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Original Article

OCT outcomes as biomarkers for disease status, visual function, and prognosis in diabetic macular edema

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Objective: To evaluate disorganization of retinal inner layers (DRIL), as detected on spectral-domain optical coherence tomography (OCT) images, as a biomarker for diabetic macular edema (DME) activity, visual function, and prognosis in eyes with DME.

Design: Longitudinal prospective.

Methods: Post hoc correlation analyses were performed on data from a phase 2 clinical trial. Seventy-one eyes of 71 patients with treatment-naive DME received either suprachoroidally administered CLS-TA (proprietary formulation of a triamcinolone acetonide injectable suspension) combined with intravitreal aflibercept or intravitreal aflibercept with a sham suprachoroidal injection procedure. DRIL area, maximum horizontal extent of DRIL, ellipsoid zone (EZ) integrity, and the presence and location of subretinal (SRF) and intraretinal fluid (IRF) were evaluated at baseline and week 24 by certified reading centre graders.

Results: At baseline, the area and maximum horizontal extent of DRIL were negatively correlated with best-corrected visual acuity (BCVA; $r = -0.25$, $p = 0.05$ and $r = -0.32$, $p = 0.01$, respectively). Mean baseline BCVA progressively worsened with each ordinal drop in EZ integrity, improved with the presence of SRF, and was invariant to the presence of IRF. DRIL area and maximum extent were significantly decreased at week 24 (-3.0 mm^2 [$p < 0.001$] and -775.8 mm [$p < 0.001$], respectively. At week 24, decreases in the area and maximum horizontal extent of DRIL were positively correlated with increases in BCVA ($r = -0.40$, $p = 0.003$ and $r = -0.30$, $p = 0.04$). Improvements in BCVA at week 24 were no different between patients showing improvement in EZ, SRF, or IRF and those showing no improvement or worsening from baseline.

Conclusions: DRIL area and DRIL maximum horizontal extent were demonstrated to be novel biomarkers for macular edema status, visual function, and prognosis in eyes with treatment-naive DME.

Objectif: Évaluer la désorganisation des couches rétiniennes internes (DRIL) sur les images obtenues à la tomographie par cohérence optique (OCT) en domaine spectral à titre de biomarqueur de l'activité de l'œdème maculaire diabétique (OMD), de la fonction visuelle et du pronostic dans des yeux présentant un OMD.

Nature: Étude longitudinale prospective.

Méthodes: Des analyses de corrélation a posteriori ont été réalisées sur des données issues d'une étude clinique de phase 2 au cours de laquelle 71 yeux de 71 patients présentant un OMD jamais traité ont été répartis en 2 groupes de traitement : administration suprachoroïdienne de CLS-TA (préparation brevetée de suspension d'acétonide de triamcinolone injectable) en association à l'aflibercept par voie intravitréenne ou aflibercept par voie intravitréenne en association à une injection suprachoroïdienne factice. L'aire de la DRIL, l'étendue horizontale maximale de la DRIL, l'intégrité de la zone ellipsoïde (ZE) ainsi que la présence et la localisation de liquide sous-rétinien (LSR) et de liquide intrarétinien (LIR) ont été mesurées au départ et à la semaine 24 par des évaluateurs agréés du centre de lecture d'images.

Résultats: Au départ, on a observé une corrélation négative entre l'aire de la DRIL et l'étendue horizontale maximale de la DRIL, d'une part, et la meilleure acuité visuelle corrigée (MAVC), d'autre part ($r = -0{,}25$; $p = 0{,}05$ et $r = -0{,}32$; $p = 0{,}01$, respectivement). La MAVC moyenne initiale s'est détériorée progressivement avec chaque baisse de l'intégrité de la ZE sur l'échelle ordinale, s'est améliorée en présence de LSR et est demeurée la même malgré la présence de LIR. L'aire de la DRIL et l'étendue maximale de la DRIL avaient significativement diminué à la semaine 24 ($-3{,}0$ mm^2 [$p < 0{,}001$] et $-775{,}8$ mm [$p < 0{,}001$], respectivement). Toujours à la semaine 24, ont a noté une corrélation positive entre les diminutions de l'aire et de l'étendue horizontale maximale de la DRIL et l'amélioration de la MAVC ($r = -0{,}40$; $p = 0{,}003$ et $r = -0{,}30$; $p = 0{,}04$). On n'a pas noté de différence quant à l'amélioration de la MAVC à la semaine 24 entre les patients chez qui la ZE, le LSR ou le LIR s'étaient améliorés et ceux chez qui ces variables sont demeurées inchangées ou se sont détériorées par rapport aux valeurs de départ.

Conclusions: Notre étude nous a permis de constater que l'aire de la DRIL et l'étendue horizontale maximale de la DRIL sont de nouveaux biomarqueurs de l'état de l'œdème maculaire, de la fonction visuelle et du pronostic en présence d'un OMD jamais traité.

Diabetic macular edema (DME) is a leading cause of visual impairment in the working-age population of most developed nations.[1] Approximately 40% of eyes with DME are nonresponders or poor responders to anti–vascular endothelial growth factor (anti-VEGF) therapy,[2–5] and consequently, biomarkers for DME activity (by presence of intraretinal fluid), visual function, and prognosis are of high clinical relevance.

