemorrhages, which

remained significant when considered individually, though the ma's did not (though with a trend, $P = .06$). Mean CWS surface area was significantly ($P = .03$) higher in progressed eyes compared with nonprogressed eyes, whereas the IRMA surface area did not significantly differ ($P > .05$) (Table 1).

Lesion distance from the ONH center. H/Ma's were also significantly ($P = .04$) further from the center of the ONH in progressed eyes (15.22 ± 5.14 mm) compared with nonprogressed eyes (10.91 ± 8.28 mm). Similarly, CWSs were also significantly ($P = .01$) further from the ONH in progressed eyes when compared to eyes that did not progress. There was no significant difference, however, in the IRMA distances from the ONH center between the groups (Table 1).

• **REGRESSION ANALYSIS:** The results of the univariate and multivariate models are shown in Table 2. In the univariate analysis, PDR progression was associated with the presence of severe nonproliferative diabetic retinopathy at baseline ($P = .008$), hemorrhage count ($P = .001$), H/ma count ($P < .001$), hemorrhage surface area ($P = .001$), H/ma surface area ($P = .001$), hemorrhages' distance from the ONH center ($P = .02$), H/ma distance from the ONH center ($P = .046$), and CWS distance from the ONH center ($P = .02$). Significant factors with a $P \leq .10$ were included in the multivariate analysis. In multivariate logistic regression, baseline severe nonproliferative diabetic retinopathy (odds ratio [OR] 12.26, 95% confidence interval [CI] 1.08-138.70, $P = .04$), hemorrhage total surface area (OR 2.63, 95% CI 1.25-5.53, $P = .01$), and surface area of H/ma's (OR 7.44, 95% CI 1.73-32.01, $P = .007$) seemed to have a significantly increased risk of progression to PDR. Eyes with H/Ma's farther away from the ONH were also at significantly higher risk of progression to PDR at 4 years (OR 1.06, 95% CI 1.04-1.17, $P = .049$). Demographic and systemic factors such as age, gender, hemoglobin A_{1c}, and duration of diabetes mellitus were not significant risk factors for progression, though there was a trend for higher HbA_{1c} over 2 years to predict progression. In the multivariable analysis, DR lesion frequencies and counts no longer remained as independent risk factors for PDR progression. Distance of CWSs also no longer remained in the multivariate model. No IRMA characteristics, including frequency, surface area, or distance, were identified as significant risk factors in the regression analysis (Table 2). The regression model only including the quantitative retinal imaging parameters is shown in Supplementary Table S1 and revealed similar findings.

DISCUSSION

IN THIS STUDY, WE USED A QUANTITATIVE APPROACH TO evaluate the importance of specific DR lesions throughout the fundus on the risk of progression to PDR. Our analysis revealed that both the number and surface area of intraretinal H/ma, and in particular hemorrhages further from the foveal or ONH center, appeared to be associated with an increased risk for progression. Interestingly, when this quantitative scoring approach was used, more peripheral CWSs, but not IRMAs, were identified as a potential risk factor for progression to PDR. It should be noted, however, that CWSs have been used as a marker for subtle IRMA, and the association with CWSs may reflect subtle IRMA that were not detected during grading of the UWF images.

Fundus risk factors for progression to PDR have been well-established for several decades based on a number of landmark clinical studies including the DRS and ETDRS studies. EDTRS investigators identified that the severity of IRMA, H/ma's, and venous beading were the most important, independent characteristics that appeared to predict the risk of progression from NPDR to PDR.[25] These observations were critical in the development of a severity scale for DR that has been the criterion standard for clinical practice and for subsequent clinical trials. Notably, the definition of severe NPDR (on both the ETDRS scale and the simplified International Clinical Diabetic Retinopathy scale) is based on these 3 key DR features.

The DRSS and staging system built on these risk factors has become even more critical in recent years as new pharmacotherapeutic agents have been shown to have an impact on these same features that appear to confer risk for progression. For example, in the RISE and RIDE trials, Ip and associates noted a significant regression in DR (improvement by ≥2 or ≥3 steps on the EDTRS DRSS) in ranibizumab-treated eyes compared to sham-treated eyes at 2 years.[26] Wykoff and associates[27] demonstrated that DR regression was most pronounced or frequent in eyes with more severe levels of NPDR at baseline, with a ≥2-step improvement in 78.4% of eyes with moderate-severe to severe NPDR compared with only 10.3% in eyes with mild-moderate NPDR. Importantly, in the open label extension of the RISE and RIDE studies, more than 70% of patients were able to maintain the improvement in DRSS level after converting to as-needed therapy between years 3 and 4.[28] Secondary and post hoc analyses of the VIVID and VISTA trials also demonstrated similar regression of DR in aflibercept-treated patients.[29] These initial observations were made in patients who were undergoing treatment with anti–vascular endothelial growth factor therapy for diabetic macular edema, but they highlighted the possibility of early intervention and treatment of DR before progression to high-risk PDR. The effectiveness of such an approach was evaluated in the phase 3 PANORAMA study, which was a randomized, double-masked clinical trial comparing aflibercept 2 mg given every 8 or 16 weeks versus sham in eyes with moderate-severe or severe NPDR (DRSS level 47 and 53). At 1 year (results presented by Wykoff C, Angiogenesis

