OPTICAL COHERENCE TOMOGRAPHY BASELINE PREDICTORS FOR INITIAL BEST-CORRECTED VISUAL ACUITY RESPONSE TO INTRAVITREAL ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR TREATMENT IN EYES WITH DIABETIC MACULAR EDEMA

The CHARTRES Study

ANA R. SANTOS, MSc,*† MIGUEL Â. COSTA, MSc,* CHRISTIAN SCHWARTZ,* DALILA ALVES, MSc,* JOÃO FIGUEIRA, MD, PhD,*‡§ RUFINO SILVA, MD, PhD,*‡§ JOSE G. CUNHA-VAZ, MD, PhD*§

Purpose: To identify baseline optical coherence tomography morphologic characteristics predicting the visual response to anti-vascular endothelial growth factor therapy in diabetic macular edema.

Methods: Sixty-seven patients with diabetic macular edema completed a prospective, observational study (NCT01947881-CHARTRES). All patients received monthly intravitreal injections of Lucentis for 3 months followed by PRN treatment and underwent best-corrected visual acuity measurements and spectral domain optical coherence tomography at Baseline, Months 1, 2, 3, and 6. Visual treatment response was characterized as good ($\geq$10 letters), moderate (5–10 letters), and poor ($<$5 or letters loss). Spectral domain optical coherence tomography images were graded before and after treatment by a certified Reading Center.

Results: One month after loading dose, 26 patients (38.80%) were identified as good responders, 19 (28.35%) as Moderate and 22 (32.83%) as poor responders. There were no significant best-corrected visual acuity and central retinal thickness differences at baseline ($P = 0.176$; $P = 0.573$, respectively). Ellipsoid zone disruption and disorganization of retinal inner layers were good predictors for treatment response, representing a significant risk for poor visual recovery to anti-vascular endothelial growth factor therapy (odds ratio = 10.96; $P < 0.001$ for ellipsoid zone disruption and odds ratio = 7.05; $P = 0.034$ for disorganization of retinal inner layers).

Conclusion: Damage of ellipsoid zone, higher values of disorganization of retinal inner layers, and central retinal thickness decrease are good predictors of best-corrected visual acuity response to anti-vascular endothelial growth factor therapy.

Key words: anti-VEGF, BCVA predictors, diabetic macular edema, optical coherence tomography.

Diabetic macular edema (DME) is the major cause of visual acuity impairment in patients with diabetic retinopathy (DR).[1] Vascular endothelial growth factors (VEGFs) play an important role in the alterations of vascular permeability and development of DME. It interferes with the "tight junctions" of the endothelium of the retinal vessels leading to a breakdown of the BRB and consequent leakage to the retinal tissue.[2] Based on this concept, the administration of intravitreal (IVT) anti-VEGFs in DME has been widely demonstrated to be efficient in macular thickness improvement and consequent increase of best-corrected visual

acuity (BCVA),[3,4] although these results may not be permanent and multiple injections may be required to maintain treatment efficacy. Furthermore, in some cases, the resolution of DME is not followed by recovery of visual function. According to Elman et al,[5] after 24 months of treatment with ranibizumab and deferred laser, 49% of the subjects had a BCVA gain ≥10 letters (good responders), 22% had a BCVA gain between 5 and 10 letters (responders), and 29% had a BCVA gain <5 letters or a decrease in BCVA (poor responders). Massin et al[6] and Mitchell et al[7] refer that after 12 months of treatment with ranibizumab in monotherapy, 40% to 60% of the subjects had a BCVA gain ≥10 letters, 30% had a BCVA gain between 5 and 10 letters, and 10% to 30% had a BCVA gain <5 letters or a decrease in BCVA. Moreover, Gonzalez et al,[8] in a post hoc analysis of Diabetic Retinopathy Clinical Research Network's Protocol I data, showed that the mean change in BCVA from Month 3 to Month 12 is lower than 5 letters indicating that the response to the loading dose (3 initial monthly injections) seems to determine the final visual recovery at 1 and 3 years.

It is, therefore, of major importance to characterize the baseline features that may identify the different visual outcomes observed in different eyes after the initial three monthly injections of anti-VEGF in DME and if any of these characteristics can predict poor response to treatment.

Damage in the inner/outer segments of the photoreceptor layer (IS/OS), currently termed as ellipsoid zone (EZ),[9] or in the retinal pigment epithelium have been reported to predict the visual response to treatment with anti-VEGF injections, as well as the extent of disorganization of the retinal inner layers (DRIL).[10–12] However, most of these studies were performed retrospectively or in patients previously treated with IVT corticosteroids or anti-angiogenic drugs.

In this study, we sought to analyze and quantify the DME morphologic features that could correlate with BCVA response in the initial stage of anti-VEGF treatment response (after the loading dose) and up to 6 months, in a prospective study of well-characterized naive patients with DME that has clinical indication for ranibizumab treatment. Using a detailed grading of spectral domain optical coherence tomography (SD-OCT) images, we assessed not only the central retinal thickness (CRT) response to therapy but also the baseline morphologic characteristics of outer and inner retinal layers, as well as size and location of cystoid spaces, and their relationship with visual acuity outcomes.