Historically, optical coherence tomography (OCT)–measured central retinal thickness has served as a standard

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parameter for diagnosis and follow-up of DME.[6,7] However, this marker has only modest correlation with baseline visual acuity (VA) or VA change after DME treatment in clinical trials, and therefore, other OCT biomarkers that better reflect visual function need to be identified.[8–10] Recently, disorganization of retinal inner layers (DRIL) has been reported to be associated with VA both at baseline and after resolution DME.[11–13] Specifically, the extent of DRIL in the central 1 mm foveal zone correlates with VA changes after treatment and, in 1 study, showed better correlation than other OCT biomarkers, including retinal thickness, cone outer-segment tips status, external limiting membrane, and ellipsoid zone (EZ) integrity.[13]

This study assesses the relationship between BCVA and OCT variables in a DME data set from a phase 2 clinical trial, with monitor-verified diagnoses per eligibility criteria, protocol refractions, study-certified imagers, and spectral-domain OCT evaluation at a centralized masked reading centre. This clinical trial assessed CLS-TA (Clearside Biomedical, Alpharetta, Ga.), an investigational formulation of the corticosteroid triamcinolone acetonide for suprachoroidal injection, but this report focuses on BCVA and OCT anatomic correlations instead of treatment efficacy and is agnostic to the randomized treatment assignment. Specifically, the relationship between best-corrected VA (BCVA) and DRIL, maximum horizontal extent of DRIL, EZ integrity, and the presence and location of subretinal (SRF) and intraretinal fluid (IRF) were correlated with VA. Correlation analyses were performed to describe the relationship at baseline and between the change from baseline at week 24.

Methods

This post hoc analysis was performed on data from TYBEE (ClinicalTrails.gov identifier NCT03126786), a randomized, double-masked, parallel-group, controlled multicentre phase 2 study of 24 weeks' duration comparing the efficacy and safety of CLS-TA (4 mg [0.1 mL of 40 mg/mL]) administered suprachoroidally in conjunction with intravitreal aflibercept (2 mg [50 μL]) with intravitreal aflibercept plus a sham suprachoroidal injection procedure (with a needleless hub). Enrolled patients were type 1 and 2 diabetics of at least 18 years of age diagnosed with treatment-naive DME, Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA between 20 and 70 letters, and central subfield thickness >300 μm in the study eye at the time of screening. The original clinical trial protocol was approved by the institutional review board at each site, and the trial was performed in compliance with the tenets of the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice guidelines, and applicable local regulations. Written informed consent was obtained from all the patients before enrolment in the study. The study details are summarized further in Barakat et al.[14]

Spectral-domain OCT image acquisition

Measurements of the area and maximum horizontal extent in disorganization of retinal inner layers (DRIL) were obtained via spectral-domain OCT (SD-OCT) using either the Heidelberg Spectralis device (Heidelberg Engineering, Heidelberg, Germany) or the Cirrus HD-OCT device (Carl Zeiss Meditec, Dublin, Calif.). Patients assessed via the Heidelberg device underwent 20- × 20-degree SD-OCT macular cube scans centered on the foveal centre after pupillary dilation. The volume scans were captured in high-resolution mode using a 49-line horizontal raster pattern with an average of 16 automatic real-time tracking images per B-scan.[11,12] Patients assessed via the Cirrus device underwent pupillary dilation and then 6 × 6 mm SD-OCT volume cubes (512 A-scans × 128 B-scans) were acquired centred on the fovea.[15]

All images were captured by clinical study-certified ophthalmic photographers using certified SD-OCT instruments at all follow-up visits. Baseline volume scans were registered to image the same location in the subsequent visits.

Image grading

Calculation of the DRIL parameters was done in a novel fashion, as described below, though we adopted the definition described by Sun et al.[12]; specifically, DRIL in each B-scan was recognized by the presence of a region where the boundaries could not be identified between the ganglion cell–inner plexiform layer complex, inner nuclear layer, and outer plexiform layer.[12] DRIL was assessed irrespective of the presence of retinal edema and intraretinal cysts.

To calculate the total area of DRIL, grading software previously developed and validated by the Doheny Image Reading Center (3D-OCTOR) was used to perform quantitative analysis on macular cube B-scans imported from the SD-OCT device. The 3D-OCTOR software allows manual segmentation of boundaries of various retinal layers to isolate and analyze areas of interest[16,17] (Fig. 1).

We generated an area map for the non-DRIL portion of the inner retinal layers by snapping the outer plexiform layer segmentation up to the internal limiting membrane segmentation at the edge of the identified DRIL in each B-scan. DRIL area was then simply calculated by subtracting the non-DRIL portion of the inner retinal layers from the total retinal area. We also measured the maximum horizontal extent of DRIL.

EZ integrity, IRF, and SRF were assessed via inspection of the OCT scans in the central subfield, which was defined as a circular area of 1 mm diameter around the centre point of the macula. The integrity of the EZ in the central subfield was graded as "normal," "abnormal but continuous," "abnormal discontinuous (patchy)," or "absent." Presence of intraretinal fluid was graded as "absent," "questionable," "definite (outside centre subfield)," "definite (centre subfield involved)," or "cannot grade." Presence of subretinal fluid was graded as "absent," "questionable," "definite (outside

