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Association of Disorganization of Retinal Inner Layers With Visual Acuity Response to Anti–Vascular Endothelial Growth Factor Therapy for Macular Edema Secondary to Retinal Vein Occlusion

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IMPORTANCE Disorganization of retinal inner layers (DRIL) has demonstrated significant correlations with visual acuity (VA) in center-involved diabetic macular edema. In patients with retinal vein occlusion (RVO) and secondary macular edema, DRIL may be a useful biomarker in determining VA outcomes.

OBJECTIVE To examine whether DRIL at baseline and after treatment is associated with VA in RVO.

DESIGN, SETTING, AND PARTICIPANTS A retrospective review of records of 147 patients 18 years or older with treatment-naive branch RVO (BRVO), central RVO (CRVO), or hemispheric RVO (HRVO), with a minimum of 12 months of follow-up, who presented to a tertiary ophthalmic center from December 1, 2010, to January 1, 2016, was conducted. Data collection continued through January 2017. Exclusion criteria included active confounding retinal or ocular disease, history of pars plana vitrectomy, or prior intravitreal injections. Two masked graders calculated a DRIL score based on DRIL presence in 3 predefined regions on spectral-domain optical coherence tomography at baseline, 6 months, and 12 months. A third masked grader was used for discrepancies.

EXPOSURES Anti–vascular endothelial growth factor (AVF) therapy (ranibizumab, aflibercept, or bevacizumab) determined by the treating physician.

MAIN OUTCOMES AND MEASURES The DRIL score at baseline for determining VA outcomes and correlation of VA with changes in DRIL burden in response to AVF therapy.

RESULTS In the 147 patients (mean [SD] age, 68.9 [13.1] years; 75 [51.0%] female), baseline DRIL was seen in 91 eyes (61.9%). In the BRVO group but not the CRVO group, baseline DRIL was associated with lower baseline Early Treatment Diabetic Retinopathy Study (ETDRS) score (score of 66.7 for no DRIL vs 54.6 for DRIL, $P = .002$). Absence of DRIL at baseline in the CRVO/HRVO group correlated with greater VA gains at 6 months, adjusting for baseline VA (score change of 19.50 for no DRIL vs 12.72 for DRIL; $P = .04$). During 12 months, continued DRIL presence in BRVO was associated with less VA gain up to 6 months (score change of 6.2 for the DRIL increase group vs 18.6 for the DRIL decrease group vs 2.9 for the DRIL stable group; $P = .02$). Increasing DRIL scores in CRVO/HRVO were associated with reduced VA improvement at 6 months (score change of −0.12 for the DRIL increase group vs 16.90 for the DRIL decrease group vs 8.45 for the DRIL stable group; $P = .002$) and 12 months (score change of −1.91 for the DRIL increase group vs 17.83 for the DRIL decrease group vs 6.97 for the DRIL stable group; $P < .001$).

CONCLUSIONS AND RELEVANCE Baseline DRIL presence and DRIL burden changes with AVF therapy for macular edema secondary to RVO may be useful biomarkers of ETDRS score improvements.

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Retinal vein occlusion (RVO) is the second most common form of retinal vascular disease; obstructions can occur in the central retinal vein or one of its branches.[1] As vascular permeability increases, secondary intraretinal fluid and subretinal fluid commonly accumulate in the outer plexiform layer and subretinal space, respectively, and can lead to long-term disorganization of the ellipsoid zone.[2] These findings, along with other complications such as macular ischemia and neovascularization, threaten visual acuity (VA).

Disorganization of retinal inner layers (DRIL) is the extent to which there is a failure in recognition of any of the demarcations between the ganglion cell-inner plexiform layer complex (evaluated as a single layer complex because the interface between the ganglion cell layer and the inner plexiform layer is not easily visible on retinal scans), inner nuclear layer, and outer plexiform layer.[3] Foveal DRIL is further defined as the inability to distinguish boundaries between any 2 of these inner retinal layers in more than 50% of the foveal 1-mm zone. Foveal DRIL can be present with or without other macular pathologic findings, such as cystoid macular edema.

Disorganization of retinal inner layers has demonstrated a correlation with VA in patients who have current or resolved center-involving diabetic macular edema.[3-6] Its utility as a biomarker of VA in these patients is greater than other markers, such as retinal thickness or glycemic status.[7] The proposed mechanism is that DRIL indicates specific anatomical damage of the visual data transmission structure in the retinal layers.[5] In addition, the presence of DRIL detects macular capillary nonperfusion in patients with severe nonproliferative and proliferative diabetic retinopathy. This evidence and the identification of DRIL in other ischemia-related pathologic conditions (ie, acute retinal necrosis[8] and closed globe trauma[9]) suggest that DRIL is a sign of poor inner retinal circulation.[6]

Disorganization of retinal inner layers has demonstrated importance as a biomarker in patients with diabetic retinopathy, macular edema, and, to a lesser extent, RVO.[7,10] This study further explores the association of DRIL and VA before and during treatment with anti-vascular endothelial growth factor (AVF) agents in patients with macular edema secondary to RVO using a novel method of DRIL quantification for analysis. The purpose of this study was to determine the value of DRIL as a possible biomarker in patients with macular edema secondary to RVO, specifically, association of VA with DRIL presence at baseline and in response to treatment with AVF agents.

