Long-Term Visual Outcomes and Morphologic Biomarkers of Vision Loss in Eyes With Diabetic Macular Edema Treated With Anti-VEGF Therapy

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• PURPOSE: To analyze the morphological characteristics and long-term visual outcomes in eyes with diabetic retinopathy (DR) and diabetic macular edema (DME) treated with anti−vascular endothelial growth factor (anti-VEGF) therapy.

• DESIGN: Retrospective clinical cohort study.

• METHODS: Patients with a long-term follow-up and evidence of resolved DME in at least 1 visit (study visit) after 5 years of follow-up after the initiation of anti-VEGF therapy were included. At the study visit, structural optical coherence tomography (OCT) scans were reviewed for qualitative features reflecting a distress of the neuroretina or retinal pigment epithelium (RPE). A quantitative topographical assessment of the inner and outer retinal thicknesses was also provided.

• RESULTS: A total of 61 eyes (50 patients) were included and were divided into 2 subgroups according to visual acuity (VA) at the study visit, yielding a group of 24 eyes with a VA <20/40 ("poor/intermediate vision" group), and 37 eyes with a VA ≥20/40 ("good vision" group). The external limiting membrane (ELM) and RPE bands were more frequently disrupted or absent in the poor/intermediate vision group ($P = .003$ and $P = .019$). Similarly, disorganization of retinal inner layers was more prevalent in the poor/intermediate vision group ($P = .013$). The foveal and parafoveal outer retinal thicknesses were reduced in eyes with poor/intermediate vision ($P = .022$ and $P = .044$). Multivariate stepwise linear regression analysis demonstrated that VA was associated with appearances of the RPE and ELM ($P < .0001$ and $P = .048$), foveal and parafoveal outer retinal thicknesses ($P = .046$ and $P = .035$).

• CONCLUSIONS: Modifications in the outer retina and RPE represent OCT biomarkers of long-term visual outcomes in eyes with DME treated with anti-VEGF. (Am J Ophthalmol 2022;235: 80–89. © 2021 Elsevier Inc. All rights reserved.)

Diabetic retinopathy (DR) is the leading cause of blindness in working-aged people worldwide.[1] Diabetic macular edema (DME) represents a frequent complication of diabetes and occurs in nearly 12% of patients with DR.[2]

The accumulation of intraretinal fluid occurring in DME may result in a significant reduction in visual acuity. In 1985, the Early Treatment Diabetic Retinopathy Study (ETDRS) reported on the use of laser photocoagulation to treat DME.[3] The trial enrolled 1122 patients with DME and demonstrated that laser treatment is associated with a reduced risk of moderate vision loss. Therefore, laser treatment had been considered as the treatment of choice in eyes with DME before the debut of intravitreal anti−vascular endothelial growth factor (anti-VEGF) therapy. A number of authors have proposed that anti-VEGF drugs represent an effective and safe treatment in patients with DME.[4-9] More importantly, anti-VEGF therapy has demonstrated better improvement in visual acuity in comparison with focal/grid laser. It is noteworthy, however, that in a number of patients, anti-VEGF therapy may be unsatisfactory, and in these cases a switch to other treatments, including intravitreal dexamethasone, has been demonstrated to be potentially efficacious.[10] In the long term (ie, >5 years), visual outcomes for patients with DME and who are treated with anti-VEGF have been variable. Overall, studies on trial data have demonstrated that anti-VEGF treatment is associated with better long-term visual outcomes.[11,12]

Structural optical coherence tomography (OCT) is widely used as an objective and reproducible imaging tool in the diagnosis and follow-up of patients with DME. More importantly, qualitative and quantitative analyses of the morphological features in with DME eyes detected with structural OCT have begun in earnest and may provide important biomarkers of disease progression and visual outcomes. The relationship between impairment of visual acuity and outer retinal layer injury has been investigated in a longi-

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tudinal study over a period of 12 months.[13] In that study, the authors investigated 62 DME eyes treated with intravitreal ranibizumab injections and demonstrated that VA improvement was positively correlated with shortening of the disrupted external limiting membrane (ELM) and ellipsoid zone (EZ) at 12 months. Structural OCT was also used to detect the presence of disorganization of the retinal inner layers (eg, inability to separately identify inner retinal layers in 1 mm central fovea—disorganization of retinal inner layers [DRIL]).[14,15] Importantly, the presence of foveal DRIL proved to be associated with reduced visual acuity in patients with resolved DME at 12 months.[16]

What is lacking is information concerning the relationship among the morphological characteristics detected with structural OCT, the clinical features of this disease, and long-term visual outcomes in patients with DME. Previous reports were indeed limited in the assessment of patients with short follow-up (ie, 1-2 years). Therefore, in this longitudinal study, we performed a qualitative and quantitative analysis on OCT images from DME eyes obtained more than 5 years after the initiation of anti-VEGF treatment to characterize morphologic characteristics correlating with good and poor long-term visual outcomes.