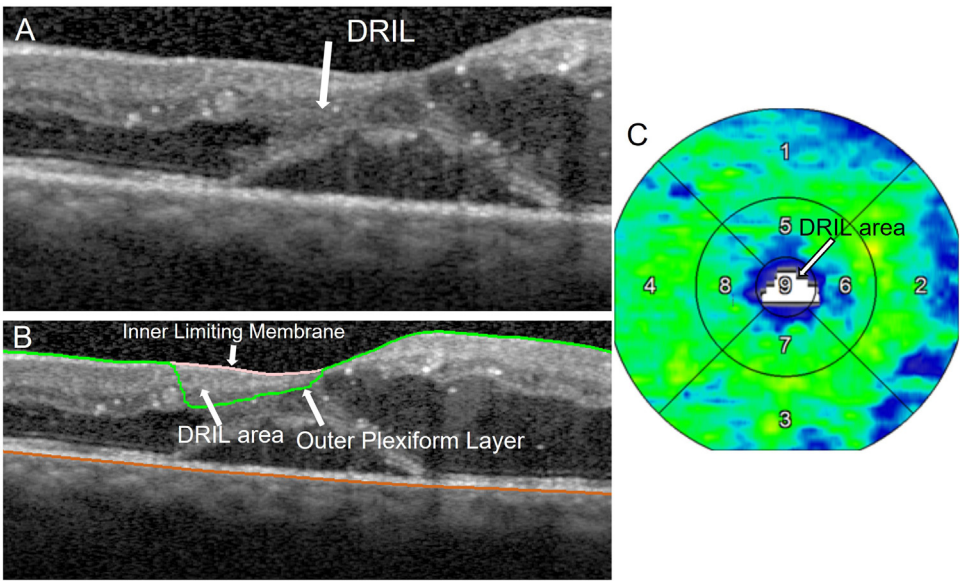

Fig. 1—(A) Optical coherence tomography (OCT) B-scan showing disorganization of retinal inner layers (DRIL). (B) Illustration of OCT B-scan showing DRIL area segmentation. (C) DRIL area map from volume scans (central white region).

centre subfield)," "definite (center subfield involved)," or "cannot grade." A grade of "cannot grade" was indicative of insufficient image quality to perform the assessment.

Grading of DRIL features was performed by 2 independent masked certified Doheny Image Reading Center graders (M.G.N. and S.V.B.) to assess the repeatability of all measurements. Analysis and interpretation of EZ, IRF, and SRF were performed by 2 independent masked certified MERIT CRO (formerly Eyekor, Inc.) reading centre graders.

Best-corrected visual acuity

BCVA was assessed following the ETDRS protocol, including ETDRS eye charts and standardized lighting and lanes. All BCVA assessments were performed by trained staff who were certified on the study procedures. BCVA assessment preceded any examination that required contact with the eye, with results being reported as the total number of letters read correctly following refraction.

Statistical analysis

This post hoc analysis was performed with data from the controlled phase 2 TYBEE trial examining patients with treatment-naive DME. All participants in this study received suprachoroidally administered CLS-TA in conjunction with intravitreal aflibercept or intravitreal aflibercept in conjunction with a sham suprachoroidal injection procedure. The purpose of this post hoc analysis was to assess the correlations between BCVA and OCT anatomic features. Therefore, analyses were performed on data from all patients regardless of randomized treatment assignment or receipt of rescue medications.

Correlation analyses were performed on data collected prior to administration of study treatment at baseline and separately on the change from baseline after 24 weeks of follow-up. Only patients with complete data, that is, BCVA and OCT data collected on the same date, were included in the analysis. OCT anatomic features included the area of DRIL, maximum horizontal extent of DRIL, EZ integrity, and presence of IRF and SRF. The area of DRIL was measured in millimeters squared (mm^2), and the maximum horizontal extent of DRIL was measured in microns (μm). EZ integrity and the presence of subretinal fluid were each graded into 4 levels of severity, whereas the presence of IRF was graded into 5 levels. Images of insufficient quality were assigned values of "cannot grade" and were excluded from the analyses. Pooling of grades was performed in cases where the sample size of patients within a severity grade was small.

The linear relationships between BCVA and the area and maximum horizonal extent of DRIL at baseline, between change from baseline in BCVA at week 24 and baseline area and maximum horizonal extent of DRIL, and between change from baseline in BCVA and area and maximum horizonal extent of DRIL at week 24 were assessed using Pearson's correlation coefficients, 95% confidence intervals using Fisher's z transformation. Then p values from the 2-sided test for zero linear correlation were calculated.

To compare BCVA between the severity grades of EZ, IRF, and SRF at baseline, a 1-way analysis of covariance model was used. Because of sample size limitations in IRF grading at baseline, patients graded as "absent," "questionable," or "definite (outside centre subfield)" were pooled. Likewise, in SRF grading at baseline, patients graded as "absent" or "questionable" were pooled, and patients graded as "definite (outside centre subfield)" or "definite (centre subfield involved)" were pooled. The analysis-of-covariance

models included baseline BCVA as the dependent variable, baseline anatomy grade as the independent variable, and baseline central subfield thickness (CST) and age as the covariates. For the EZ analysis, adjustments for multiple comparisons were made using the method by Tukey–Kramer.

To assess the relationship between baseline anatomic grade (normal vs not normal with respect to EZ; pooled grades with respect to IRF and SRF) on the change from baseline in BCVA at week 24, an analysis of covariance was performed. These models included the change from baseline BCVA as the dependent variable, baseline anatomic grade as the independent variable, and baseline BCVA, baseline CST, and age as the covariates. The relationship between the change from baseline in BCVA and the change in anatomic grade at week 24 was assessed using an analysis-of-covariance model with change from baseline in BCVA as the dependent variable, the status of anatomy (categorized as either showing any improvement vs no improvement or worsening) as the independent variable, and baseline BCVA, baseline CST, and age as the covariates. The category of "no improvement" reflected no change in grading between the baseline and week 24 assessments, whereas the category of "worsening" indicated that the condition of the anatomic feature had progressed from the baseline state to a more severe anomalous state.

For the continuous variables, that is, BCVA and area and maximum extent of DRIL, the change from baseline values were calculated by subtracting the baseline values from the week 24 values. Values for missing data were not inputted. Thirty percent of eyes (~20 eyes) were regraded for DRIL area and DRIL maximum extent measurement in masked method by the second grader (M.G.N.). Reproducibility data were analyzed using intraclass correlation coefficient (ICC). Unless otherwise stated, reported *p* values were not adjusted to account for multiple testing and were compared to a nominal 2-sided significance level of 0.05. Statistical analysis was conducted using SAS Software version 9.4 (SAS Institute, Cary, NC).