Methods

Study Design

A prospective, exploratory, and observational study (NCT01947881-CHARTRES) was conducted in diabetic Type 2 patients receiving the same interventional treatment after clinical practice guidelines. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional review board and ethics committee of AIBILI, Coimbra, Portugal. Written informed consent was obtained from all study patients. Patients were treated and followed according to the standard practice for DME treatment with ranibizumab IVT injections as described in the Summary of Product Characteristics (SmPC): Loading dose of three monthly injections followed by PRN regimen.

Sample Calculation

The previously mentioned authors[5–7] showed that it is expected to have 40% to 60% of good responders, 20% to 30% of responders, and 20% to 30% of poor responders after 12 and 24 months of IVT treatment with ranibizumab for DME. Therefore, focusing on the initial 3 months of treatment (the loading dose of three monthly IVT injections), and taking into account that at least one of the three groups may only represent 20% of the sample, the inclusion of 70 subjects was considered appropriate to cover the extreme situation of 42 good responders (60%), 14 responders (20%), and 14 poor responders (20%).

Study Participants

Naive patients with indication for treatment with ranibizumab injections for DME in the investigator's

From the *AIBILI, Association for Innovation and Biomedical Research on Light and Image Coimbra, Portugal; Orthoptics Scientific Area, †School of Allied Health Technologies, Polytechnic Institute of Porto, Porto, Portugal; ‡Department of Ophthalmology, Coimbra University Hospital Center, Coimbra, Portugal; and §Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

This study was financially supported by Novartis.

R. Silva and J. Figueira are consultants of Bayer, Novartis, Alimera Sciences, Alcon and Allergan. R. Silva is also consultant of Thea. J. G. Cunha-Vaz reports grants from Carl Zeiss Meditec, outside the submitted work and is consultant for Alimera Sciences, Allergan, Bayer, Gene Signal, Novartis, Pfizer, Precision Ocular Ltd., Roche, Sanofi-Aventis, Vifor Pharma and Carl Zeiss Meditec. The remaining authors have no any conflicting interests to disclose.

Trial Registration: ClinicalTrials.gov (NCT01947881-CHARTRES).

Reprint requests: Ana R. Santos, MSc, AIBILI, Association for Innovation and Biomedical Research on Light and Image, Azinhaga Sta. Comba, Celas, 3000-548 Coimbra, Portugal; e-mail: asantos@aibili.pt

opinion and fulfilling the following inclusion criteria 1) Type 2 Diabetes Mellitus; 2) center-involved DME, confirmed by OCT and defined as a baseline SD-OCT central subfield retinal thickness $\geq$300 μm[13,14]; 3) visual impairment due to DME with BCVA $\geq$ 20/160 and $\leq$ 20/40 ($\geq$39 letters and $\leq$73 letters); 4) glycated hemoglobin (HbA1C) $\leq$12% at screening visit. Exclusion criteria: 1) presence of proliferative DR; 2) previous laser photocoagulation (panretinal or focal) in the study eye within 6 months before study inclusion; 3) previous treatments with IVT injections of triancinolone or anti-VEGF drugs in the study eye; 4) previous vitrectomy surgery; 6) other chorioretinal diseases such as central serous chorioretinopathy, high myopia, chorioretinitis, or any other fundus disease associated with morphologic or functional changes.

Study Procedures

All included patients performed an initial visit (V1) with the following procedures: clinical history (medical history, demographics, and concomitant medications); vital signs, metabolic analysis; biomicroscopy; intraocular pressure with Goldmann tonometry; ophthalmoscopy; BCVA (using ETDRS method); color fundus photography—FP (7 ETDRS fields); SD-OCT (HD-OCT Cirrus, Zeiss Meditec); and fluorescein angiography–FA (Topcon TRC 50DXTM).

After baseline visit (V1), all patients were treated with three monthly IVT injections of anti-VEGF ranibizumab during 3 months (loading dose—V2, V3 and V4) and monitored at each visit before injection with BCVA and OCT measurements. One month after the last injection of the loading dose period (V5), i.e., 3 months after the first injection, BCVA, OCT, CFP, and FA procedures were repeated, and patients received more monthly injections after a PRN regimen if the central retinal thickness remained $\geq$300 μm. Patients were monitored monthly with BCVA, and OCT examinations and the final visit (V6) was performed 6 months after the first injection. Best-corrected visual acuity, OCT, CFP, and FA were performed as well. Optical coherence tomography, CFP, and FA images were graded by certified graders in a reading center—Coimbra Ophthalmology Reading Centre (CORC). Quality control of CFP and FA grading was ensured by double grading in 8% of all cases with an observed agreement of 93.8% between graders.

Optical Coherence Tomography Acquisition and Grading

A Macular Cube 512 $\times$ 128 scan and 2 macular 5 HD lines (at 180° and 90°) were acquired in all patients using HD-OCT Cirrus 5000 (Zeiss Meditec, Dublin). A detailed OCT double grading was done in all visits by two CORC independent graders. The observed agreement between the 2 graders was 93.6%. All disagreement cases were resolved by mutual agreement. Central retinal thickness, perifoveal, and parafoveal retinal thicknesses were quantified using Macular Cube maps. Diabetic macular edema was classified as diffuse or cystoid (cystoid macular edema);[15] cystoid macular edema was also classified according the location of cystoid spaces in the retinal layers (inner, outer, or overall cystoid spaces) and severity (mild, moderate, or severe cystoid spaces),[16] The integrity of both inner and outer retinal layers was also accessed (Figure 1). Disorganization of the retinal inner layers (DRIL) was defined as the horizontal extent in microns for which any boundaries between the ganglion cell–inner plexiform layer complex, inner nuclear layer, and outer plexiform layer could not be identified.[11] The DRIL extent was measured using the equipment software calliper in each of the five horizontal HD B-scans, and these measurements were averaged across five scans to derive a global DRIL area for each eye at baseline. Disruption of external retinal membrane (ELM), EZ and retinal pigment epithelium complex were defined as the horizontal extent with loss of the hyperreflective signal that characterizes each layer.[17] The disruption of these layers was also measured in the central 1 mm of the five consecutive horizontal scans of five HD Line protocol. Presence of neurosensorial serous detachment, vitreo-macular traction, and epiretinal membrane was also analyzed.

