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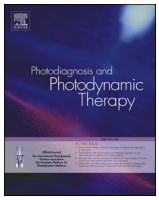

Research Paper

Optical coherence tomography biomarkers indicating visual enhancement in diabetic macular edema resolved through anti-VEGF therapy OCT biomarkers in resolved DME

Pasquale Viggiano [a,*], Stela Vujosevic [b,c], Francesca Palumbo [a], Maria Oliva Grassi [a], Giacomo Boscia [a], Enrico Borrelli [d,e], Michele Reibaldi [a,d,e], Luigi Sborgia [a], Teresa Molfetta [a], Federica Evangelista [f], Giovanni Alessio [a], Francesco Boscia [a]

[a] Department of Translational Biomedicine Neuroscience, University of Bari "Aldo Moro", Bari, Piazza Giulio Cesare,11, Italy
[b] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy.
[c] Eye Clinic, IRCCS MultiMedica, Milan, Italy
[d] Department of Ophthalmology, "City of Health and Science" Hospital, Turin, Italy
[e] Department of Surgical Sciences, University of Turin, Turin, Italy
[f] Department of Ophthalmology, Ente Ecclesiastico Ospedale Generale Regionale "F. Miulli", Acquaviva delle Fonti, Bari, Italy

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ABSTRACT

Purpose: to investigate the structural features and extended visual results in eyes affected by diabetic retinopathy (DR) and diabetic macular edema (DME) that have been successfully treated with anti-vascular endothelial growth factor (VEGF) therapy.
Methods: Individuals (39 eyes of 39 patients) who had undergone long-term follow-up and demonstrated evidence of resolved DME after at least 2 years of follow-up following the initiation of anti-VEGF therapy were included. During the ""study visit"", structural OCT scans were examined to assess qualitative features indicative of neuroretina or retinal pigment epithelium distress. Additionally, a quantitative assessment of the inner and outer retinal thicknesses was conducted for topographical analysis.
Results: The most robust qualitative association observed with BCVA at the "study visit" was linked to the presence of DRIL ($p = 0.043$) and the appearance of the ELM. ($p = 0.045$). Regarding quantitative parameters, a strong correlation was noted between the visual acuity during the "study visit" and the foveal and parafoveal thicknesses of both the inner and outer retina ($p < 0.001$).
Conclusions: Changes in the status of ELM, the presence of DRIL, and the thicknesses of the foveal and parafoveal regions can act as OCT biomarkers, signifying prolonged visual improvements in eyes that have experienced resolved DME after undergoing anti-VEGF therapy.

1. Introduction

Diabetic macular edema (DME) stands as the leading cause of vision loss among individuals with diabetes [1]. The disruption of the blood-retinal barrier (BRB) in DME can lead to the buildup of plasma proteins, lipids, and extracellular fluid within the macula [2]. The BRB is composed of two distinct components: the inner BRB and the outer BRB. The inner BRB is characterized by tight junctions formed between the endothelial cells of retinal capillaries. On the other hand, the outer BRB is established through tight junctions between the cells of the retinal pigment epithelium (RPE). Supporting the proper function of the inner BRB are astrocytes, Müller cells, and pericytes [3,4]. In cases of diabetic retinopathy (DR), retinal hypoxia triggers the breakdown of the inner BRB. This breakdown results in the buildup of fluid within both the outer plexiform layer (OPL) and the inner nuclear layer (INL) of the retina, ultimately leading to the onset of cystoid macular edema.

Several treatment approaches, such as laser photocoagulation [5], intravitreal steroids [6], and intravitreal anti-vascular endothelial growth factor (VEGF) medications [6], have demonstrated effectiveness in the management of DME. Multiple research studies have indicated

* Corresponding author.
E-mail address: pasquale.viggiano90@gmail.com (P. Viggiano).

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that anti-VEGF drugs represent a feasible and secure treatment choice for individuals withDME [7,8]. In instances where this treatment approach may not be fully effective, transitioning to other therapies, like intravitreal dexamethasone, has demonstrated potential effectiveness [9].

Structural Optical Coherence Tomography (OCT) is extensively utilized as an objective and highly reproducible imaging tool for diagnosing and monitoring patients with DME. What's even more significant is the ongoing effort to conduct qualitative and quantitative analyses of morphological characteristics observed in DME cases using structural OCT. These analyses hold the potential to offer valuable biomarkers for tracking disease progression and predicting visual outcomes. Currently, spectral domain OCT (SD-OCT) is employed to identify previously imperceptible morphological alterations within the retina. These changes include assessing the status of the RPE, and the integrity of the inner segment/outer segments (IS/OS) and the external limiting membrane (ELM), all of which are crucial for preserving optimal vision. Indeed, prior reports have indicated a potential link between the status of the inner segment/outer segments and visual acuity in DME eyes [10]. Nonetheless, the connection between the status of the ELM and visual acuity (VA) in diabetic patients receiving intravitreal injections is not fully comprehended. Structural OCT was also utilized to identify the existence of disruption in the layers of the retina, such as the inability to differentiate any inner retinal layers in the 1 mm central fovea-known as DRIL [11,12]. Importantly, the occurrence of foveal DRIL was found to be linked to decreased visual acuity in patients who had resolved DME at the 12-month mark [13].