Meeting, Miami, February 2019), 65.2% and 79.9% of patients showed a ≥2-step improvement in DRSS level in the groups receiving aflibercept every 8 or 16 weeks, respectively, compared with only 15% in the sham-treated patients. These results (not yet published) led to FDA clearance for NPDR as an approved indication for aflibercept.

Despite the widespread clinical utilization and impact of the DRSS established by the EDTRS, it is important to recognize the limitations of this scale, which in large part are based on the limitations of the available imaging technology of that era (eg, analog/film-based, limited field of view). In particular, the severity or extent of specific DR features was based on comparison to standard or reference photographs. Thus, the number of different severity standards, in large part, dictated the number of possible severity levels for specific features. When tracking the history of these standard photos back to the original Airlie House system, the number of severity standards was somewhat arbitrarily chosen.[5] In addition, as the system was reference photo based, the incremental difference in severity was almost assuredly not linear. It is also uncertain as to whether the system was sufficiently granular or had sufficient dynamic range to differentiate all levels of DR progression risk that may be of clinical importance. A more granular or precise quantitative scoring system may be of particular interest at present to aid in monitoring DR regression after anti–vascular endothelial growth factor therapy.

Another significant limitation of the EDTRS-based DRSS staging system is that it is based on a presumed representative sample of the retina. It is well established that extensive DR lesions may be present beyond the 7 standard ETDRS fields, and that DR lesions may not be uniformly distributed across the retina.[7] Silva and associates[8,9] highlighted the potential importance of these peripheral lesions in a pilot study that demonstrated a more than 4 times higher risk of progression to PDR in eyes with predominantly peripheral disease. The present study in fact utilizes this previously reported data set for the new quantitative analysis. DRCR.net protocol AA is in progress and will ultimately confirm or refute the importance of these peripheral DR lesions.

Determination of predominantly peripheral DR, however, is a subjective assessment and may not fully describe or capture the importance of more peripherally positioned lesions in DR. We have already demonstrated that this determination may vary based on subtle changes in the definition such as the use of lesion number vs lesion area, or any field vs all fields.[10] Also, it seems unlikely that the location of the border between the ETDRS field and the corresponding peripheral field is fortuitously the optimal position in the retina to differentiate a peripheral vs a central lesion. It would seem logical that a continuous measure of the eccentricity of a particular lesion would be preferred. Given that our retinal images are digital and automated techniques are becoming available to analyze DR images, a more quantitative approach to assessing DR severity would appear to be feasible. In addition, if lesions throughout the fundus are considered, the relative importance and relevance of specific DR lesions may be impacted. For example, IRMA are generally considered to be a more severe or advanced lesion compared to H/ma's. However, most peripheral DR lesions are H/ma's, and inclusion of these peripheral lesions may increase the relative importance of H/ma's to the overall risk prediction. Indeed, some DR lesions that appear to be predictive when only the 7 standard fields are considered may no longer remain as independent predictors when the entire retina is assessed. For example, extent (count or area) of IRMA was not associated with an increased risk of progression to DR in our analysis. We must acknowledge, however, that this may be an artifact of the relatively small number of cases graded to have IRMA in our analysis, and will need to be confirmed in future larger studies.

Silva and associates used an automated tool to count H/ma's on UWF images and standard ETDRS photos and showed good agreement between the UWF and ETDRS assessment of H/ma severity within the region covered by the 7 standard fields.[16] Counting numbers of lesions is an easier task than precisely segmenting the border of individual lesions, which is a prerequisite if the size and area of lesions is to be assessed. We did not find the performance of existing automated tools to be satisfactory for precise lesion border segmentation, but we expect such tools to become available with continued progress in artificial intelligence–based segmentation systems for planar images.[30,31] In the interim, for the present analysis, we used an exhaustive manual segmentation approach to quantify H, ma's, CWSs, and IRMA lesions.