Data Analysis

One month after the loading dose, at Visit 5, patients were categorized according to their BCVA evolution from baseline and were stratified in three treatment response groups: good responders ($\geq$10 ETDRS letters gained), moderate responders ($\geq$5 & $<$10 ETDRS letters gained) or poor responders ($<$5 ETDRS letters gained or loss of visual acuity). Morphologic SD-OCT characteristics were compared between treatment response groups by univariate analysis performed with analysis of variance or Kruskal–Wallis test and Fisher's exact test. Subsequently, multinomial logistic regression was performed to identify possible treatment response predictors. The multinomial logistic regression generates an odds ratio (OR) for each category of the dependent variable in relation to the reference category.

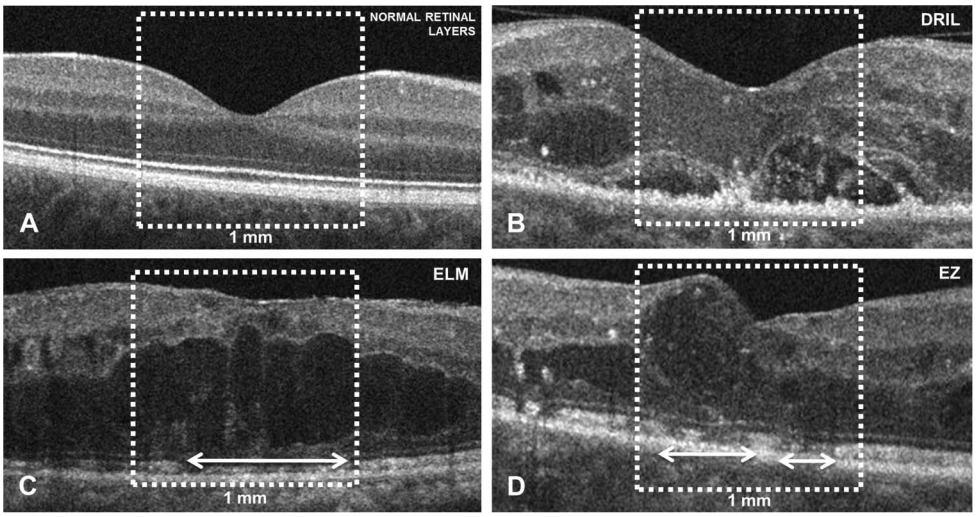

Fig. 1. Representative images of the presence and extension of DRIL and disruption of EZ and ELM layers, measured in the 1-mm central area (white box): (**A**) Normal retinal layers without disruption; (**B**) Severe DRIL–retina inner layers boundaries cannot be identified in almost half of the 1-mm central area (white circle and white arrow); (**C**) Severe ELM disruption showed by absence of reflectivity in the ELM layer (white arrow); (**D**) Severe EZ disruption showed by the presence of several areas with no hyperreflective signal (white arrows).

The OR value includes the confidence interval (CI 95%) allowing estimating the degree of accuracy. To analyze associations between variables, Spearman's correlation coefficient and the respective statistical significance were computed. A receiver operating characteristic (ROC) analysis was performed to identify the best predictors for a more than five ETDRS letters improvement in BCVA. All tests were two-sided and significance was set at 0.05. Statistical analysis was performed with Stata 12.1 SE (College Station, TX: StataCorp LP).

Results

A total of 71 patients were included in this study, and 67 were considered for analysis. Four patients (5.6%) discontinued the study, 2 voluntarily and 2 because of health complications unrelated to the study (Figure 2).

Response to Anti-Vascular Endothelial Growth Factor Treatment According to Final Best-Corrected Visual Acuity

According to BCVA changes from baseline to Visit 5 (after three monthly injections of ranibizumab), 26 patients (38.81%) were considered good responders, 19 patients (28.35%) were considered moderate responders, and 22 patients (32.84%) were considered poor responders.

Baseline Characteristics of Study Patients by Treatment Response

Baseline characteristics (demographics, vital signs, metabolic factors, diabetes duration, BCVA, CRT, and ETDRS DR level) for all study population, and by treatment response, are summarized in Table 1. No significant differences were found at baseline between treatment response groups, even after using age and diabetes duration as adjusting factors.

Central Retinal Thickness Decrease as a Predictor for Best-Corrected Visual Acuity Treatment Response

Despite no significant CRT differences between groups at baseline, a higher and significant CRT decrease was found in the treatment groups with better response at Visit 5 (1 month after loading dose) and Visit 6 (6 months after initiating treatment), respectively ($P < 0.001$) (Figure 3).