Key Points

Question Is disorganization of inner retinal layers a biomarker of visual acuity at baseline and treatment response in retinal vein occlusion?

Findings In this study of 147 eyes of 147 patients with retinal vein occlusion, the presence or absence of disorganization of inner retinal layers at baseline was a biomarker of visual acuity improvement; furthermore, increasing disorganization of inner retinal layers burden in patients with central retinal vein occlusion was associated with reduced visual acuity gains.

Meaning These results suggest that disorganization of inner retinal layers may be a useful biomarker for visual acuity prognostication at baseline and during treatment in patients with retinal vein occlusion.

Methods

All data for this retrospective review were collected and analyzed at Cole Eye Institute, Cleveland, Ohio. Because of the retrospective nature of the study, written informed consent was not required. All data were deidentified. All study-related procedures were performed in accordance with good clinical practice (International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use E6), applicable US Food and Drug Administration regulations, and the Health Insurance Portability and Accountability Act. The study was approved by the Cleveland Clinic Investigational Review Board.

Study Participants

Patients presenting to Cole Eye Institute from December 1, 2010, to January 1, 2016, with a new diagnosis based on *International Classification of Diseases, Ninth Revision* codes of central RVO (CRVO) (code 362.35), hemispheric RVO (HRVO) (code 362.36), or branch RVO (BRVO) (code 362.37) and spectral-domain optical coherence tomography (SD-OCT) (Zeiss Inc) at the time of diagnosis were included in this study. Data collection continued through January 2017. Patients were required to be 18 years or older with a minimum follow-up time of 12 months and treated with AVF agents (aflibercept, bevacizumab, or ranibizumab). Exclusion criteria included the presence of active confounding retinal or ocular disease (eg, diabetic retinopathy, exudative macular degeneration, macular hole, or amblyopia), history of pars plana vitrectomy, and any prior intravitreal injection treatment in the study eye.