PATIENTS AND METHODS

The San Raffaele Ethics Committee was notified about this retrospective cohort study. The study adhered to the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the study.

• **PATIENTS:** In this study, patients 18 years of age and older with center-involved DME in at least 1 eye were identified from the medical records of a medical retinal practice at the San Raffaele Scientific institute. In detail, subjects were included in the initial study cohort if they had a history of DME treated with anti-VEGF therapy. Diagnosis of DME was determined by clinical examination, structural OCT, and fluorescein angiography evaluation, as previously described.[17] Exclusion criteria included the following: history of macular laser treatment in the study eye after the initiation of anti-VEGF therapy; history of vitreoretinal surgery; history of amblyopia; and history or evidence of other retinal and optic nerve disorders, including the presence of vitreoretinal diseases (ie, vitreo-macular traction and epiretinal membrane), because they may affect the qualitative and quantitative OCT analysis. The population fulfilling the inclusion and exclusion criteria was the starting cohort for this analysis (n = 456 of 582 DME patients in our database).

All of the patients received anti-VEGF intravitreal injections of bevacizumab, ranibizumab, or aflibercept and were treated using a pro re nata (PRN) regimen. During the follow-up, a switch to the dexamethasone intravitreal implant (Ozurdex(Allergan, an AbbVie Company, Irvine, CA, USA)) was at the discretion of the treating physician.

To be included, patients were also required to have a minimum of 3 yearly retinal visits covering a study period of 5 years after the first anti-VEGF injection. Furthermore, included eyes were required to have evidence of resolved DME (ie, restoration of the foveolar depression with a central macular thickness <315 μm)[18] in at least 1 visit after 5 years of follow-up visits after the initiation of anti-VEGF therapy. The last visit with OCT evidence of resolved DME was considered as the study visit and was used for morphologic analysis. Our analysis was performed at this visit to avoid DME from confounding the OCT qualitative and quantitative analysis.[19] After this further evaluation, 50 patients (61 eyes) were potentially includable in our analysis.

Structural OCT imaging was performed with the spectral domain (SD) Heidelberg Spectralis HRA+OCT device (Heidelberg Engineering). Each set of SD-OCT scans consisted of 19 B-scans, each of which comprised 24 averaged scans, covering approximately a 5.5 × 4.5-mm area centered on the fovea. A minimum signal strength of 25 was required for the OCT images to be included, as recommended by the manufacturer.[20]

Visual acuities at baseline (ie, visit immediately preceding the initiation of anti-VEGF therapy) and at the study visit (>5 years) were measured with Snellen charts and then converted to logarithm of the minimal angle of resolution (LogMAR) equivalents. The analyzed study cohort was divided into 2 subgroups according to the visual acuity at the study visit, yielding a group of eyes with a best corrected visual acuity (BCVA) < 20/40 Snellen ("poor/intermediate vision" group) and a group of eyes with a BCVA ≥20/40 Snellen ("good vision" group).[12]

• **OCT GRADING:** Structural OCT images at the study visit were first reviewed for eligibility by 2 independent and experienced readers (E.B. and R.S.). Eligible eyes were thus independently graded for qualitative features by the same readers who were masked as to the visual outcomes. In cases in which the 2 graders did not agree on a single consensus result, the final decision was made by the senior author (G.Q.).

In details, OCT images at the study visit were graded for qualitative features previously proposed as correlating with visual outcomes in patients with DME, as follows (Figure 1):

- Structural alterations of the outer retina within the foveola (ie, region within a 100-μm radius from the fovea center): the EZ and ELM bands were assessed for their integrity. These bands were graded as being absent or discontinuous or intact.[21]
- (2) Alterations of the RPE within the foveola: OCT images were also reviewed for the integrity of the RPE. The RPE was also graded as being absent or discontinuous or intact.[22]