On a ROC analysis, CRT decrease 1 month after the first injection is not a statistically significant predictor

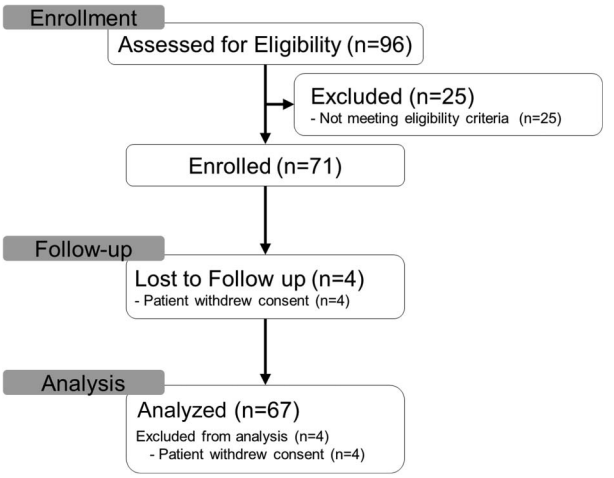

Fig. 2. Flow chart of the study.

Table 1. Baseline Characteristics of Patients

	Study Population (n = 67)	Good Responders (n = 26)	Moderate Responders (n = 19)	Poor Responders (n = 22)	*P*
Demographics					
Age, mean ± SD, years	64.4 ± 10.3	64.9 ± 7.5	60.5 ± 15.7	67.2 ± 5.4	0.106
Females, n (%)	26 (38.8)	7 (26.9)	6 (31.6)	13 (59.1)	0.056
Vital signs					
Heart rate, mean ± SD, bpm	73.5 ± 10.6	74.3 ± 9.9	74.1 ± 12.2	72.0 ± 10.1	0.702
Systolic blood pressure, mean ± SD, mmHg	142.5 ± 10.3	141.9 ± 9.4	139.4 ± 10.5	145.7 ± 10.6	0.177
Diastolic blood pressure, mean ± SD, mmHg	72.6 ± 9.0	72.7 ± 9.5	74.1 ± 8.5	71.3 ± 9.0	0.608
Metabolic factors					
HbA1C, mean ± SD, %	7.8 ± 1.5	7.9 ± 1.7	8.1 ± 1.2	7.5 ± 1.5	0.385
Total cholesterol, mean ± SD, mg/dL	189.7 ± 50.9	191.8 ± 42.3	197.8 ± 69.5	179.7 ± 40.7	0.701
HDL cholesterol, mean ± SD, mg/dL	47.4 ± 10.8	48.2 ± 11.7	46.5 ± 11.6	47.2 ± 9.3	0.586
LDL cholesterol, mean ± SD, mg/dL	127.1 ± 39.1	129.0 ± 35.8	134.7 ± 51.6	117.9 ± 28.7	0.542
Triglycerides, mean ± SD, mg/dL	155.1 ± 92.2	155.5 ± 76.7	163.5 ± 135.8	146.9 ± 106.1	0.682
Disease characteristics					
Diabetes duration, mean ± SD, years	18.1 ± 7.7	18.1 ± 7.6	15.4 ± 5.7	20.5 ± 8.8	0.333
DME duration, median (IQR), months	7.94 ± 23.62	7.81 ± 28.57	6.47 ± 15.81	9.36 ± 23.74	0.744
CRT, mean ± SD, μm	427 ± 107	421 ± 101	463 ± 144	404 ± 64	0.573
BCVA, mean ± SD, letters	63.4 ± 9.2	63.6 ± 8.5	65.7 ± 9.0	61.3 ± 10.0	0.176
Snellen equivalent	20/63	20/50	20/50	20/63	
DR level (ETDRS scale), n (%)					
35 (C–F)	48 (71.6)	17 (65.4)	11 (57.9)	20 (90.9)	0.062
53 (A–B)	14 (20.9)	6 (23.1)	7 (36.8)	1 (4.6)	
57 (A–D)	5 (7.5)	3 (11.5)	1 (5.3)	1 (4.6)	

HbA1C, glycated hemoglobin; HDL, high-density liprotein; LDL, low-density liprotein; *P*, *P* value; Age and diabetes duration were adjusted in this analysis.

for treatment response. However, using a threshold of 8.7% for CRT decrease, we were able to distinguish 73.3% of patients who recovered more than 5 BCVA letters after 3 monthly injections despite a high percentage of false negatives (sensitivity 73.3%, specificity 50.0%, ROC AUC 0.581) (Figure 4).