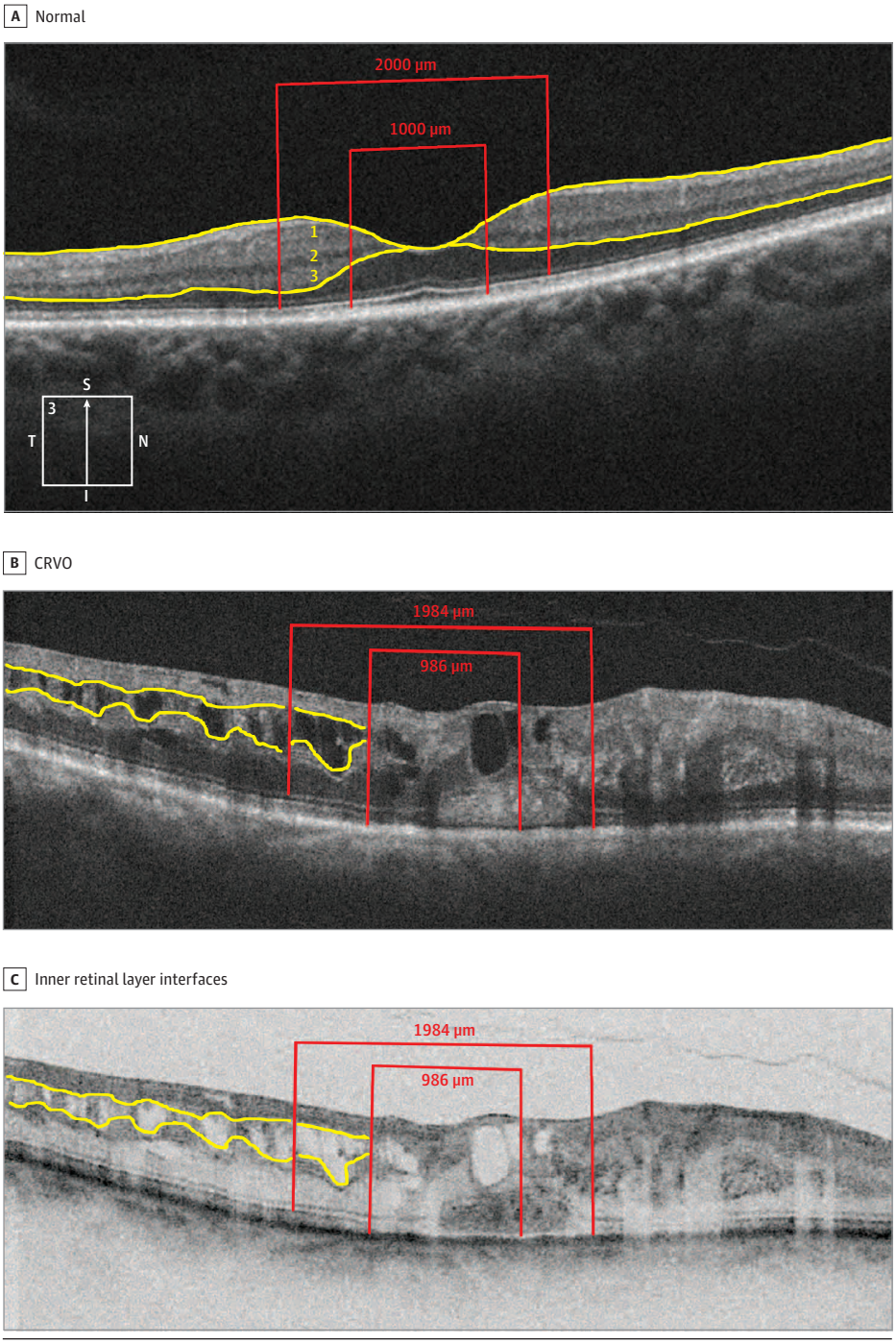
Data Collection

Medical files of all eligible patients were reviewed at baseline for demographic data. At baseline, 6 months, and 12 months, the best-corrected VA was recorded, as well as the SD-OCT signal quality, subretinal fluid, and number and type of AVF interventions. Macular cube readings, including central subfield thickness (CST), cube volume, and cube mean thickness, were also recorded at these time points.

Image Analysis and Study End Points

All patients were assessed using SD-OCT (Zeiss Cirrus HD-OCT, Fundus Finder) captured by certified ophthalmic photographers. Three B-scans were evaluated, including the scan that passed through the foveal center and a single scan above and below the center. For this study, only the central line scan that passed through the fovea was analyzed. It was divided into 3 concentric zones of 500 μm to represent the central 1 mm, the central 2 mm excluding the central 1 mm, and the area outside the central 2 mm (**Figure**). Each region was evaluated for any presence of DRIL and was assigned a DRIL score of 0 to 3 based on DRIL presence (+1) or absence (0) at baseline, 6 months, and 12 months.

Figure. Spectral-Domain Optical Coherence Tomography (SD-OCT) of the Inner Retina and Regional Division in a Normal Eye and Areas of Disorganization of Retinal Inner Layers (DRIL) in a Representative Case



A, Normal SD-OCT showing the inner retina (yellow lines) and the concentric zones surrounding the fovea (red lines) to create 3 regions for DRIL detection and scoring. Numbers represent the inner retinal layer interfaces: (1) ganglion cell–inner plexiform layer complex (evaluated as a single layer complex because the interface between the ganglion cell layer and the inner plexiform layer is not easily visible on retinal scans), (2) inner nuclear layer, and (3) outer plexiform layer. B, Patient with a central retinal vein occlusion (CRVO) exhibiting intraretinal fluid and DRIL in all 3 regions on SD-OCT and reverse grayscale SD-OCT. C, The yellow lines highlight the inner retinal layer interfaces, which disappear in the areas of DRIL.

Disorganization of retinal inner layers was positively identified if either of the interfaces between the ganglion cell layer-inner plexiform layer complex and inner nuclear layer and/or the inner nuclear layer and outer plexiform layer could not be distinguished despite the presence of other macular pathologic findings (ie, cystoid macular edema). Assessment of the interfaces was enhanced using reverse grayscale and increased contrast present on the imaging software (Zeiss Cirrus HD-OCT Review Software, version 9.5.1.13585) (Figure).

Each SD-OCT was graded by 2 masked independent graders (M.H., F.F.C.) and were then adjudicated and confirmed by a masked third experienced investigator (A.S.B.). All investigators were masked to all clinical information during their assessments.

Changes in DRIL scores were calculated from baseline to 6 months, from 6 months to 12 months, and from baseline to 12 months. In each period, patients were assigned to one of the following groups: (1) no DRIL throughout, (2) an increased pres-

Table 1. Comparison of Demographic Variables for the Study Patients[a]

Variable	Overall (N = 147)	HRVO/CRVO (n = 75)	BRVO (n = 72)	*P* Value (Test)
Eye				
Left	76 (51.7)	37 (49.3)	39 (54.2)	.56 (Pearson χ^2)
Right	71 (48.3)	38 (50.7)	33 (45.8)	
Age, mean (SD), y	68.9 (13.1)	69.5 (13.2)	68.2 (13.1)	.52 (ANOVA)
Sex				
Male	72 (49.0)	36 (48.0)	36 (50.0)	.81 (Pearson χ^2)
Female	75 (51.0)	39 (52.0)	36 (50.0)	
Diabetes	46 (31.5)[b]	25 (33.8)[c]	21 (29.2)	.55 (Pearson χ^2)
Glaucoma	35 (24.0)[b]	20 (27.0)[c]	15 (20.8)	.38 (Pearson χ^2)
Lens status				
Pseudophakic	40 (27.2)	22 (29.3)	18 (25.0)	.56 (Pearson χ^2)
Phakic	107 (72.8)	53 (70.7)	54 (75.0)	
Macular edema present on initial OCT	138 (93.9)	67 (89.3)	71 (98.6)	.03 (Fisher exact)
FA on presentation	93 (63.3)	50 (66.7)	43 (59.7)	.38 (Pearson χ^2)
Ischemic status				
No peripheral ischemia	118 (80.3)	60 (80.0)	58 (80.6)	.93 (Pearson χ^2)
Peripheral Ischemia noted	29 (19.7)	15 (20.0)	14 (19.4)	
Fellow eye with RVO	11 (7.5)	5 (6.7)	6 (8.3)	.70 (Pearson χ^2)
Fellow eye with macular edema	13 (8.8)	6 (8.0)	7 (9.7)	.71 (Pearson χ^2)
Previous PRP	1 (0.7)	1 (1.3)	0	.99 (Fisher exact)
Previous focal laser	1 (0.7)	0	1 (1.4)	.49 (Fisher exact)

Abbreviations: ANOVA, analysis of variance; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; FA, fluorescein angiography; HRVO, hemispheric retinal vein occlusion; OCT, optical coherence tomography; PRP, peripheral panretinal photocoagulation; RVO, retinal vein occlusion.