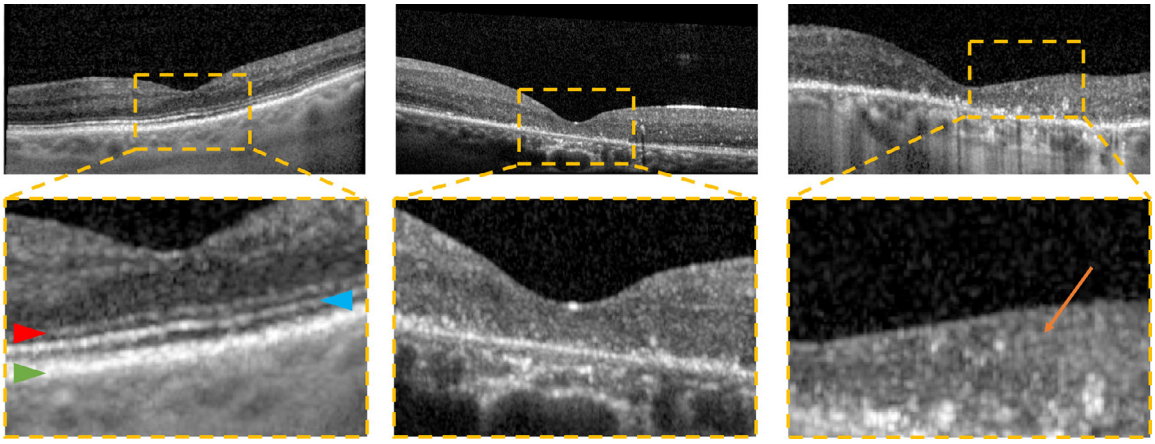

FIGURE 1. Clinical images showing optical coherence tomography (OCT) features that were qualitatively graded. Representative horizontal OCT B-scan images through the fovea from 3 eyes with resolved diabetic macular edema (above). A magnified visualization of the foveal region is reported in the bottom row. The OCT images were graded for qualitative features previously proposed as signs of neuroretina damage. In detail, the appearances of the external limiting membrane (ELM; red arrowhead), ellipsoid zone (EZ; blue arrowhead), and retinal pigment epithelium (RPE; green arrowhead) were graded for integrity. These 3 OCT bands were intact in the first case (left) and absent in the second case (middle). The presence of disorganization of the retinal inner layers (DRIL; highlighted with the orange arrow) was also graded (right).

- (3) Structural alterations of the inner retina within the 1-mm central fovea: OCT images were graded for the presence of DRIL, as previously described.[23]

Images were also graded for the following quantitative measurements:

- Subfoveal choroidal thickness: this assessment was performed on the horizontal OCT B-scan through the fovea, as previously described in eyes of individuals with diabetes.[24]
- Inner and outer retinal thicknesses: the retinal thickness within the circle of the ETDRS-grid centered over the fovea was measured using Spectralis built-in software.[25] Measurements are automatically averaged across each of the following subfields: the central fovea subfield within the inner 1-mm-diameter circle, the inner circle subfield between the inner and middle 3-mm-diameter circles, and the outer circle subfield between the middle and outer 6-mm-diameter circles. This algorithm automatically identifies and calculates the difference between the nerve fiber layer and outer plexiform layers inner boundary segmentations, yielding a combined thickness of the inner retina. The outer retina was identified and measured as the combination of the outer plexiform and outer nuclear layers. Before computing the thickness values, the graders evaluated all B-scans and manually corrected any segmentation or decentration errors.

• **STATISTICAL ANALYSIS:** To detect departures from normality distribution, the Shapiro–Wilk test was performed for all variables. Measurement values between groups were compared using the Student *t* test for independent samples. Qualitative variables were compared using the Fisher exact test. Considering the whole cohort, visual acuities at baseline and study visits were compared by conducting a related-samples Wilcoxon signed rank test.

The mean change in visual acuity (Δ BCVA = BCVA at the study visit – BCVA at baseline) was considered as the main outcome in our study, and a stepwise multiple regression analysis with Δ BCVA as the dependent variable was calculated to assess associations with other variables. Furthermore, a stepwise multiple regression analysis with visual acuity at the study visit as the dependent variable was computed. Relationships between variables associated with worse long-term visual outcomes (dependent variables) and relevant clinical aspects including number of anti-VEGF injections (ie, a surrogate of DME relapses in a PRN regimen) and occurrence of subfoveal neuroretinal detachment (ie, an established factor associated with greater damage of the outer retina)[26] were evaluated using linear regression analysis.

The unweighted *k* statistic test[27] (intact vs disrupted/absent for ELM, EZ, and RPE appearances) was performed to evaluate the agreement between graders in the assessment of OCT qualitative features.

Statistical calculations were performed using Statistical Package for Social Sciences (version 23.0, IBM SPSS). The chosen level of statistical significance was $P < .05$.