Baseline Optical Coherence Tomography Morphologic Features by Treatment Best-Corrected Visual Acuity Response

As described above, baseline morphologic features of DME were analyzed on OCT, as well as the degree of disruption and disorganization of inner and outer retinal

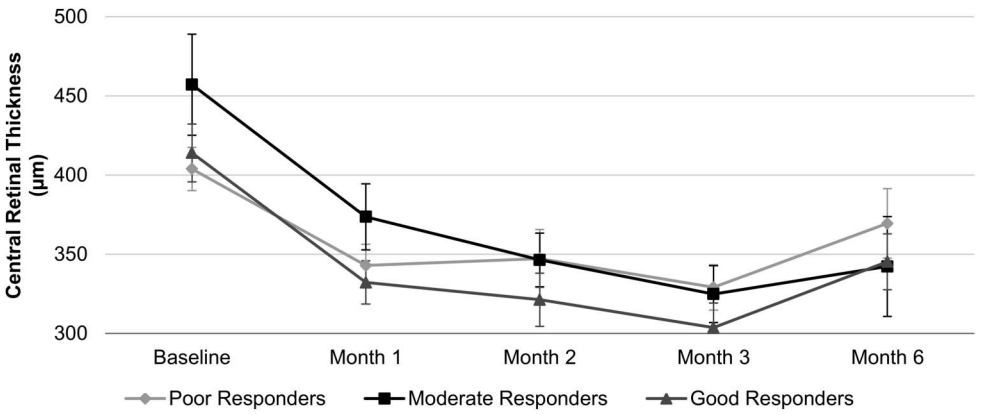

Fig. 3. Decrease of retinal thickness by treatment response from Baseline to Month 1 (after first injection), Month 2 (after second injection), Month 3 (after third injection), and Month 6.

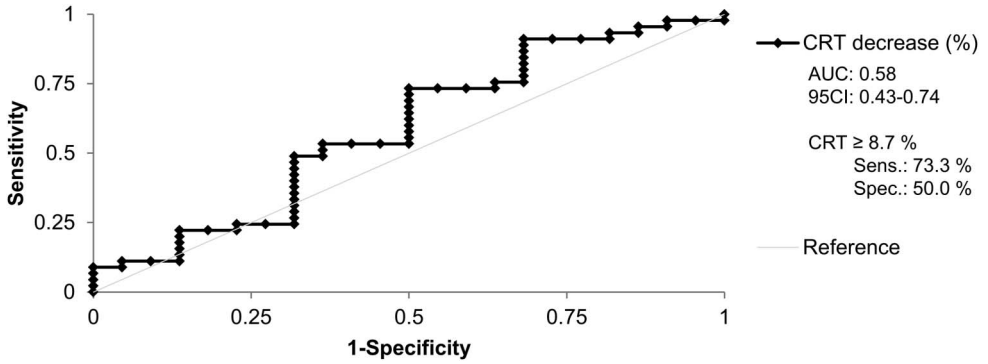

Fig. 4. ROC analysis for CRT decrease as a threshold for BCVA improvement.

layers to evaluate the possibility of predicting different treatment responses. Significant differences were found among treatment response groups regarding DRIL area (P = 0.021) and EZ and ELM disruption area (P = 0.006 and P = 0.003, respectively), at baseline. Likewise, cystoid spaces severity seems also to be associated with a poor response to anti-VEGF treatment. Poor responders group have higher percentage of moderate cystoid spaces (57.14%), whereas good responders group showed higher percentage of mild cystoid spaces (23.08%). However, these differences were not statistically significant (P = 0.252) (Table 2).

Poor responders presented, at baseline, a greater extent of EZ and ELM disruption (P = 0.003 and 0.006, respectively). Extension of DRIL area was also significantly higher in this group (P = 0.021) (Figure 5).

Moreover, a correlation was found between EZ and ELM disruption area and DRIL area with the treatment response. The presence of EZ disruption was the morphologic characteristic with a stronger relation to a poor response to treatment (Table 3).

Optical Coherence Tomography Predictors for Treatment Best-Corrected Visual Acuity Response

To identify the best OCT morphologic predictors for an improvement of more than 5 letters in BCVA after treatment, a ROC analysis was

Table 2. Baseline OCT Morphologic Features of DME for the Study Population and by Treatment Response Group

	Study Population (n = 67)	Good Responders (n = 26)	Moderate Responders (n = 19)	Poor Responders (n = 22)	*P*
OCT morphologic features, n (%)					
Diffuse macular edema	58 (86.57)	22 (84.62)	17 (89.47)	19 (86.36)	0.999
Cystoid macular edema	67 (100)	26 (100)	19 (100)	22 (100)	—
Outer cystoid spaces	64 (96.97)	25 (96.15)	18 (94.74)	21 (100)	0.745
Inner cystoid spaces	63 (95.45)	25 (96.15)	18 (94.74)	20 (95.24)	0.999
Overall cystoid spaces	20 (30.3)	7 (26.92)	9 (47.37)	4 (19.05)	0.159
Severity of cystoid spaces, n (%)					
Mild	14 (21.21)	6 (23.08)	5 (26.32)	3 (14.29)	0.252
Moderate	31 (46.97)	14 (53.85)	5 (26.32)	12 (57.14)	
Severe	21 (31.82)	6 (23.08)	9 (47.37)	6 (28.57)	
Cystoid spaces size, n (%)					
Small ($<$250 μm)	6 (9.09)	4 (15.38)	1 (5.25)	1 (4.76)	0.107
Medium ($\geq$250 & $<$500 μm)	39 (59.09)	12 (46.15)	10 (52.63)	17 (80.95)	
Large: $\geq$500 μm	21 (31.82)	10 (38.46)	8 (42.11)	3 (14.29)	
Disruption of retinal layers, μm^2					
DRIL, mean ± SD	367.8 ± 211.0	278.6 ± 191.7	404.4 ± 158.9	441.6 ± 240.5	**0.021**
EZ, mean ± SD	314.7 ± 249.2	196.1 ± 201.2	327.3 ± 219.1	444.0 ± 266.2	**0.003**
ELM, mean ± SD	103.4 ± 158.8	30.2 ± 64.7	135.8 ± 177.9	161.9 ± 189.6	**0.006**
RPE, mean ± SD	31.2 ± 74.2	14.2 ± 57.6	16.4 ± 46.0	63.9 ± 99.2	0.148
Other OCT features, n (%)					
NSD	22 (32.84)	6 (23.08)	7 (36.84)	9 (40.91)	0.394
Epiretinal membrane	22 (32.84)	8 (30.77)	4 (21.05)	10 (45.45)	0.263
Vitreo-retinal traction	3 (4.48)	1 (3.85)	2 (10.53)	0	0.224