Table 1—Demographics and baseline characteristics

Characteristic	TYBEE study
No. of participants	71
Mean age (SD), y	59.5 (11.51)
Women, n (%)	21 (29.6)
Phakic, n (%)	59 (83.1)
Duration of disease:	
Mean (SD)	85.8 days (212.19 days)
Median	20.0 days
BCVA, mean (SD)	
ETDRS letters	57.6 (12.13)
Snellen equivalent	20/80 (20/40–20/320)
Mean CST, μm (SD)	501.3 (149.25)
Mean HbA1c, % (SD)	7.66 (1.722)

BCVA, best-corrected visual acuity; CST, central subfield retinal thickness; ETDRS, Early Treatment Diabetic Retinopathy Study; HbA1c, hemoglobin A1c

Results

A total of 71 eyes of 71 patients were randomized into the TYBEE study. Across all patients, the mean age was 59.5 years, 21 of 71 patients (29.6%) were female, and most eyes were phakic (59 of 71 eyes; 83.1%). Mean BCVA and CST at baseline were 57.6 letters and 501.3 μm, respectively. OCT images from 71 eyes were assessed for the presence of DRIL. At baseline, 67 eyes were assessed as having DRIL, with the remaining 4 either not having DRIL (n = 3) or not having a gradable image (n = 1). Of the 67 eyes with the presence of DRIL, the area and maximum extent of DRIL could not be graded in 3 eyes. Of the 64 eyes with gradable DRIL, the mean DRIL area was 4.1 mm^2 (range, 0–13.44 mm^2), and mean maximum extent of DRIL was 2092.5 μm (range, 0–5874 μm), respectively. Demographic and baseline characteristics data are presented in Table 1.

BCVA and OCT anatomy at baseline

Correlation analysis between baseline BCVA and area and maximum horizontal extent of DRIL included data from 64 eyes. Table 2 summarizes the correlation coefficients and associated 95% confidence intervals. Low but

Table 2—Correlation analysis between best-corrected visual acuity and optical coherence tomography features

					Pearson correlation statistics		
Variable	Mean (SD)	Correlated with variable	Mean (SD)	n	Sample correlation	95% CI	*p* value
Baseline BCVA	59.0 (11.52)	Baseline area of DRIL (mm^2)	4.06 (6.04)	64	−0.251	(−0.467, −0.004)	0.045
Baseline BCVA	59.0 (11.52)	Baseline maximum horizontal extent of DRIL (μm)	2092.5 (1226.58)	64	−0.320	(−0.522, −0.077)	0.010
Change from baseline in BCVA at week 24	11.8 (9.08)	Baseline area of DRIL (mm^2)	4.3 (6.39)	56	0.288	(0.025, 0.510)	0.031
Change from baseline in BCVA at week 24	11.8 (9.08)	Baseline maximum horizontal extent of DRIL (μm)	2014.2 (1207.02)	56	0.072	(−0.195, 0.329)	0.597
Change from baseline in BCVA at week 24	12.3 (9.01)	Change from baseline in area of DRIL at week 24 (mm^2)	−3.0 (5.83)	53	−0.395	(−0.599, −0.136)	0.003
Change from baseline in BCVA at week 24	12.3 (9.20)	Change from baseline in maximum horizontal extent of DRIL at week 24 (μm)	−775.8 (892.45)	50	−0.295	(−0.527, −0.015)	0.037

BCVA, best-corrected visual acuity; DRIL, disorganization of retinal inner layers
Note: Pearson correlation coefficient and 95% CI generated using Fisher's *z* transformation. *p* Value for H_0: $\rho = 0$.

statistically significant negative correlates were found between BCVA and the area of DRIL ($r = -0.25$, $p = 0.05$; Fig. 2A) and between BCVA and the maximum horizontal extent of DRIL ($r = -0.32$, $p = 0.01$; Fig. 2B).

Analysis between baseline BCVA and baseline OCT anatomy included data from 41 eyes with gradable EZ integrity, 71 eyes with gradable IRF, and 70 eyes with gradable SRF. Mean BCVA at baseline was greater in eyes with an EZ grade of "normal" (61.7 letters) and progressively worsened as EZ integrity deteriorated to a low of 37.5 letters in eyes with an EZ status of "absent" (Fig. 2C). As shown in Table 3, eyes with normal EZ integrity had a significantly better baseline BCVA compared with eyes graded as "abnormal discontinuous (patchy)" and "absent" ($p \leq 0.05$ for both) after adjusting for multiple comparisons. Similar results were noted between eyes graded as "abnormal but continuous" and both "abnormal discontinuous (patchy)" and "absent" groups.

With respect to the presence of IRF at baseline, most eyes (67 of 71) showed definite centre subfield involvement, with or without cystoid spaces. Mean BCVA at baseline differed little between the 2 pooled groups ($p = 0.90$, averaging 57–58 letters; Table 4 and Fig. 2D).

Thirty-nine of 70 eyes showed an absent or questionable central subfield SRF at baseline. Mean BCVA at baseline (Fig. 2E) was greater in eyes with a definite presence of SRF (61.4 letters) compared with eyes with no or questionable SRF (55.2 letters); the difference between these 2 groups was statistically significant ($p = 0.02$), as detailed in Table 5.

24-Week change from baseline in BCVA and OCT anatomy

Analyses between change from baseline BCVA at week 24 and the area and maximum horizontal extent of DRIL at baseline included data from 56 eyes. The mean difference for DRIL area and maximum extent was -3.0 mm^2 and -775.8 mm at week 24. Correlation coefficients and associated 95% confidence intervals are shown in Table 2. A low but statistically significant positive linear correlation was found between change in BCVA and the area of DRIL at baseline ($r = 0.29$, $p = 0.03$; Fig. 3A). There was an absence of linear correlation between change in BCVA and the maximum horizontal extent of DRIL at baseline ($r = 0.07$, $p = 0.60$; Fig. 3B).