RESULTS

• **CHARACTERISTICS OF PATIENTS INCLUDED IN THE ANALYSIS:** Of the initial cohort of 582 consecutive patients scheduled for anti-VEGF therapy because of DME, 50 patients (61 eyes) were finally included in our analysis. Table 1 summarizes the demographics and clinical charac-

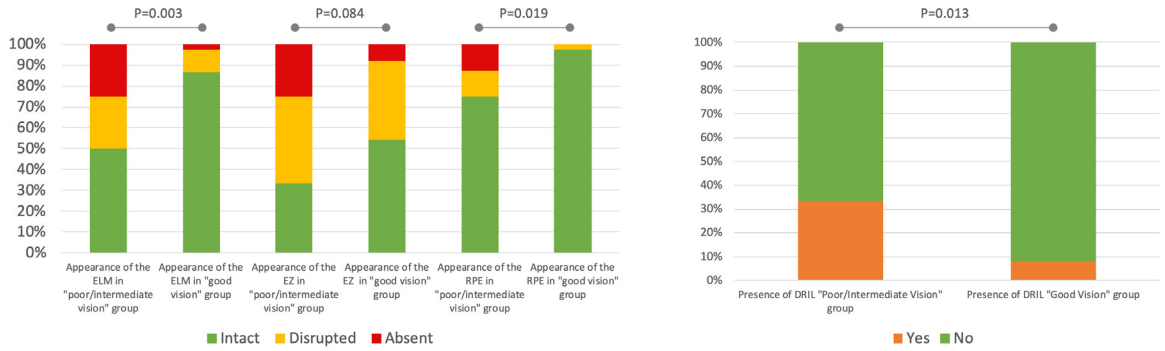

FIGURE 2. Grouped column chart showing the relative frequencies of optical coherence tomography (OCT) qualitative findings in the study cohort. The chart shows the relative frequencies of eyes graded with a specific OCT finding. The relative frequencies are given as a percentage of patients with a specific characteristic in a distinct group. On the X axis, columns are grouped on the basis of the presence of each OCT finding and vision groups.

TABLE 1. Demographic and Clinical Characteristics of Enrolled Patients.

No. of patients, n	50
No. of eyes, n	61
Age, y, mean ± SD	63.8 ± 13.2
Sex, n	
Male	31
Female	19
BCVA at baseline (LogMAR), mean ± SD	0.31 ± 0.30
BCVA at the study visit (LogMAR), mean±SD	0.29 ± 0.29
Type of diabetes, n	
Type 1	9
Type 2	41

BCVA = best corrected visual acuity; LogMAR = logarithm of the minimum angle of resolution.

teristics of this study cohort. Mean ± SD visual acuity was 0.31 ± 0.30 LogMAR at baseline and 0.29 ± 0.29 LogMAR at the study visit ($P = .310$).

This cohort of 61 eyes was divided into 2 subgroups according to the visual acuity at the study visit, yielding a group of 24 eyes (39.3%) with a BCVA <20/40 Snellen (poor/intermediate vision group), and 37 eyes (60.7%) with a BCVA ≥20/40 Snellen (good vision group). No differences in terms of clinical characteristics were detected between these 2 groups (Table 2).

• **QUALITATIVE OCT ANALYSIS:** At the study visit, the ELM band was disrupted in 6 eyes (25.0%) and absent in 6 eyes (25.0%) in the poor/intermediate vision group, whereas it was graded as disrupted in 4 eyes (10.8%) and absent in 1 eye (2.7%) in the good vision group ($P = .003$) (Table 2, Figure 2). Similarly, the EZ band was graded as disrupted and absent in 10 eyes (41.7%) and 6 eyes (25.0%) with poor/intermediate vision, and was disrupted and absent in 10 eyes (27.0%) and 6 eyes (16.2%) with good vision ($P = .084$) (Table 2, Figure 2). A total of 6 poor/intermediate vision eyes (25.0%) showed a disrupted/absent RPE, whereas only 1 eye in the good vision group displayed the presence of a foveolar disrupted RPE ($P = .019$) (Table 2, Figure 1). DRIL was found in 8 eyes (33.3%) within the poor/intermediate vision group and in 3 eyes (8.1%) within the good vision group ($P = .013$) (Table 2, Figure 2).

• **QUANTITATIVE OCT ANALYSIS:** The foveal outer retinal thickness was 89.2 ± 17.4 μm in poor/intermediate vision eyes and 101.9 ± 23.2 μm in good vision eyes ($P = .022$) (Table 3, Figure 3). The parafoveal outer retinal thickness was 90.8 ± 14.1 μm and 98.9 ± 14.6 μm in the poor/intermediate vision and good vision groups, respectively ($P = .044$) (Table 2, Figure 3). In the perifoveal region, the outer retinal thickness was 83.23 ± 11.8 μm in the poor/intermediate vision eyes and 89.4 ± 13.1 μm in the good vision eyes ($P = .075$) (Table 2, Figure 3). No differences in inner retinal thickness were detected between the 2 groups (Table 2, Figure 3).