ELM, external limiting membrane; NSD, neurosensorial serous detachment; *P*, *P* value (values in bold are significant for $p<0.05$); RPE, retina pigment epithelium.

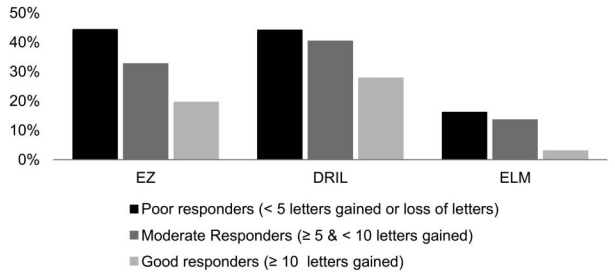

Fig. 5. Disruption of retinal inner layers (DRIL) area and disruption of EZ and external limiting membrane (ELM) retinal layers by treatment response group.

performed (Table 4), showing that the best predictor for this visual improvement was the EZ disruption area (ROC AUC 0.71-sensitivity 59%, specificity 80%).

The predictive value of OCT morphologic features to treatment response after the loading dose was analyzed with univariate multinomial logistic regression. Then, multivariate multinomial logistic regression was performed with the following parameters: EZ disruption area and DRIL area; because these two variables showed statistically significant differences between good responders and poor responders in the univariate logistic analysis. Being the primary treatment outcomes, BCVA and CRT were also analyzed (Table 5).

Ellipsoid zone disruption area seems to have an important contribution to a poor functional outcome to anti-VEGF treatment, with an OR of 10.96 (CI = 2.94–40.8; $P < 0.001$) for poor responders versus good responders, which means that the higher the EZ disruption area, the worst is expected to be the functional treatment recovery. Disorganization of retinal inner layer area seems also to be a risk factor for BCVA response to treatment with an OR of 7.05 (CI = 1.16–42.89; $P = 0.034$) for poor responders versus good responders. These results are similar at Month 6 (V6), with an OR of 7.86 (CI = 2.10–29.43; $P = 0.002$) for EZ and an OR of 8.05 (CI = 1.20–54.01; $P = 0.032$) for DRIL.

A subanalysis was performed excluding patients who received laser therapy 6 months before inclusion (32.8%) to evaluate the possible impact of this treatment in the present biomarkers. No significant changes were found and DRIL and EZ remained the major predictors of poor treatment response (results not shown).

Table 3. Correlation Between Retinal Layers Disruption and BCVA Treatment Response

	Correlation	*P*
EZ disruption area	$r_s = -0.56$; CI = −0.70 to −0.36	<0.001
ELM disruption area	$r_s = -0.52$; CI = −0.67 to −0.32	<0.001
DRIL area	$r_s = -0.39$; CI = −0.58 to −0.17	0.001

P, *P* value; r_s, Spearman correlation coefficient.

Table 4. Area Under the Curve and Sensitivity and Specificity for the DRIL Area and EZ and ELM Disruption Areas

	ROC AUC	Sensitivity at 80% Specificity
DRIL area	0.65 (CI 95% = 0.50–0.81)	55
EZ disruption area	0.71 (CI 95% = 0.56–0.85)	59
ELM disruption area	0.64 (CI 95% = 0.50–0.78)	45

CRT and RPE disruption area did not show acceptable accuracy to discriminate poor responders.

AUC, area under the curve; RPE, retinal pigment epithelium.

Discussion

Regular IVT treatment with ranibizumab in patients with DME decreases CRT and improves BCVA.[7,18] In this study, we prospectively observed 67 patient eyes with naive DME after initiating a loading dose of three monthly IVT ranibizumab injections followed by PRN treatment for up to 6 months. A mean CRT decrease of 107.93 μm (−25%) was obtained immediately after the loading dose (three monthly injections), with a significant recovery of BCVA (+6.78 letters; $P < 0.001$). These results are in accordance with other clinical trials such as RESTORE, RISE, and RIDE,[7,18] where significant CRT decreases and BCVA increases were achieved after the same regimen of ranibizumab therapy.