[a] Data are presented as number (percentage) of patients unless otherwise indicated.

[b] N = 146.

[c] n = 74.

ence of DRIL, (3) a decreased presence of DRIL, or (4) stable DRIL scores. These measurements together were then compared with Early Treatment Diabetic Retinopathy Study (ETDRS) scores to establish the ability of DRIL to characterize VA changes at baseline and in the evolution of disease during treatment with AVF therapy.

Statistical Analysis

Categorical variables were described using numbers (percentages); continuous variables were summarized using means (SDs). Comparisons that involved categorical variables were assessed using Pearson χ^2 tests, Fisher exact tests, or Kruskal-Wallis tests (for ordered categorical variables only). Pairwise comparisons were performed with significant class variables that had more than 2 groups. Comparisons that involved continuous variables were assessed using analysis of variance (ANOVA) tests. Multirater agreement was assessed using κ statistics based on the Fleiss method and compared against no agreement beyond chance using the McNemar test. Comparisons of baseline characteristics with changes in ETDRS scores at 6 months used ANOVA tests (categorical variables) or Pearson correlation (continuous variables). Multivariable models identifying change in ETDRS scores at 6 months were fit using a stepwise procedure. Factors with $P < .20$ were entered in the model and remained if significance was $P > .05$. Disorganization of retinal inner layers at baseline was then added to the best model to determine whether the factor remained significant after adjustment for other factors. All *P* values are 2-sided and unadjusted. Analyses were performed using SAS statistical software, version 9.4 (SAS Institute Inc).

Results

A total of 147 eyes of 147 patients (mean [SD] age, 68.9 [13.1] years; 75 [51.0%] female) were included in this study. Baseline demographics and characteristics are given in **Table 1**. Mean (SD) baseline VA was 56.6 (17.8) ETDRS letters, and across all patients with RVO at 12 months, a mean VA change of +11.8 ETDRS letters was seen. All 147 eyes were treatment naive and had baseline, 6-month (±1 month), and 12-month (±2 months) follow-up with SD-OCT at each visit; assessments included DRIL identification, CST, cube volume, and cube mean thickness.

Interrater agreement for DRIL measures at baseline, 6 months, and 12 months for BRVO revealed moderate agreement between readers for all 3 regions of DRIL measures (κ range = 0.28-0.69) except for DRIL inside the central 2-mm region and outside the central 1-mm region at 12 months, which had only slight agreement (κ = 0.18). In the CRVO/HRVO group, findings were similar to the BRVO group in that there was moderate agreement among readers at baseline, 6 months, and 12 months for all 3 regions of DRIL measures (κ range = 0.27-0.76) except for DRIL inside the central 2-mm region and outside the central 1-mm region at 12 months, which also revealed only slight agreement (κ = 0.13).

The mean (SD) number of injections was 5.2 (2.7) in the BRVO group and 5.1 (2.9) in the CRVO/HRVO group. The number of injections given shows a significant decrease in treatment from months 6 to 12 compared with months 0 to 6 in both groups. In the BRVO group, the mean number of injections

given in months 0 to 6 was 3.47 and decreased to 1.77 in months 6 to 12 ($P < .001$). This finding was again demonstrated in the CRVO/HRVO group, in which the mean number of injections given in months 0 to 6 was 3.28 and decreased to 1.80 in months 6 to 12 ($P < .001$).

Association of Baseline DRIL and VA

Table 2 outlines DRIL measures at each time point in both groups. Across all patients with RVO, the baseline presence of any DRIL was seen in 91 of 147 eyes (61.9%). In the BRVO group, baseline DRIL was seen in 41 of 72 patients (56.9%), and in the CRVO group, baseline DRIL was identified in 50 of 75 patients (66.7%). The DRIL scores at baseline across all 147 patients with RVO included absence of DRIL in 56 (38.1%), a score of 1 in 28 (19.0%), a score of 2 in 39 (26.5%), and a score of 3 in 24 (16.3%). The most common region for DRIL detection at baseline was within the central 2 mm excluding the central 1 mm (77 of 147 eyes) followed by the region outside the 2-mm range (66 of 147 eyes), and least detection in the central 1 mm (35 of 147 eyes).

In the BRVO group, the presence of any DRIL at baseline was correlated with lower baseline ETDRS scores (scores of 66.7 for no DRIL vs 54.6 for DRIL, $P = .002$), and patients in the BRVO group who experienced a 10-letter gain or more were more likely to have had DRIL at baseline compared with those who had less than a 10-letter gain (score change of 26 for DRIL at baseline vs 12 for no DRIL at baseline; $P = .04$).