• **REGRESSION ANALYSIS:** Results of multivariate linear regressions are summarized in Table 3 and Table 4. The strongest associations with Δ BCVA were with appearance of the RPE ($P = .030$), appearance of the ELM ($P = .036$) and parafoveal outer retinal thickness ($P = .045$). The strongest associations with final BCVA were with appearance of the RPE ($P < .0001$), parafoveal outer retinal thickness ($P = .035$), foveal outer retinal thickness ($P = .046$), and appearance of the ELM ($P = .048$). In univariate analysis, the occurrence of subfoveal neuroretinal detachment during the follow-up (independent variable) was significantly associated with foveal outer retinal thickness ($R^2 = 0.276$, $P = .045$) and appearance of the ELM ($R^2 = 0.277$, $P = .044$). In our study cohort, the number of anti-VEGF injections was not associated with outer retinal and RPE changes.

• **REPEATABILITY:** The unweighted k values for intergrader repeatability were 0.88 (58 of 61) for ELM appear-

TABLE 2. Clinical and Morphologic Characteristics at Study Visit

Characteristics	Groups		*P*
	Poor/Intermediate Vision (n=24)	Good Vision (n=37)	
Total follow-up after anti-VEGF therapy initiation, y	5.3 (0.9)	5.8 (1.4)	.180[a]
Diagnosis of PDR, eyes, n (%)	7 (29.2%)	8 (21.6%)	.542[b]
Previous treatment with macular laser, eyes, n (%)	8 (33.3%)	16 (43.2%)	.338[b]
Laser treatment of retinal periphery, eyes, n (%)	17 (70.8%)	19 (51.3%)	.098[b]
Pseudophakia, eyes, n (%)	12 (50.0%)	19 (51.3%)	1.0[b]
No. of anti-VEGF injections (no. of eyes)	7.6 (4.4)	8.4 (7.1)	.658[a]
No. of intravitreal treatments (anti-VEGF + DEX implant) (no. of eyes)	9.3 (4.4)	9.9 (7.1)	.978[a]
Subfoveal choroidal thickness, μm	240.6 (90.5)	251.5 (91.7)	.661[a]
Appearance of ELM, eyes, n	Intact = 12 Disrupted = 6 Absent = 6	Intact = 32 Disrupted = 4 Absent = 1	.003[b]
Appearance of EZ, eyes, n	Intact = 8 Disrupted = 10 Absent = 6	Intact = 20 Disrupted = 14 Absent= 3	.084[b]
Appearance of RPE, eyes, n	Intact = 18 Disrupted = 3 Absent = 3	Intact= 36 Disrupted= 1 Absent= 0	.019[b]
Presence of DRIL, eyes, n (%)	8 (33.3%)	3 (8.1%)	.013[b]
Foveal inner retinal thickness, μm	74.0 (20.4)	80.5 (17.8)	.236[a]
Foveal outer retinal thickness, μm	89.2 (17.4)	101.9 (23.2)	.022[a]
Parafoveal inner retinal thickness, μm	145.2 (26.1)	154.0 (23.3)	.206[a]
Parafoveal outer retinal thickness, μm	90.8 (14.1)	98.9 (14.6)	.044[a]
Perifoveal inner retinal thickness, μm	146.8 (22.8)	144.2 (28.2)	.708[a]
Perifoveal outer retinal thickness, μm	83.3 (11.8)	89.4 (13.1)	.075[a]

DEX = dexamethasone, DRIL = disorganization of retinal inner layers, ELM = external limiting membrane, EZ = ellipsoid zone, PDR = proliferative diabetic retinopathy, RPE = retinal pigment epithelium, VEGF = vascular endothelial growth factor;

[a] *t* Test

[b] Fisher exact test.Quantitative values are expressed in mean ± SD.

ance, 0.93 (59 of 61) for EZ appearance, 1.0 (61 of 61) for RPE appearance, and 0.81 (58 of 61) for presence of DRIL. Agreement was reached for all discrepancies after adjudication between graders.