In this current study, our aim was to examine at final visit both quantitative and qualitative factors through structural OCT in eyes that had experienced resolved DME two years after initiating intravitreal therapy. The primary outcome was to identify the parameters that displayed the strongest correlation with BCVA.

2. Methods

The retrospective cohort study received approval from the Ethics Committee of the University of Bari "Aldo Moro." The study adhered to the principles outlined in the 1964 Helsinki Declaration, as well as its subsequent amendments. We reached out to all enrolled individuals to secure their written informed consent for the retrospective utilization of their clinical information.

2.1. Study participants

In this study, we identified individuals aged 18 and older who had center-involved DME in at least one eye by reviewing the medical records of a medical retinal practice affiliated with the Department of Translational Biomedicine Neuroscience at the University of Bari "Aldo Moro." Specifically, individuals were included in the initial study cohort if they had a documented history of DME and had received treatment with anti-VEGF therapy. The diagnosis of DME was established through a fundus examination and further confirmed by fluorescein angiography and SD-OCT [14].

Exclusion criteria encompassed the following: (i) history of amblyopia; (ii) a history or observable evidence of other retinal and optic nerve disorders, including the presence of vitreoretinal diseases such as vitreo-macular traction and epiretinal membrane (iii) history of vitreoretinal surgery (iv) a history of macular laser treatment in the study eye following the initiation of anti-VEGF therapy (v) presence of advanced cataract causing substantial impairment in visual acuity. The population that met both the inclusion and exclusion criteria constituted the initial cohort for this analysis, with final 39 individuals out of the 698 DME patients recorded in our database. Throughout the two years of study analysis, all patients are undergoing regular endocrinological monitoring for the control of diabetes.

All patients in the study received anti-VEGF intravitreal injections, including ranibizumab or aflibercept, and were managed under a PRN treatment regimen. Throughout the follow-up period, the decision to switch to the dexamethasone intravitreal implant (Ozurdex®) was made at the discretion of the treating physician.

Inclusion criteria for patients required that they exhibit evidence of resolved DME, which was defined as the restoration of the foveolar depression and a central macular thickness (CMT) measuring less than 315 μm in at least one visit after two years of follow-up following the initiation of anti-VEGF therapy. The final visit where OCT confirmed the resolution of DME was considered the ""study visit"" and served as the basis for our morphological analysis. This approach was adopted to ensure that the presence of DME did not introduce confounding factors into the qualitative and quantitative analysis of OCT findings.

We conducted a retrospective review of the clinical records of patients who underwent examinations using Spectralis OCT, (Heidelberg, Spectralis, Germany). Poor quality images (signal strength <25) were excluded [15]. Clinical and OCT data were collected in July 2023, and for each patient, we analyzed the most recent OCT image available up to that date, along with visual acuity measurements obtained on the same day.

Visual acuities were assessed at two key time points: the baseline visit (which occurred immediately before the initiation of anti-VEGF therapy) and the ""study visit"" (which took place after a period of two years). These assessments were conducted using Snellen charts and subsequently converted to logarithm of the minimal angle of resolution (LogMAR) equivalents.

2.2. OCT grading

Two independent and experienced retina specialists (PV and EB) evaluated structural OCT images. In instances where these two graders did not reach a unanimous consensus on a single result, the final determination was made by the senior author (FB).

The OCT images obtained during the study examination were analyzed for specific qualitative aspects, as exemplified in Fig. 1. These aspects include:

1. Structural alterations in the outer retina within the foveola, which denotes the area within a 100 μm radius from the fovea's center. The integrity of the EZ and ELM bands was evaluated and classified as absent, discontinuous, or intact [16].
2. Changes in the RPE within the foveola. Evaluation of the RPE's integrity in the OCT images led to its classification as absent, discontinuous, or intact [16].
3. Structural modifications in the inner retina within the 1 mm central fovea. The OCT images were examined for the presence of DRIL, as previously described in the literature [11].

Quantitative measurements were also conducted on the images, encompassing the following parameters: Inner and outer retinal thicknesses: The thickness of the retina within the circle of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, which is centered over the fovea, was measured using the built-in software of the Spectralis device [16,17].