In the CRVO/HRVO group, the presence of DRIL at baseline was not statistically significantly associated with lower baseline ETDRS scores (score of 58.6 for no DRIL vs 51.0 for DRIL, $P = .09$). However, absence of DRIL at baseline in this group was associated with greater VA gains at 6 months when adjusting for baseline VA (score change of 19.50 for no DRIL vs 12.72 for DRIL; $P = .04$).

Association of DRIL Score Changes and VA

During the 12-month period in all 147 patients with RVO, there was reduced DRIL in 51 (34.7%), increased DRIL in 34 (23.1%) despite treatment, no change in DRIL score from baseline in 20 (13.6%), and no DRIL identified in 42 (28.6%). At 6 months, all regions demonstrated a decrease in DRIL detection. The largest reductions in DRIL were in the region outside the central 2 mm, excluding the central 1 mm which demonstrated a decrease from 77 eyes at baseline to 41 eyes, followed by the region outside the central 2 mm, which decreased from 66 eyes to 41 eyes; the central 1 mm had a minimal change, with a reduction of 35 eyes to 26 eyes. At 12 months, all regions revealed a modest increase in DRIL detection was the greatest in the central 1-mm region (40 eyes), followed by the region inside the central 2 mm and outside the central 1 mm (46 eyes), and lastly in the region outside the central 2 mm (48 eyes).

In the BRVO group, the continued presence of DRIL was an indicator of fewer VA gains with treatment up to 6 months (score change of 6.2 for the DRIL increase group vs 18.6 for the DRIL decrease group vs 2.9 for the DRIL stable group; $P = .02$), but this finding did not persist through the 12-month period (score change of 9.8 for the DRIL increase group vs 17.0 for the DRIL decrease group vs 5.2 for the DRIL stable group; $P = .15$) and was not significant after adjusting for baseline VA at 6 months (score change of 7.45 for the DRIL increase group vs 15.06 for the DRIL decrease group vs 2.70 for the DRIL stable group; $P = .08$) or 12 months (score change of 11.23 for the DRIL increase group vs 13.04 for the DRIL decrease group vs 4.97 for the DRIL stable group; $P = .40$). Among those patients with DRIL at baseline who had no change in their DRIL score or who saw an increase in their DRIL score at 6 months, those in the BRVO group (57.3 letters) had a higher baseline VA than those in the CRVO/HRVO group (38.5 letters) ($P = .01$) and were more likely to reach a VA of 20/32 at month 6 than those in the CRVO/HRVO group (1 eye in the HRVO/CRVO group vs 7 eyes in the BRVO group; $P = .04$). Likewise, patients with baseline DRIL and stable or increased DRIL scores at 12 months demonstrated that those in the BRVO group had higher VA at month 12 than those in the CRVO/HRVO group (letter score of 50.8 in the HRVO/CRVO vs 70.8 in the BRVO group; $P = .001$) and were more likely to obtain a VA of 20/32 at 12 months than those in the CRVO/HRVO group (3 eyes in the HRVO/CRVO group vs 10 eyes in the BRVO group; $P = .01$).

In the CRVO/HRVO group after adjusting for baseline ETDRS, pairwise comparisons show that increasing DRIL scores at 6 months led to less change in ETDRS scores at both 6 months (score change of −0.12 for the DRIL increase group vs 16.90 for the DRIL decrease group vs 8.45 for the DRIL stable group; $P = .002$) and 12 months (score change of −6.25 for the DRIL increase group vs 15.52 for the DRIL decrease group vs 2.96 for the DRIL stable group; $P = .002$) compared with those without DRIL at both time points and those in whom the DRIL score decreased. Furthermore, this association held statistical significance after adjusting for CST change at 6 months ($P<.001$) and 12 months ($P<.001$). This significance was again demonstrated for increasing DRIL scores at 12 months. After adjusting for baseline VA, patients with increasing DRIL scores at 12 months had significantly less change in ETDRS scores compared with those without DRIL at both time points and those with decreasing DRIL scores from baseline to 12 months (score change of −1.91 for the DRIL increase group vs 17.83 for the DRIL decrease group vs 6.97 for the DRIL stable group; $P<.001$). In addition, after adjusting for CST change at 12 months, the association remained statistically significant at 12 months ($P<.001$).

Multivariable Models

Table 3 demonstrates the association between change in ETDRS score at 6 months and possible biomarkers for the BRVO and CRVO/HRVO cohorts. **Table 4** presents the results from the multivariable models to determine change in ETDRS score at 6 months in the BRVO and CRVO/HRVO groups. There were not enough events to potentially include macular edema in these models.

Discussion

Multiple studies[3-6] have now validated the utility of DRIL in current and resolved diabetic macular edema. Disorganization of inner retinal layers in eyes with macular edema secondary to RVO has been evaluated in a single recent study by

Table 2. Comparison of DRIL Measures at Each Time Point for the BRVO and HRVO/CRVO Groups[a]