DISCUSSION

In this study, we reported the long-term (ie, >5 years) visual outcomes in patients with DME treated with anti-VEGF therapy. In a real-world setting, long-term visual outcomes are generally favorable, and many eyes maintain better than 20/40 vision after >5 years from the initiation of anti-VEGF therapy. Notably, results of the present study demonstrated a significant relationship between morphological alterations detected using structural OCT and long-term visual outcomes. Among the demographic and clinical factors, age, number of anti-VEGF injections, switch to intravitreal dexamethasone therapy, time interval between initiation of therapy and study visit, and previous macular laser treatment did not have a significantly impact on long-term visual outcomes. Because the purpose of our study was to investigate the associations between long-term visual outcomes and morphological macular structural changes detected using OCT, the assessment of which may be affected in the presence of coexisting conditions or intraretinal fluid, we did not include patients in whom DME was not resolved after 5 years of follow-up in at least 1 visit, as well as patients with evidence of vitreomacular disorders or a history of vitreoretinal surgery.

In this study, the presence of specific qualitative changes in the outer retina on structural OCT images was associated with worse long-term visual outcomes. Several previous reports using structural OCT have proved that eyes with resolved DME may be characterized by an attenuation of the outer retinal layers.[28-31] Assuming that reflection signal arising from the EZ is thought to originate from the photoreceptor inner segment ellipsoids,[32-34] which are densely packed with mitochondria, a reduction in EZ visualization may reflect a functional or structural impairment in photoreceptors. Conversely, the ELM is an intercellular junction between Müller and pho-

TABLE 3. Results of Stepwise Multiple Regression Analysis of Association Between Δ Visual Acuity and Other Variables

Characteristic	Δ VA as Dependent Variable	
	Standardized β Coefficient (SE)	*P*
Age	0.160 (0.004)	.482
Total follow-up after anti-VEGF therapy initiation	–0.075 (0.031)	.670
Diagnosis of PDR	0.282 (0.085)	.111
Previous treatment with macular laser	0.065 (0.079)	.716
Laser treatment of retinal periphery	0.163 (0.073)	.318
Pseudophakia	–0.542 (0.094)	.016
No. of anti-VEGF injections	–1.141 (0.019)	.060
No. of intravitreal treatments (anti-VEGF + DEX implant)	1.023 (0.019)	.087
Subfoveal choroidal thickness	0.061 (0.000)	.762
Appearance of ELM	0.322 (0.021)	.036[a]
Appearance of EZ	0.070 (0.081)	.786
Appearance of RPE	0.370 (0.019)	.030[a]
Presence of DRIL	0.079 (0.109)	.691
Foveal inner retinal thickness	–0.004 (0.003)	.986
Foveal outer retinal thickness	–0.312 (0.003)	.303
Parafoveal inner retinal thickness, μm	–0.100 (0.003)	.752
Parafoveal outer retinal thickness, μm	–0.552 (0.005)	.045[a]
Perifoveal inner retinal thickness, μm	0.205 (0.003)	.483
Perifoveal outer retinal thickness, μm	–0.033 (0.003)	.873

DEX = dexamethasone, DRIL = disorganization of retinal inner layers, ELM = external limiting membrane, EZ = ellipsoid zone, PDR = proliferative diabetic retinopathy, RPE = retinal pigment epithelium, VEGF = vascular endothelial growth factor.

[a]Significant.