Analysis between the 24-week change from baseline in BCVA and baseline OCT anatomy included data from 36 eyes with gradable EZ integrity, 63 eyes with gradable IRF, and 62 eyes with gradable SRF. Figure 3C–E summarizes the relationship between these baseline central subfield anatomic features and the change from baseline in BCVA at week 24. Mean change from baseline in BCVA at week 24 was greater but not statistically different in eyes with a normal EZ grade compared with eyes considered not normal by the reading centre (14.0 letters vs 12.8 letters, respectively; $p = 0.75$).

Eyes with definite IRF (with or without cystoid) at baseline showed less improvement in BCVA at week 24 compared with eyes graded as "absent," "questionable," or

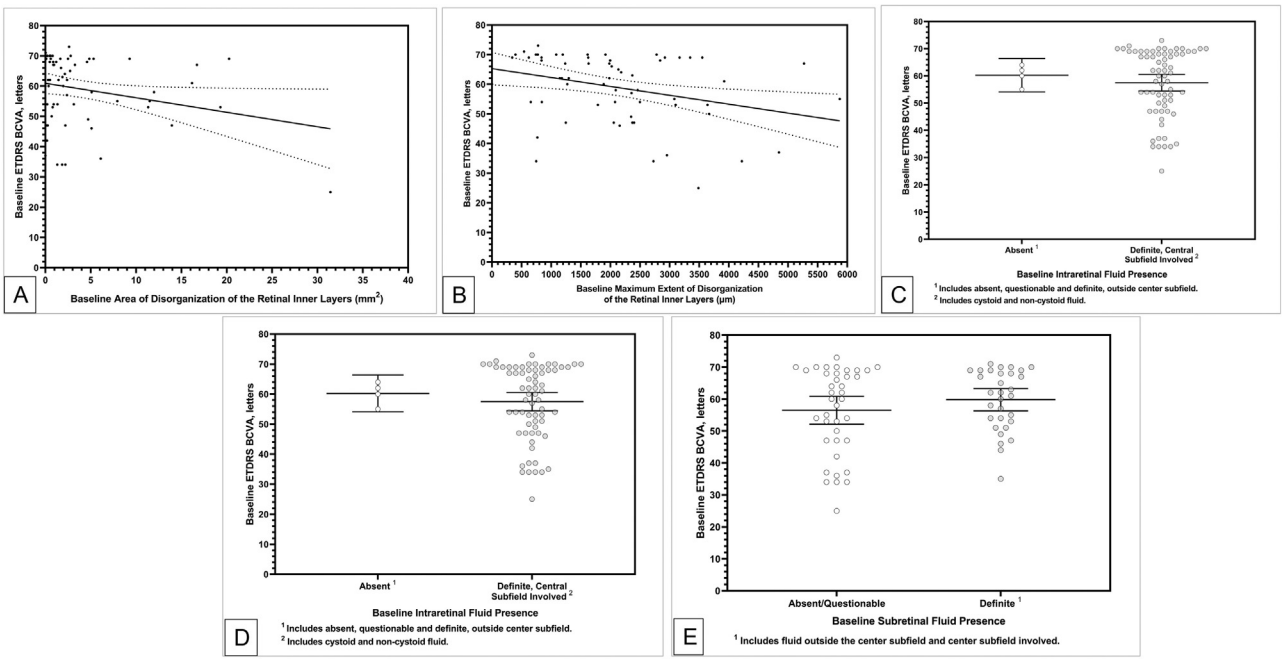

Fig. 2—Plots showing relationships between baseline anatomic features and best-corrected visual acuity (BCVA). (A) Area of disorganization of retinal inner layers (DRIL) and Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA at baseline. (B) Maximum horizontal extent of DRIL and ETDRS BCVA at baseline. (C) Ellipsoid zone status and ETDRS BCVA at baseline. (D) Presence of intraretinal fluid and ETDRS BCVA at baseline. (E) Presence of subretinal fluid and ETDRS BCVA at baseline. Scatterplots show linear regression lines (solid) plotted along with lines (dashed) outlining 95% confidence intervals for anatomic features measured on a continuous scale or means and the associated 95% confidence intervals for anatomic features assessed by severity grade.

Table 3—Association between best-corrected visual acuity and ellipsoid zone integrity

	Baseline ellipsoid zone status			
Baseline BCVA, letters	Normal (1)	Abnormal but continuous (2)	Abnormal discontinuous (patchy) (3)	Absent (4)
No. of patients	25	6	7	3
Mean (standard error)*	61.7 (1.93)	61.1 (3.68)	43.8 (3.57)	37.5 (5.71)
Difference (95% CI) vs (1)†		0.6 (−10.8, 12.0)	17.9 (6.4, 29.4)	24.2 (7.2, 41.2)
p Value vs (1)		0.999	<0.001	0.003
Difference (95% CI) vs (2)			17.3 (3.7, 29.4)	23.6 (5.6, 41.5)
p Value vs (2)			0.008	0.006
Difference (95% CI) vs (3)				6.3 (−10.8, 23.3)
P-value vs. 3				0.755
	Baseline ellipsoid zone			
Change from baseline in BCVA at week 24	**Normal**	**Not normal**		
No. of patients	24	12		
Mean (standard error)*	14.0 (1.91)	12.8 (2.89)		
Difference (95% CI)‡		1.2 (−6.5, 8.9)		
p Value		0.754		
		Change from baseline in ellipsoid zone at week 24		
Change from baseline in BCVA at week 24	**Any improvement**	**No improvement or worsening**		
No. of patients	5	30		
Mean (standard error)*	11.7 (4.07)	14.0 (1.58)		
Difference (95% CI)§		−2.3 (−11.4, 6.8)		
p Value		0.606		

BCVA, best-corrected visual acuity; CST, central subfield retinal thickness

Note: Spectral-domain optical coherence tomography images with reading centre grades of "cannot grade" are excluded from the analysis.

*Values represent the least-squares mean and standard error within each group.

†Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with baseline BCVA as the dependent variable, baseline ellipsoid zone grade as the independent variable, and baseline CST and age as covariates. *p* Values are adjusted using the Tukey−Kramer method for multiple comparisons.

‡Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, the baseline ellipsoid zone grade as the independent variable, and baseline BCVA, baseline CST, and age as covariates.

§Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, change from baseline in ellipsoid zone as the independent variable, and baseline BCVA, baseline CST, and age as covariates.

Table 4—Association between best-corrected visual acuity and intraretinal fluid presence

	Intraretinal fluid presence at baseline	
Baseline BCVA, letters	Absent/questionable/definite, outside centre subfield	Definite/central subfield involved
No. of patients	4	67
Mean (standard error)*	57.0 (5.35)	57.7 (1.30)
Difference (95% CI)†		−0.7 (−11.7, 10.3)
p Value		0.902
	Baseline intraretinal fluid	
Change from baseline in BCVA at week 24	**Absent/questionable/definite, outside centre subfield**	**Definite/central subfield involved**
No. of patients	4	59
Mean (standard error)*	16.1 (4.62)	12.7 (1.19)
Difference (95% CI)‡		3.4 (−6.1, 13.0)
p Value		0.478
	Change from baseline in intraretinal fluid at week 24	
Change from baseline in BCVA at week 24	**Any improvement**	**No improvement or worsening**
No. of patients	10	51
Mean (standard error)*	17.8 (3.01)	12.3 (1.25)
Difference (95% CI)§		5.5 (−1.1, 12.2)
p Value		0.102

BCVA, best-corrected visual acuity; CST, central subfield retinal thickness

Note: Spectral-domain optical coherence tomography images with reading centre grades of "cannot grade" are excluded from the analysis.

*Values represent the least-squares mean and standard error within each group.

†Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with baseline BCVA as the dependent variable, baseline intraretinal fluid presence grade as the independent variable, and baseline CST and age as covariates.

‡Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, baseline intraretinal fluid presence grade as the independent variable, and baseline BCVA, baseline CST, and age as covariates.

§Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, change from baseline in intraretinal fluid presence as the independent variable, and baseline BCVA, baseline CST, and age as covariates.

Table 5—Association between best-corrected visual acuity and subretinal fluid presence

	Subretinal fluid presence at baseline	
Baseline BCVA, letters	Absent/questionable	Definite (outside centre subfield and centre subfield involved)
No. of patients	39	31
Mean (standard error)*	55.2 (1.67)	61.4 (1.88)
Difference (95% CI)†		−6.1 (−11.3, −1.0)
p Value		0.020
	Baseline subretinal fluid	
Change from baseline in BCVA at week 24	**Absent/questionable**	**Definite (outside centre subfield and centre subfield involved)**
No. of patients	34	28
Mean (standard error)*	13.6 (1.63)	12.0 (1.81)
Difference (95% CI)‡		1.6 (−3.5, 6.6)
P-value		0.539
	Change from baseline in subretinal fluid at week 24	
Change from baseline in BCVA at week 24	**Any improvement**	**No improvement or worsening**
No. of patients	27	33
Mean (standard error)*	13.8 (1.81)	12.7 (1.62)
Difference (95% CI)§		1.1 (−3.9, 6.2)
p Value		0.650

BCVA, best-corrected visual acuity; CST, central subfield retinal thickness
Note: Spectral-domain optical coherence tomography images with reading centre grades of "cannot grade" are excluded from the analysis.
*Values represent the least-squares mean and standard error within each group.
†Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with baseline BCVA as the dependent variable, baseline subretinal fluid presence grade as the independent variable, and baseline CST and age as covariates.
‡Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, baseline subretinal fluid presence grade as the independent variable, and baseline BCVA, baseline CST, and age as covariates.
§Between-group differences and 95% CIs are based on the least-square means derived from an analysis-of-covariance model with change from baseline in BCVA at week 24 as the dependent variable, change from baseline in subretinal fluid presence as the independent variable, and baseline BCVA, baseline CST, and age as covariates.

"definitely outside the centre subfield" (12.7 letters vs 16.1 letters, respectively; $p = 0.478$). In a consistent manner but to a lesser extent, eyes with definite SRF at baseline showed less vision improvement when compared with eyes graded by the reading centre as having no or questionable SRF (12.0 letters vs 13.6 letters, respectively; $p = 0.539$).