Factor	Total (N = 147)	HRVO/CRVO (n = 75)	BRVO (n = 72)	*P* Value (Test)
Baseline				
DRIL outside the central 2 mm				
No	81 (55.1)	35 (46.7)	46 (63.9)	.04 (Pearson χ^2)
Yes	66 (44.9)	40 (53.3)	26 (36.1)	
DRIL inside central 2 mm and outside central 1 mm				
No	70 (47.6)	35 (46.7)	35 (48.6)	.81 (Pearson χ^2)
Yes	77 (52.4)	40 (53.3)	37 (51.4)	
DRIL in the central 1 mm				
No	112 (76.2)	60 (80.0)	52 (72.2)	.27 (Pearson χ^2)
Yes	35 (23.8)	15 (20.0)	20 (27.8)	
DRIL at baseline				
0	56 (38.1)	25 (33.3)	31 (43.1)	.48 (Kruskal-Wallis)
1	28 (19.0)	16 (21.3)	12 (16.7)	
2	39 (26.5)	23 (30.7)	16 (22.2)	
3	24 (16.3)	11 (14.7)	13 (18.1)	
Baseline IOP,[b] mean (SD), mm Hg	16.7 (4.0)	17.1 (4.5)	16.3 (3.2)	.25 (ANOVA)
Baseline CST, mean (SD), μm	457.9 (153.7)	502.4 (171.0)	411.4 (117.4)	<.001 (ANOVA)
Baseline cube volume, mean (SD), mm^3	11.5 (2.1)	11.7 (2.4)	11.3 (1.6)	.17 (ANOVA)
Baseline cube thickness, mean (SD), μm	321.4 (57.8)	329.0 (67.7)	313.4 (44.3)	.10 (ANOVA)
Month 6				
DRIL outside the central 2 mm				
No	106 (72.1)	54 (72.0)	52 (72.2)	.98 (Pearson χ^2)
Yes	41 (27.9)	21 (28.0)	20 (27.8)	
DRIL inside central 2 mm and outside central 1 mm				
No	111 (75.5)	57 (76.0)	54 (75.0)	.89 (Pearson χ^2)
Yes	36 (24.5)	18 (24.0)	18 (25.0)	
DRIL in the central 1 mm				
No	121 (82.3)	64 (85.3)	57 (79.2)	.33 (Pearson χ^2)
Yes	26 (17.7)	11 (14.7)	15 (20.8)	
DRIL at 6 mo				
0	88 (59.9)	47 (62.7)	41 (56.9)	.50 (Kruskal-Wallis)
1	30 (20.4)	16 (21.3)	14 (19.4)	
2	14 (9.5)	2 (2.7)	12 (16.7)	
3	15 (10.2)	10 (13.3)	5 (6.9)	
Month 6 IOP, mean (SD), mm Hg[b]	16.6 (3.6)	16.9 (4.3)	16.3 (2.8)	.33 (ANOVA)
Month 6 CST, mean (SD)	323.8 (113.2)	334.5 (136.1)	312.5 (82.5)	.24 (ANOVA)
Month 6 cube volume, mean (SD)	10.7 (1.4)	10.6 (1.7)	10.8 (1.1)	.58 (ANOVA)
Month 6 cube thickness, mean (SD)	297.1 (39.7)	295.5 (46.4)	298.8 (31.4)	.61 (ANOVA)
Month 12				
DRIL outside the central 2 mm				
No	99 (67.3)	48 (64.0)	51 (70.8)	.38 (Pearson χ^2)
Yes	48 (32.7)	27 (36.0)	21 (29.2)	
DRIL inside central 2 mm and outside central 1 mm				
No	101 (68.7)	53 (70.7)	48 (66.7)	.60 (Pearson χ^2)
Yes	46 (31.3)	22 (29.3)	24 (33.3)	
DRIL in the central 1 mm				
No	107 (72.8)	56 (74.7)	51 (70.8)	.60 (Pearson χ^2)
Yes	40 (27.2)	19 (25.3)	21 (29.2)	

(continued)

Table 2. Comparison of DRIL Measures at Each Time Point for the BRVO and HRVO/CRVO Groups[a] (continued)

Factor	Total (N = 147)	HRVO/CRVO (n = 75)	BRVO (n = 72)	*P* Value (Test)
DRIL at 12 mo				
0	79 (53.7)	40 (53.3)	39 (54.2)	.99 (Kruskal-Wallis)
1	28 (19.0)	15 (20.0)	13 (18.1)	
2	14 (9.5)	7 (9.3)	7 (9.7)	
3	26 (17.7)	13 (17.3)	13 (18.1)	
Month 12 IOP, mean (SD), mm Hg	16.2 (2.9)	16.2 (2.9)	16.2 (3.0)	.92 (ANOVA)
Month 12 CST, mean (SD)	329.7 (126.0)	344.8 (155.7)	314.1 (83.1)	.14 (ANOVA)
Month 12 cube volume, mean (SD)	10.7 (1.7)	10.7 (1.9)	10.7 (1.3)	.89 (ANOVA)
Month 12 cube thickness, mean (SD)	296.7 (46.2)	296.3 (53.7)	297.1 (37.2)	.92 (ANOVA)
DRIL change at 6 mo				
No DRIL	46 (31.3)	22 (29.3)	24 (33.3)	.40 (Pearson χ^2)
Increased DRIL	24 (16.3)	10 (13.3)	14 (19.4)	
Decreased DRIL	59 (40.1)	35 (46.7)	24 (33.3)	
Stable DRIL	18 (12.2)	8 (10.7)	10 (13.9)	
DRIL change at 12 mo				
No DRIL	42 (28.6)	19 (25.3)	23 (31.9)	.73 (Pearson χ^2)
Increased DRIL	34 (23.1)	17 (22.7)	17 (23.6)	
Decreased DRIL	51 (34.7)	29 (38.7)	22 (30.6)	
Stable DRIL	20 (13.6)	10 (13.3)	10 (13.9)	

Abbreviations: ANOVA, analysis of variance; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; CST, central subfield thickness; DRIL, disorganization of retinal inner layers; HRVO hemispheric retinal vein occlusion; IOP, intraocular pressure.