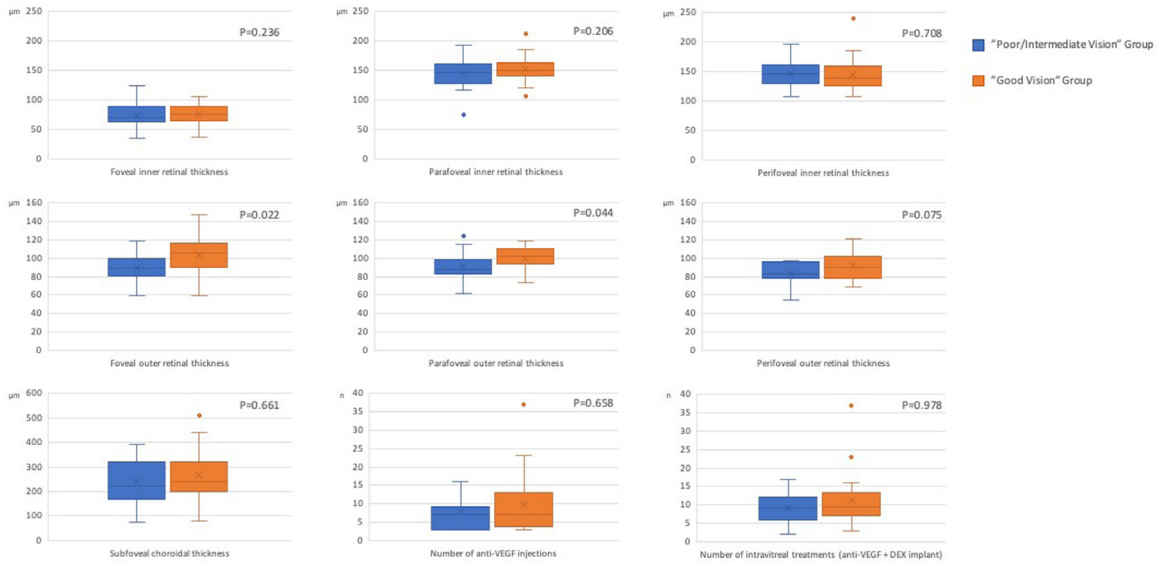

FIGURE 3. Box and whisker plots showing analyzed optical coherence tomography (OCT) measurements in diabetic macular edema (DME) patients. Each box shows median (central horizontal line), mean (cross inside the box), and interquartile range (horizontal extremes of the box) values for each variable. The ends of the whiskers represent the minimum and maximum values. Dots not included in whiskers represent outliers. Each graph shows values of a different metric in each of the 2 groups. *P* values for each comparison are reported. Details on pairwise comparisons are presented in Table 2.

TABLE 4. Results of Stepwise Multiple Regression Analysis of the Association Between Final Visual Acuity and Other Variables.

Characteristics	VA as Dependent Variable	
	Standardized β Coefficient (SE)	*P*
Age	0.039 (0.324)	.747
Total follow-up after anti-VEGF therapy initiation	–0.209 (1.328)	.192
Diagnosis of PDR	0.062 (0.507)	.614
Previous treatment with macular laser	–0.086 (0.706)	.483
Laser treatment of retinal periphery	0.170 (1.416)	.163
Pseudophakia	–0.046 (0.375)	.709
No. of anti-VEGF injections	0.067 (0.552)	.583
No. of intravitreal treatments (anti-VEGF + DEX implant)	0.087 (0.638)	.526
Subfoveal choroidal thickness	–0.026 (0.211)	.834
Appearance of ELM	0.282 (1.992)	.048[a]
Appearance of EZ	0.122 (0.874)	.386
Appearance of RPE	0.440 (3.757)	<.0001[a]
Presence of DRIL	0.117 (0.952)	.346
Foveal inner retinal thickness	–0.065 (0.490)	.626
Foveal outer retinal thickness	–0.234 (1.839)	.046[a]
Parafoveal inner retinal thickness, μm	–0.075 (0.588)	.559
Parafoveal outer retinal thickness, μm	–0.253 (2.160)	.035[a]
Perifoveal inner retinal thickness, μm	0.137 (1.130)	.264
Perifoveal outer retinal thickness, μm	–0.135 (1.126)	.265

DEX = dexamethasone, DRIL = disorganization of the retinal inner layers, ELM = external limiting membrane, EZ = ellipsoid zone, PDR = proliferative diabetic retinopathy, RPE = retinal pigment epithelium, VEGF: vascular endothelial growth factor.

[a]Significant.

toreceptor cells with barrier properties against macromolecules.[35] The ELM damage occurring in DME has been speculated to be secondary to a mechanical compression by intracellular fluid.[36] More importantly, an ELM injury may permit blood components to migrate into the outer retinal layers and aggravate the photoreceptor damage.[37]

Previous reports demonstrated that a damage of the outer retina was associated with decreased visual acuity in these eyes.[28-31,36] However, these studies provided a qualitative and quantitative assessment of the outer retina in DME patients undergoing a variety of treatments (including pars plana vitrectomy) and were limited in outlining short- and medium-term associations with visual outcomes. Among these reports, Muftuoglu et al[36] performed a retrospective study with 59 eyes of 48 patients with resolved DME after treatment. In the latter study, the mean duration of DME was 19.32 ± 15.7 weeks (range 4-73 weeks). Structural OCT analysis in these patients revealed a disrupted ELM in 16 eyes (27.2%), whereas the EZ was disrupted in 21 eyes (35.5%). Similarly, our analysis revealed a disrupted/absent ELM and EZ in 17 eyes (27.9%) and 33 eyes (54.1%), respectively. It is noteworthy that we found that long-term visual acuity is significantly reduced in DME eyes with a disrupted/absent ELM rather than a disrupted/absent EZ. The latter finding is in agreement with previous studies showing that the status of the ELM may be considered as a better predictor of visual acuity in DME eyes.[36,38] Given that a disrupted/absent appearance on OCT images was more prevalent for the EZ than the ELM layer, as well as that an ELM impairment is thought to exacerbate photoreceptor damage,[37] it seems rational that a loss of the ELM may assume a more advanced form of photoreceptor injury. The latter aspect might partially account for the absence of a significant association between visual acuity and EZ status in eyes with long-term DME. Conversely, the functional importance of the ELM in DME eyes would appear to be well established also in long-term outcomes.