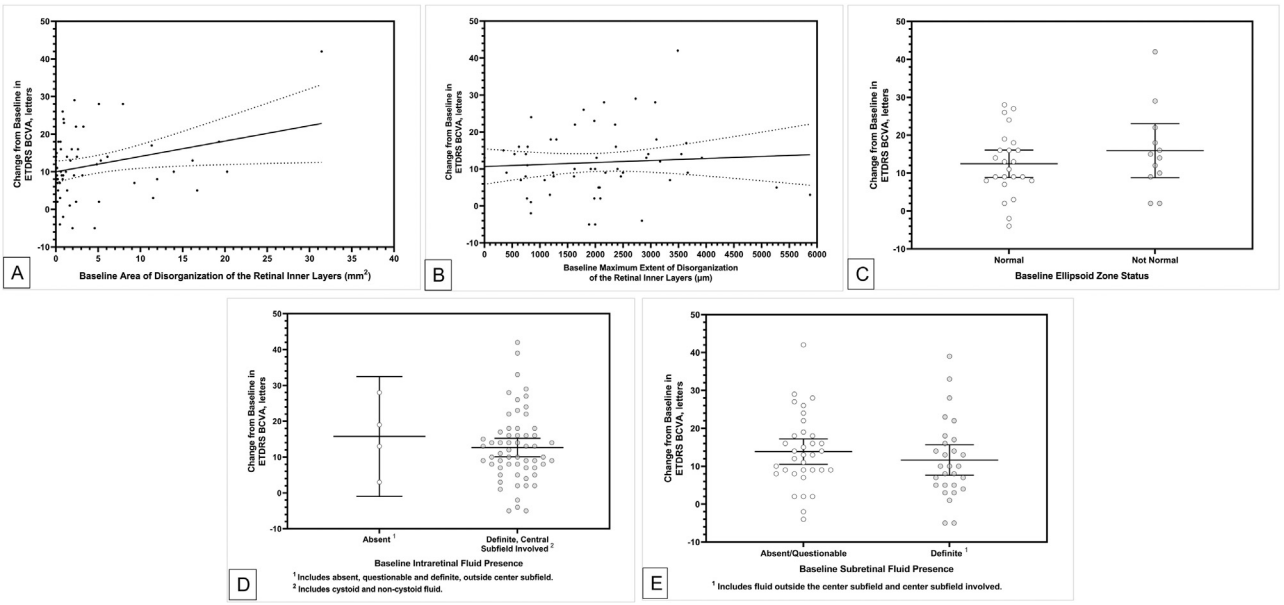

Fig. 3—Plots showing relationships between baseline anatomic features and change from baseline in best-corrected visual acuity (BCVA) at week 24. (A) Area of disorganization of retinal inner layers (DRIL) at baseline and change in Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA at week 24. (B) Maximum horizontal extent of DRIL at baseline and change in ETDRS BCVA at week 24. (C) Ellipsoid zone status at baseline and change in ETDRS BCVA at week 24. (D) Presence of intraretinal fluid at baseline and change in ETDRS BCVA at week 24. (E) Presence of subretinal fluid at baseline and change in ETDRS BCVA at week 24. Scatterplots show linear regression lines (solid) plotted along with lines (dashed) outlining 95% confidence intervals for anatomic features measured on a continuous scale or means and the associated 95% confidence intervals for anatomic features assessed by severity grade.

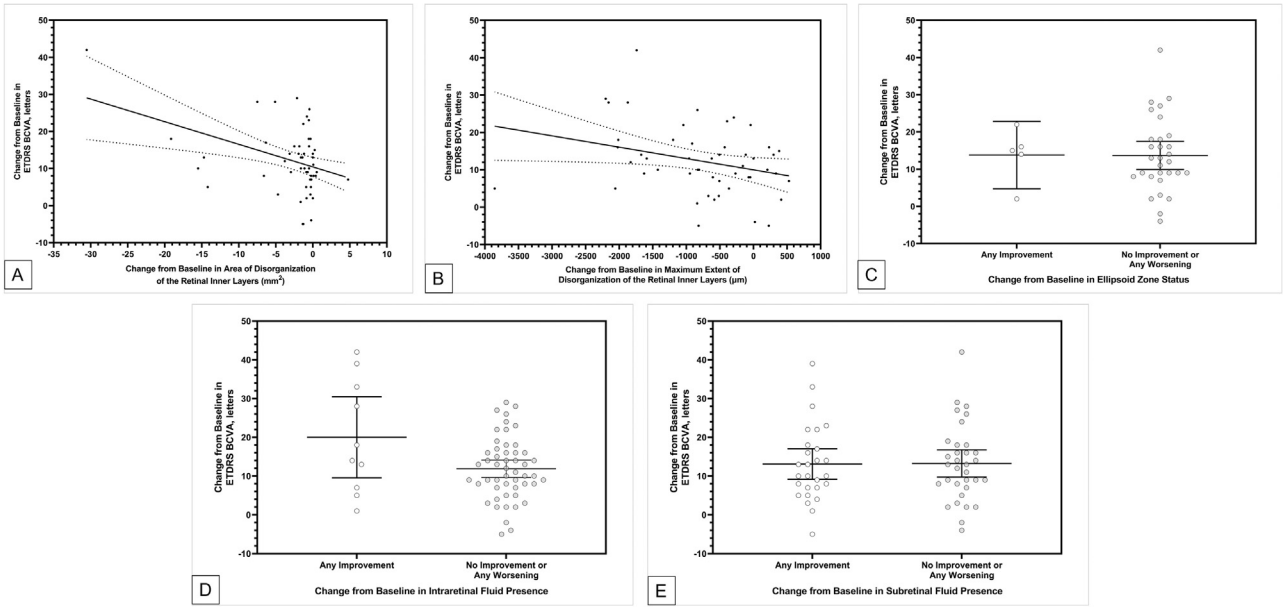

Fig. 4—Plots showing relationships between change from baseline in anatomic features at week 24 and 24 week change from baseline in best-corrected visual acuity (BCVA). (A) Change in area of disorganization of retinal inner layers (DRIL) and change in Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA at week 24. (B) Change in maximum horizontal extent of DRIL and change in ETDRS BCVA at week 24. (C) Change in ellipsoid zone status and change in ETDRS BCVA at week 24. (D) Change in presence of intraretinal fluid and change in ETDRS BCVA at week 24. (E) Change in presence of subretinal fluid and change in ETDRS BCVA at week 24. Scatterplots show linear regression lines (solid) plotted along with lines (dashed) outlining 95% confidence intervals for anatomic features measured on a continuous scale or means and the associated 95% confidence intervals for anatomic features assessed by severity grade.

24-Week changes in BCVA and 24-week changes in OCT anatomy

At week 24, there were 53 eyes with BCVA and area of DRIL data and 50 eyes with both BCVA and data for the maximum horizontal extent of DRIL. Linear correlation coefficients and associated 95% confidence intervals are detailed in Table 2. Low negative correlations were found between change in BCVA and change in area of DRIL ($r = -0.40$, $p = 0.003$; Fig. 4A) and change in maximum horizontal extent of DRIL at week 24 ($r = -0.30$, $p = 0.04$; Fig. 4B).