[a] Data are presented as number (percentage) of patients unless otherwise indicated.

[b] Data not available for all patients (baseline IOP data missing in 2 patients and month 6 IOP data in 1 patient).

Table 3. Association Between ETDRS Score Change at 6 Months and Possible Biomarkers for the BRVO and CRVO/HRVO Cohorts

	No. of Patients		Estimate (95% CI) or Mean (SD)[a]		*P* Value	
Factor	BRVO	CRVO/HRVO	BRVO	CRVO/HRVO	BRVO	CRVO/HRVO
Age	72	75	0.04 (−0.19 to 0.27)	−0.33 (−0.52 to −0.11)	.73	.003
Sex						
Male	36	36	10.1 (17.6)	17.7 (17.7)	.62	.18
Female	36	39	12.0 (15.2)	12.4 (16.4)		
Diabetes						
No	51	49	10.7 (17.7)	15.3 (16.0)	.78	.64
Yes	21	25	11.9 (12.8)	13.3 (19.0)		
Glaucoma						
No	57	54	9.7 (16.0)	14.2 (16.7)	.19	.69
Yes	15	20	15.9 (17.2)	15.9 (18.2)		
FA on presentation						
No	29	25	9.5 (21.1)	10.7 (14.1)	.53	.13
Yes	43	50	12.0 (12.4)	17.1 (18.2)		
Baseline ETDRS score	72	75	−0.54 (−0.68 to −0.35)	−0.62 (−0.74 to −0.46)	<.001	<.001
Baseline cube mean thickness	72	75	−0.15 (−0.37 to 0.09)	0.11 (−0.12 to 0.33)	.22	.35
Baseline CST	72	75	0.15 (−0.08 to 0.37)	−0.04 (−0.27 to 0.18)	.20	.71
Baseline cube volume	72	75	−0.14 (−0.36 to 0.09)	0.11 (−0.12 to 0.33)	.24	.35
Baseline IOP	70	75	0.02 (−0.22 to 0.25)	0.01 (−0.22 to 0.24)	.88	.93

Abbreviations: BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; CST, central subfield thickness; ETDRS, Early Treatment Diabetic Retinopathy Study; FA, fluorescein angiography; HRVO, hemispheric retinal vein occlusion; IOP, intraocular pressure.

[a] Analysis of variance or Pearson correlation coefficient.

Mimouni et al,[10] which demonstrated that DRIL extent after the first 3-monthly injections was a biomarker of VA improvement or decline. In this study, baseline presence of DRIL and changes in DRIL burden during treatment with AVF agents for macular edema secondary to RVO demonstrated potential utility as biomarkers of ETDRS score improvement. In patients with BRVO, significant differences were seen in baseline ETDRS scores when any DRIL was present. In the CRVO/HRVO group, those without DRIL had significantly greater increases in ETDRS scores at 6 months after adjusting for differences in baseline ETDRS scores. This difference was also apparent in the CRVO/HRVO group after adjusting for baseline VA and

Table 4. Results From the Multivariable Model to Identify Change in ETDRS Scores at 6 Months for the BRVO and CRVO/HRVO Cohorts

	Estimate (95% CI)		*P* Value	
Factor	BRVO	CRVO/HRVO	BRVO	CRVO/HRVO
Baseline cube mean thickness	−0.06 (−0.14 to 0.009)	NA	.09	NA
Baseline CST	NA	−0.017 (−0.03 to 0.0006)	NA	.06
Baseline ETDRS score	−0.54 (−0.75 to −0.32)	−0.60 (−0.76 to −0.44)	<.001	<.001
Age	NA	−0.33 (−0.55 to −0.10)	NA	.005
DRIL at baseline	−0.72 (−7.77 to 6.34)	7.64 (1.40 to 13.87)	.84	.02

Abbreviations: BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; CST, central subfield thickness; DRIL, disorganization of retinal inner layers; ETDRS, Early Treatment Diabetic Retinopathy Study; HRVO, hemispheric retinal vein occlusion; NA, not applicable.

changes in CST when evaluating the association between DRIL score change at 6 and 12 months and ETDRS score changes at 6 and 12 months.

The association of capillary macular nonperfusion and DRIL as well as foveal avascular zone enlargement in eyes with diabetic retinopathy has been established.[6] A positive association between foveal avascular zone area and DRIL was also recognized in a study by Balaratnasingam et al.[11] Previous studies[3,5,10] evaluating DRIL were limited to the central 1-mm region and central 1.5-mm[3] foveal region. Given this positive association of DRIL and nonperfusion, we thought it reasonable to assess the entire 3-mm horizontal scan for DRIL. In addition, DRIL extent by length measurement can be cumbersome and is a less practical approach for clinical use. Instead, we evaluated each scan with a presence or absence approach based on 3 concentric regions surrounding the fovea.