To quantify the damage of the neuroretina, we also performed a topographic quantitative analysis of the inner and outer retinas. In agreement with the qualitative investigation discussed above, we showed that the foveal and parafoveal outer retinal layers' thicknesses were reduced in patients with worse long-term visual outcomes.

Importantly, the present study highlights the distinctive relationship between outer retina damage and visual acuity in DME eyes: considering the final visual acuity as dependent variable, we observed a direct relationship with variables defining damage of the outer retina (ie, appearance of the ELM, foveal outer retina thickness, parafoveal outer retina thickness), even after accounting for confounding factors such as age and disease stage (ie, nonproliferative vs proliferative DR), which are known to modify visual acuity. Similarly, the average change in visual acuity between baseline and study visits was significantly associated with variables defining damage of the outer retina

(ie, appearance of the ELM and parafoveal outer retina thickness). In agreement with Vujosevic et al,[26] a history of subfoveal neuroretinal detachment preceding the study visit was associated with greater damage of the outer retina.

Our analysis revealed that presence of foveolar RPE atrophy was more prevalent in the poor/moderate vision group. It is noteworthy that this was the parameter that most correlated with visual acuity among the variables included in the multivariate analysis. Karst et al[22] recently performed a retrospective study with 36 eyes characterized by central retinal thinning after DME resolution. The authors demonstrated that 8 patients (22.0%) were characterized by concurrent RPE atrophy. Similarly, 7 of 61 eyes (11.5%) in our study cohort featured a disrupted/absent foveolar RPE. Among these, 5 eyes had evidence of RPE atrophy without any prior macular laser treatment. Therefore, our observations seem to confirm previous findings that DME may result in damage of the RPE regardless of a previous history of macular laser,[22] and that this structural damage is relevant to the visual outcomes of these patients.[39]

Disorganization of the inner retinal layers is a potential and well-characterized OCT finding in eyes with resolved DME. The presence of DRIL may indicate disorganization or destruction of cells within inner retinal layers, and likely represents an interruption of pathways transmitting visual information from the photoreceptors to the ganglion cells.[23] In a previous important study by Sun et al,[23] the presence of DRIL was significantly associated with worse visual acuity at baseline and at 4-month and 8-month follow-up visits. Consistently, in our study cohort of eyes with resolved DME and long-term follow-up, we demonstrated that eyes with DRIL were characterized by worse visual acuity at the final visit.

Our study has limitations that must be considered when discussing our results. First, this was a retrospective analysis that may be susceptible to selection and ascertainment bias. Moreover, our study cohort was not part of a large multicenter trial and included patients who were not followed up at regular intervals. However, we included only patients with a minimum of 3 yearly retinal visits covering a study period of 5 years after the first anti-VEGF injection, and this may have circumscribed this limitation. Second, as previously stated, our cohort consisted of patients with at least 1 visit demonstrating resolved DME after 5 years of follow-up in at least 1 eye. In addition, we did not include patients with evidence of vitreomacular disorders or a history of vitreoretinal surgery, as these may affect the qualitative and quantitative OCT analysis. Furthermore, our patients had a PRN treatment using different anti-VEGF drugs, and a number of our patients were switched to dexamethasone therapy during their follow-up, although multivariate analysis did demonstrate that a change to dexamethasone did not have an impact on long-term outcomes. As a result, our analysis can be applied only to this group of participants and may differ in other cases. Conversely, this is a real-world study, and our results may have wide applicability. Moreover, the study was not large enough to account for confounding factors such as duration of diabetes, presence of other systemic disorders, or level of blood glucose control. Our study also has strengths, including the use of standardized data accumulated over more than 5 years of follow-up, and the use of 2 masked, independent, and experienced graders.

In conclusion, this study provides OCT biomarkers associated with long-term visual outcomes in eyes with DME treated with anti-VEGF therapy. Thinning in the outer foveal and parafoveal thicknesses, as well as the absence of intact ELM/EZ bands, were associated with worse long-term visual outcomes in these patients. Early identification of these OCT findings may lead to adaptation of therapy in DME patients. Finally, our findings may be used to guide the optimal anatomic target in patients with this disorder.

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