Although CRT is widely used to evaluate and follow eyes with DME, it has been shown to be only moderately correlated with BCVA outcomes.[19] The available clinical trial data have shown that only 50% to 60% of eyes treated with anti-VEGF for DME respond with complete retinal thickening resolution or have improvement of VA to 20/20 or better.[20]

In our study, we analyzed the potential role of CRT decrease after the first injection as a predictor for BCVA. Central retinal thickness decrease does not reach statistical significance as a predictor for treatment response, but a threshold of CRT decrease of 8.7% immediately after the first anti-VEGF injection (at 1 month) was able to distinguish 73.3% of patients who recovered more than 5 BCVA letters at 3 months, with a modest specificity. Larger sample size may, in the future, help to clarify this finding. It is noteworthy

Table 5. Univariate and Multivariate Multinomial Logistic Regression Analysis of Baseline OCT Features With Influence on BCVA Treatment Outcome

Variables	Univariate Analysis				Multivariate Analysis			
	Moderate Responders		Poor Responders		Moderate Responders		Poor Responders	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
BCVA	1.03 (0.96–1.11)	0.421	0.97 (0.92–1.03)	0.399	1.09 (0.99–1.20)	0.076	1.03 (0.93–1.13)	0.589
CRT	1.00 (1.00–1.01)	0.230	1.00 (0.99–1.00)	0.526	1.00 (1.00–1.01)	0.512	0.98 (0.97–1.00)	**0.001**
DRIL area	1.03 (0.20–5.26)	0.970	**4.58 (1.18–17.79)**	**0.028**	0.88 (0.15–5.03)	0.886	**7.05 (1.16–42.89)**	**0.034**
EZ disruption area	1.93 (0.84–4.40)	0.120	**3.93 (1.72–8.94)**	**0.001**	2.72 (0.86–8.64)	0.090	**10.96 (2.94–40.8)**	**<0.001**
Cystoid spaces severity	1.50 (0.65–3.48)	0.336	1.32 (0.59–2.94)	0.501	—	—	—	—
NSD	1.94 (0.53–7.17)	0.318	2.31 (0.66–8.03)	0.189	—	—	—	—
Epiretinal membrane	0.60 (0.15–2.39)	0.469	1.88 (0.57–6.12)	0.297	—	—	—	—

(—): Variables not included in the multivariate analysis.
NSD, neurosensorial serous detachment; *P*, *P* value.

that other studies[21] have based their retreatment criteria in a similar percentage of CRT decrease (10% between visits).

However, whereas some patients have an excellent visual outcome after treatment, some others maintain a substantial visual disability. Robust predictive biomarkers for treatment response in eyes with DME are still lacking despite the large number of studies and reports dedicated to this subject.

A number of studies[10,22,23] have suggested modest associations between OCT parameters, such as presence of intraretinal cysts, hyperreflective foci, subretinal fluid, disruption of ELM, and photoreceptors layer (EZ), with BCVA in eyes with DME, but these correlations have not been strong enough to predict visual acuity reliably and most of the reported studies were conducted retrospectively in mixed treatment cohorts. Other studies[11,24] have identified DRIL on OCT as a parameter indicating highly associated with current and future vision in eyes with DME. These authors found that DRIL affecting 50% of the 1-mm central retinal zone was the only parameter consistently associated with worse BCVA in eyes with current DME and resolved DME after treatment. They also found that increasing of DRIL in the course of the treatment was associated with reduction in BCVA. But again, data from these studies were obtained retrospectively, as part of routine clinical care rather than part of a research protocol and included eyes that underwent different DME treatments before and during the study follow-up and with different DME durations.

In this prospective study, we were able to confirm that presence and extent of DRIL before treatment are correlated with BCVA outcomes to anti-VEGF therapy after the loading dose and, most important, the presence of these morphologic changes seems to be a good predictor of treatment response, representing a risk of almost 8 times higher for poor visual recovery than patients without DRIL. The mechanisms by which DRIL affects BCVA are yet to be determined, although it likely to represent signs of anatomical interruption in the visual transmission pathway from the photoreceptors to the ganglion cells.[11,25]

However, Maheshwary et al[17] found a statically significant correlation between the percentage of photoreceptors IS/OS disruption and visual acuity, which means that EZ disruption may be another significant predictor of VA in patients with DME. However, patients who gained normal vision after treatment were excluded from the analysis, which can compromise the predictive value of this feature. In our study, a significantly higher percentage of EZ and ELM disruption was found at baseline in the poor responders group compared with good responders, and a moderate

correlation was found between the presence of these features and the response to treatment. Our data have also showed that more EZ disruption at baseline represents a higher risk for a poor visual recovery (OR: 10.96; $P < 0.001$), when comparing to the presence of DRIL (OR: 7.05; $P = 0.001$).

Macular cystoid spaces have been proposed as another indicator of retinal damage. Raafay et al[10] found that their presence predicted a reduction in BCVA letter score and that the presence of large cystoid spaces seems to be more disruptive than small ones. Likewise, in our work, cystoid spaces severity seems also to be a predictor of poor response to anti-VEGF treatment.

Our study validates the importance of determination of morphologic patterns on SD-OCT and shows that the integrity of both inner (DRIL) and outer retinal layers (EZ and ELM) can be good predictive biomarkers of future BCVA in patients with DME undergoing anti-VEGF therapy.

This study is considered particularly relevant because it was conducted prospectively, in treatment naive patients who were submitted to the same regimen of ranibizumab treatment and followed up with several examinations before, during, and after therapy. Diabetes duration and DME duration were not significantly different between treatment response groups ($P = 0.333$ and $P = 0.444$, respectively) and most of the patients (70%) presented a DR severity ETDRS level of 35 at baseline which means that it is a population of relatively mild-to-moderate DR and thus, ideal to study such detailed morphologic retinal changes as DRIL, EZ, and ELM disruption with accuracy. It also means that these changes are already present in early stages of DR and, therefore, could provide a significant contribution to counseling, management, and treatment of diabetic macular edema.