This study evaluates DRIL using SD-OCT and without manual length measurements. Using this approach, we were able to positively correlate baseline VA and presence or absence of DRIL at baseline, although the DRIL score (range, 1-3) itself was not correlated. However, changes in DRIL scores at 6 and 12 months identified future VA but only in the CRVO cohort. Furthermore, this association held true after adjusting for changes in CST at 6 and 12 months as well. It is possible that in this well-matched cohort of patients with BRVO and CRVO/HRVO that the greater proportion of retina affected in CRVO leads to more reliable longitudinal DRIL measurements and, hence, DRIL scores across the entirety of the scan.

In addition, in this study, a decrease in DRIL score was seen in all regions from baseline to 6 months, but the converse was true, to a lesser extent, from 6 to 12 months. This finding could represent undertreatment of disease in the 6- through 12-month period, allowing for progression and further nonperfusion because the mean number of injections decreased by half in months 6 to 12 compared with months 0 to 6 in both groups.

Limitations

Limitations include the retrospective nature of the study, the treat-as-needed regimen with AVF agents, and the lack of histopathologic studies on DRIL, which is an OCT-derived entity. In addition, interrater variability was only moderate in all regions at all time points except in the DRIL inside the 2-mm region and outside the 1-mm region at 12 months in the CRVO/HRVO and BRVO cohorts, which may be secondary to retinal architecture changes because of persistent ischemia in this specific group. This finding underscores the difficulty in DRIL detection and measurement in a clinical scenario, and despite only moderate agreement, the results still reveal positive correlations with VA outcomes. Future studies may further our understanding of DRIL as a biomarker in retinal vascular disease. Automation of DRIL measurement by OCT software may prove useful in future treatment paradigms as well as VA determination.

Conclusions

The baseline presence of DRIL and changes in DRIL burden during treatment with AVF agents for macular edema secondary to RVO may be useful biomarkers of ETDRS score improvement when adjusting for baseline ETDRS score and even after adjusting for CST changes. In this study, a stronger association was seen in the CRVO/HRVO group than in the BRVO group. These data combined with data from other studies[3-5,10] in retinal vascular disease reveal DRIL as a potentially useful biomarker in VA outcomes. Future studies that further evaluate DRIL, as well as histopathologic studies, and perhaps automated DRIL identification and measurement may prove useful for future VA identification and treatment paradigms.

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Invited Commentary

Disorganization of Retinal Inner Layers and the Importance of Setting Boundaries

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The visualization of structural retinal changes has brought a new dimension to how vitreoretinal diseases are assessed and treated. The advent of optical coherence tomography (OCT) prompted numerous efforts to find, characterize, and validate morphologic biomarkers. These biomarkers are an important prerequisite to developing an automated image analysis that could eliminate subjective errors and provide ophthalmologists with objective measurements. In this issue of *JAMA Ophthalmology*, Babiuch et al[1] report the results of a retrospective review in which they examined whether disorganization of retinal inner layers (DRIL) at baseline and after anti-vascular endothelial growth factor (anti-VEGF) treatment in a cohort of 147 patients with branch, central, and hemispheric retinal vein occlusion (RVO) was associated with visual acuity.

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To further our understanding of any structural alteration and its functional role, it is essential to be precise in our description of the alteration. Disorganization of retinal inner layers has been characterized by Sun et al[2] as the lack of distinguishable boundaries between the ganglion cell-inner plexiform layer complex, inner nuclear layer, and outer plexiform layer. However, this definition may also lack the accuracy necessary to detect such a nonfeature reliably, a conclusion supported by findings in the present study,[1] in which interrater agreement was only slight to moderate. We have to be aware that many different features can contribute to a disorganized appearance of retinal inner layers, including the presence of hyperreflective foci, a generalized blurring of layers that leads to a homogenous mass, cystoid spaces that alter retinal boundaries, or an increase or decrease of optical intensity.[2-4] Moreover, each of these features might have different associations with visual function and different degrees of reversibility and treatment response in spatiotemporal appearance. This finding could explain why there was a decrease in DRIL detection up to month 6 in the study by Babiuch et al[1]; such a decrease could equally result from cystoid changes responding to anti-VEGF therapy.

The authors point out that Cirrus spectral-domain (SD) OCT (Zeiss Inc) was used in all patients to evaluate DRIL on a single central B-scan at baseline, month 6, and month 12. Each B-scan was divided into 3 concentric zones, and the presence or absence of DRIL in each zone was used to generate a score to be compared with Early Treatment Diabetic Retinopathy Study measurements.

Our experience indicates that DRIL not only shows local variability, with the central subfield being sporadically unaffected, but also is more easily identified using SD-OCT devices that have a higher signal to noise ratio (eg, Spectralis SD-OCT). At the Vienna Reading Center, we recently performed a large-scale structure-function analysis of numerous morphologic features in a cohort of more than 600 treatment-naive patients with RVO. By manually grading more than 8000 B-scans of the central subfield, DRIL was detected in 84% of all patients, a prevalence comparable to that in a study by Mimouni et al,[5] in which 83% of patients presented with DRIL. We were able to identify a subset of features, including central retinal thickness and subretinal fluid, that had a distinct association with visual acuity. However, in our multivariate