After the multivariable analysis, the parameters that remained significant independent predictors of progression to PDR at 4 years were total area of H/ma's and average distance of H/ma's from both the foveal center and ONH center. As an exploratory analysis, graders also attempted to differentiate ma's from hemorrhages, and the hemorrhage area and distance appeared to be the key driver of increased risk. However, we are doubtful of the ability to reliably differentiate ma's from dot hemorrhages. The relatively low observed ma counts would appear to suggest that ma's were likely significantly underestimated and likely confused for dot hemorrhages. It is also well known that many more ma's are visible by fluorescein angiography than on color photos,[32] further highlighting likely inaccuracies in pure ma-based assessments. Notably, although the number of H/ma was an important risk factor in the univariate analysis, only area of these lesions remained significant in the multivariable analysis. Because ma's are likely to contribute minimally to total area of lesions when considered in the context of large hemorrhages, underdetection of ma's may not significantly diminish the performance of risk prediction models using lesion area. The observation that H/ma lesion area was a significant independent risk factor, however, may imply that developing tools for precise

segmentation of H/ma borders may be a priority for future technical development. Importantly, H/ma and hemorrhage area remained independent predictors of progression even when the DRSS level (specifically the presence of severe NPDR) was included in the multivariable model. This suggests that lesion area is not fully accounted for in the current DRSS staging approach. This is perhaps not surprising as the DRSS level is based more on assessment of number of lesions relative to the standard photographs rather than on surface area.

More significantly, the results of our analysis would suggest that lesion location is of great importance in predicting risk of progression to PDR. Eyes with H/ma's or lesions further from the center of the ONH on average appeared to be at higher risk for progression. This finding would appear to be consistent with the previous report from Silva and associates[9] using this cohort that highlighted that predominantly peripheral disease conferred a higher risk for progression to PDR. It is notable that we observed that more "peripherally" placed CWSs, but not the extent (number of area) of CWSs, was associated with a higher risk in the univariate analysis. CWSs tend to be more centrally distributed compared to H/ma's (Table 1). However, even with this more central distribution, a more eccentric CWS would appear to confer greater risk. CWS distance, however, did not remain in the multivariable model.

The concept that more peripheral retinopathy may be associated with higher risk for progression to PDR may have important implications for the pathophysiology and management of DR. Recent data using deep-learning models to predict DR progression on clinical trial images has further emphasized the importance of the peripheral retinal fields wherein the main predictive contribution came from retinal areas away from the ONH and fovea.[15] More peripheral regions of the retina at the distal extremes of the retinal vascular blood supply may be more susceptible to ischemic injury, and may be the first to manifest large areas of ischemic retina that can drive the development of neovascularization. Increasing peripheral hemorrhages and CWSs may be a reflection of this progressive ischemia. An eye that is showing a progressive increase in the eccentricity of DR lesions may be the one that is most likely to benefit from preventative anti–vascular endothelial growth factor therapy. This hypothesis, of course, will need to be tested in future large prospective studies.

There are several limitations of our study that must be considered when assessing our findings. First, our project was based on a relatively small data set and represents a post hoc analysis of prospectively collected study. As such, this is still a pilot analysis, which requires replication and validation. For example, we had relatively few IRMA lesions graded in our cohort. This may be due to underdetection of IRMA on the UWF images or a low frequency of IRMA in these eyes. It is possible that with a larger data set including more cases with IRMA or other DR lesions, IRMA may remain as a significant risk factor for progression to PDR in the analysis. Second, only 17 eyes progressed to PDR during the course of our study, and thus our study may have been underpowered to detect weaker associations. Third, although we considered a variety of DR lesions and quantitative parameters, we did not assess or quantify venous beading. This was primarily because of uncertainty as to how to segment areas of beading. It is possible that inclusion of venous beading could improve the predictive performance of future scoring systems. However, the main purpose of the present analysis was to highlight the advantages of a quantitative approach and to demonstrate that novel measurable parameters such as lesion surface area and lesion eccentricity could be important for predicting DR progression. Another limitation of our study is that it was based on manual segmentation of the DR lesions, an approach that is clearly not practical for clinical care. On the other hand, with advances in deep learning, one would anticipate that accurate automated segmentation should be feasible in the future. Finally, although we evaluated a number of parameters, there are many other possible parameters that could be of value. For example, rather than just the distance or surface area, homogeneity or lesion density within specific fundus regions may be predictive. These additional parameters could be evaluated in future prospective data sets.

In summary, the approach to DR lesion assessment described in this report may represent the first step in developing a truly quantitative and precise DR scoring system. Lesion type, area/number, and distance can all serve as components or elements in a formula where the weights of the various elements are tuned based on outcomes from prospective studies such as the DRCR.net Protocol AA. A quantitative approach to DR severity assessment takes better and more complete advantage of the information encoded in our current digital widefield imaging tools.

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