It is noteworthy that in our study, previous laser treatment performed 6 months before inclusion did not show any significant influence in the characterization of the OCT nor any identifiable impact in treatment response to anti-VEGF treatment.

Study limitations include the fact that the study deals with a relatively small population, although chosen according to sample size calculation, and the chosen focus on the initial stage (loading dose) of IVT anti-VEGF, not allowing the evaluation of long-term effects of the anti-VEGF therapy. Larger and longer prospective studies are needed to evaluate these aspects. However, despite the focus on initial treatment response (after the loading dose), there were no significant changes in the predictive value of these biomarkers for the BCVA response at 6 months, even after PRN regimen, an observation which is in accordance with previously described studies.[5–7]

In conclusion, SD-OCT provides useful information for determining visual prognoses and outcomes in DME treatment. In naive cases of DME, DRIL, and specially EZ are confirmed as useful structural markers to evaluate retinal tissue integrity and are closely associated with final BCVA after treatment.

References

1. Kempen JH, O'Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol 2004;122:552–563.
2. Grant MB, Afzal A, Spoerri P. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opin Investig Drugs 2004;13:1275–1293.
3. Goyal S, Lavalley M, Subramanian ML. Meta-analysis and review on the effect of bevacizumab in diabetic macular edema. Graefes Arch Clin Exp Ophthalmol 2011;249:15–27.
4. Salam A, DaCosta J, Sivaprasad S. Anti-vascular endothelial growth factor agents for diabetic maculopathy. Br J Ophthalmol 2010;94:821–826.
5. Elman MJ, Bressler NM, Qin H, et al; Diabetic Retinopathy Clinical Research Network. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2011;118:609–614.
6. Massin P, Bandello F, Garweg JG, et al. Safety and efficacy of ranibizumab in diabetic macular edema (RESOLVE Study): a 12-month, randomized, controlled, double-masked, multicenter phase II study. Diabetes Care 2010;33:2399–2405.
7. Mitchell P, Bandello F, Schmidt-Erfurth U, et al. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for DME. Ophthalmology 2011;118:615–625.
8. Gonzalez V, Campbell J, Holekamp N, et al. Early and long-term responses to anti–vascular endothelial growth factor therapy in diabetic macular edema: analysis of protocol I data. Am J Ophthalmol 2016;172:72–79.
9. Staurenghi G, Sadda S, Chakravarthy U, et al. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN • OCT consensus. Ophthalmology 2014;12:1572–1578.
10. Raafay S, Na Lu, Campochiaro P. Predictors of functional and anatomic outcomes in patients with diabetic macular edema treated with ranibizumab. Ophthalmology 2015;122:1395–1401.
11. Sun J, Lin M, Lammer J, et al. Disorganization of the Retinal Inner Layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. JAMA Ophthalmol 2014;132:1309–1316.
12. Forooghian F, Stetson P, Meyer S, et al. Relationship between photoreceptor outer segment length and visual acuity in diabetic macular edema. Retina 2010;30:63–70.
13. Brown JC, Solomon S, Bressler SB, et al. Detection of diabetic foveal edema—contact lens biomicroscopy compared with optical coherence tomography. Arch Ophthalmol 2004;122:330–335.

14. Diabetic Retinopathy Clinical Research Network. Observational study of subclinical diabetic macular edema. Eye 2012;26:833–840.
15. Kim BY, Smith SD, Kaiser PK. Optical coherence tomographic patterns of diabetic macular edema. Am J Ophthalmol 2006;142:405–412.
16. Otani T, Kishi S, Maruyama Y. Patterns of diabetic macular edema with optical coherence tomography. Am J Ophthalmol 1999;127:688–693.
17. Maheshwary A, Oster S, Yuson R, et al. The association between percent disruption of the photoreceptor inner segment/outer segment and visual acuity in diabetic macular edema. Am J Ophthalmol 2010;150:63–67.
18. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for DME: results from 2-phase III randomized trials: RISE and RIDE. Ophthalmology 2012;119:789–801.
19. Diabetic Retinopathy Clinical Research Network. The relationship between OCT-measured central retinal thickness and visual acuity in diabetic macular edema. Ophthalmology 2007;114:525–536.
20. Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt vs deferred laser treatment: 3-year randomized trial results. Ophthalmology 2012;119:2312–2318.
21. Bressler S, Ayala A, Bressler N, et al. Persistent macular thickening after ranibizumab treatment for diabetic macular edema with vision impairment. JAMA Ophthalmol 2016;134:278–285.
22. Murakami T, Nishijima K, Akagi T, et al. Optical Coherence Tomography reflectivity of photoreceptors beneath cystoid spaces in diabetic macular edema. IOVS 2012;53:1506–1511.
23. Shin HJ, Lee SH, Chung H, et al. Association between photoreceptor integrity and visual outcome in diabetic macular edema. Graefes Arch Clin Exp Ophthalmol 2012;250:61–70.
24. Radwan S, Soliman A, Tokarev J, et al. Association of Disorganization of Retinal Inner Layers with vision after resolution of center-involved diabetic macular edema. JAMA Ophthalmol 2015;133:820–825.
25. Pelosini L, Hull C, Boyce J, et al. Optical Coherence Tomography may be used to predict visual acuity in patients with macular edema. IOVS 2011;52:2741–2748.