There were 35 eyes with BCVA and EZ changes from baseline values at week 24, 61 eyes with BCVA and IRF changes from baseline values, and 60 eyes with both BCVA and SRF changes from baseline values. Eyes that showed any improvement in EZ integrity at week 24 experienced less improvement from baseline in vision, on average, than eyes that did not show any change from baseline or worsened, but this difference was not statistically significant (11.7 letters vs 14.0 letters; $p = 0.61$), as shown in Table 3 and Figure 4C.

In contrast to the change in EZ integrity, eyes that showed any improvement in central subfield IRF (Table 4 and Fig. 4D) or SRF (Table 5 and Fig. 4E) showed a greater but not statistically significant improvement in BCVA at week 24 (17.8 and 13.6 letters, respectively) than eyes that did not show any change or worsened from baseline (12.3 and 12.7 letters, respectively; $p \geq 0.102$ for both).

Reproducibility analysis on 20 eyes with the masked method showed excellent reproducibility for both DRIL area (ICC = 0.98; 95% CI, 0.97–0.99), and the mean DRIL area for the first and second graders was 1.45 ± 1.43 mm^2 versus 1.50 ± 1.44 mm^2 and the absolute difference between the graders was 0.05 ± 001 mm^2. And for DRIL maximum extent, ICC = 0.97 (95% CI, 0.94–0.9), with a mean DRIL maximum extent of 1633 ± 795 μm versus 1621 ± 715 μm, and the mean absolute difference between graders was 11 ± 79 μm.

Discussion

This post hoc analysis evaluated SRF, IRF, EZ integrity, and DRIL as biomarkers for visual function and treatment response in eyes with DME from the prospective TYBEE trial. In this analysis, mean baseline BCVA progressively worsened with each ordinal drop in EZ integrity, improved with the presence of significant SRF, and was invariant to the presence of IRF. However, improvements in BCVA at week 24 were no different between patients showing any improvement in EZ integrity, SRF, or IRF compared with those showing no improvement or worsening from baseline. DRIL area was negatively correlated with BCVA at baseline, and the decrease in DRIL area was positively correlated with BCVA gain at week 24.

The observations from this study add to a growing body of evidence that DRIL is a robust biomarker for visual function

in eyes with DME. In 1 study, the relationship between DRIL and retinal function has been explored in diabetic patients by evaluation of contrast sensitivity, standard automated perimetry, frequency-doubling perimetry, and short-wavelength automated perimetry; eyes with DRIL showed a significant reduction of retinal function compared with those without DRIL.[18] Moreover, a significant association has been demonstrated between centrally located DRIL and outer retinal layer disruption, as well as diabetic retinopathy severity.[19]

With respect to treatment response, significant DRIL improvement in DME eyes has been reported after intravitreal anti-VEGF therapy,[11] as well as after corticosteroid treatment.[20] Although Wirth et al.[21] reported a nonsignificant change in DRIL after long-term anti-VEGF therapy, their study was limited by its retrospective design, evaluation of only central horizontal scans, and the fact that the study graders did not receive special training for OCT analysis. In agreement with previous reports,[11–13] we found a strong relationship between area of DRIL and VA both at baseline and after treatment. Therefore, DRIL as a microstructural biomarker may serve as a reliable predictor for functional outcome.

Despite the interest in studying DRIL, there has been inconsistency in its quantification methodology. Sun et al.[12] analyzed the DRIL extent in a 1 mm wide area centred on the fovea of the 7 central horizontal B-scans. Radwan et al.[11] analyzed a 1500 μm wide region of 5 central B-scans. In contrast, Wirth et al.[21] examined only a single horizontal foveal scan. In our study, we developed a novel method for quantification of the area of DRIL using proprietary software that allows manual segmentation of the inner retinal layers.

The exact pathogenesis of DRIL is not yet well delineated. However, it has been hypothesized that it might result from cellular destruction of inner retinal layers, including bipolar, amacrine, and horizontal cells.[12] DRIL also may represent a disruption of the visual transmission pathway due to macular thickening that exceeds the elasticity limit of bipolar axons, leading to their snapping and interruption of transmission from photoreceptors to ganglion cells.[22] The relationship between DRIL and vascular compromise has been reported previously. On fluorescein angiography, DRIL has been associated with macular ischemia.[23] Also, on OCT angiography, DRIL has been related to foveal avascular zone enlargement as well as decreased perfusion of superficial and deep vascular complexes in eyes with or without centre-involving DME.[24–26]

Our study is not without limitations, including the small sample size, because this is a post-hoc analysis of data that had been collected as part of the main TYBEE trial. Another limitation is that a subset of eyes (n = 14) was imaged using the Cirrus HD-OCT device instead of the Heidelberg Spectralis device. However, Sampani et al.[27] reported a good agreement for DRIL extent and other variables graded on a Cirrus volume cube versus Spectralis high-resolution scans. As a limitation, this study cannot exclude that the difference in BCVA was due to treatment status and not the specific biomarker investigated. However, there is no literature to consistently support differentiating effects on OCT biomarkers (such as DRIL or EZ) based on therapeutic agent used, independent of overall amelioration. And the correlation between BCVA and DRIL is poor and could potentially be influenced by outliers.

Our study also has several strengths, including imaging data collected according to a standardized prospective imaging protocol, double masking, well-defined patient cohorts, and high adherence to the study protocol. An additional strength is the grading of the OCT images by independent masked image reading centre graders with experience in diabetic retinopathy studies and OCT image analysis.

In summary, in this analysis of eyes with DME in the phase 2 TYBEE study, EZ integrity, SRF, and IRF were correlated with mean BCVA at baseline only, whereas DRIL area showed a negative correlation with BCVA at baseline, and the decrease in DRIL area was positively correlated with BCVA gain at week 24. Consequently, DRIL area and DRIL maximum horizontal extent may represent clinically useful biomarkers for disease status, visual function, and prognosis in eyes with DME.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcjo.2023.01.012.

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Footnotes and Disclosures

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