2.3. Statistical analysis

To identify deviations from a normal distribution, a Shapiro-Wilk's test was executed for all variables. Fisher's exact test was employed to compare qualitative variables. For the pairwise comparison of best-corrected visual acuity at baseline and "the "study visit"", a paired t-test was utilized with Bonferroni post hoc corrections. The specified significance level (α) is set at 0.05, and the statistical power ($1-\beta$) is determined to be 0.85.

Our study primarily focused on examining the correlations between qualitative and quantitative OCT parameters and visual acuity during

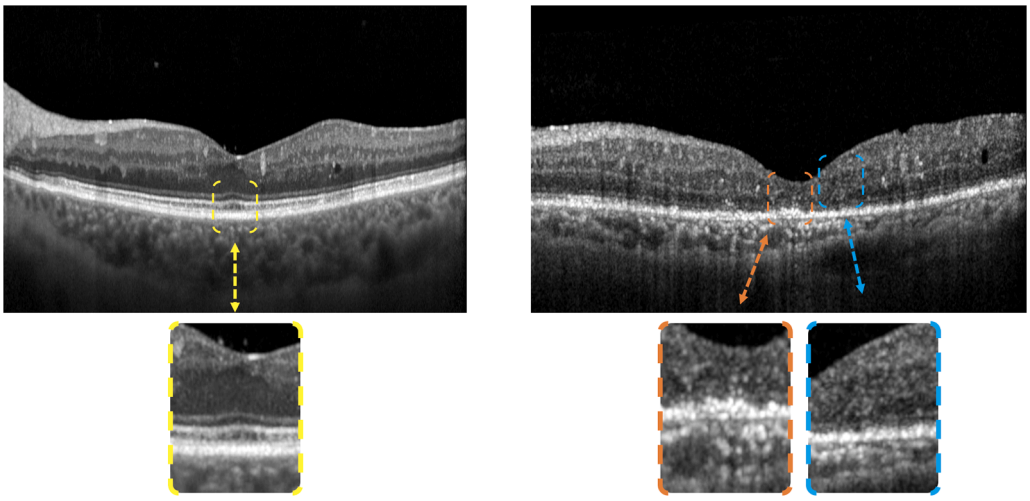

Fig. 1. displays clinical images showcasing OCT features that underwent qualitative grading at "study visit". It presents representative horizontal OCT B-scan images passing through the fovea in eyes after intravitreal injection of anti-VEGF over a two-year period, illustrating resolved diabetic macular edema. A magnified view of the foveal region is provided in the bottom row. The OCT images were subjected to grading for qualitative features previously recognized as indicators of neuroretinal damage. Specifically, the conditions of the ELM, EZ, and RPE were evaluated for their integrity. These three OCT bands were intact in the first case (left – indicated by yellow dotted brackets) and absent in the second case (right – indicated by orange dotted brackets). Additionally, the presence of DRIL was also graded (right – indicated by blue dotted brackets).

the ""study visit"". To assess the relationships with these OCT variables, a stepwise multiple regression analysis was conducted, with BCVA at the "study visit" as the dependent variable. Additionally, the study evaluated the associations between factors linked to a poorer long-term visual outcome (dependent variables) and relevant clinical factors. These factors included the number of anti-VEGF injections, transition to intravitreal dexamethasone therapy, the time gap between the initiation of therapy and the "study visit", and previous macular laser treatment. This assessment was carried out through a linear regression analysis. The unweighted Kappa statistic test was conducted to assess the agreement between graders in evaluating OCT qualitative features, specifically the intact vs disrupted/absent of ELM, EZ, and RPE. Statistical computations were carried out using the Statistical Package for Social Sciences (version 23.0, SPSS Inc., Chicago, IL, USA). The predetermined level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Characteristics of patients included in the analysis

Out of the initial cohort of 646 consecutive individuals scheduled for anti-VEGF therapy due to DME, only 39 eyes belonging to 39 subjects met the study's inclusion criteria. Table 1 presents a summary of the demographic and clinical features of this study cohort. The mean visual acuity at baseline was recorded at 0.22 ± 0.56 LogMAR, indicating a trend towards improvement to 0.15 ± 0.69 LogMAR during the "study visit", although this change did not achieve statistical significance ($p = 0.056$). Among the participants included in the study, 64 % were undergoing treatment for diabetes using oral hypoglycemics, while 36 % were receiving insulin therapy. No participant experienced the onset of a new diabetes-related nephrological disease throughout the duration of the study.

3.2. Qualitative OCT analysis

During the "study visit", the examination revealed that the ELM line was disrupted in 11 eyes (28.2 %) and absent in 1 eye (2.5 %). Similarly, the EZ band was determined to be disrupted and absent in 17 eyes (43.5 %) and 1 eye (2.5 %) with resolved DME. Furthermore, 5 eyes (12.8 %) exhibited disrupted or absent RPE. Lastly, DRIL was observed in 23 eyes (58.9 %) during the "study visit" (Table 2).

Table 1
Baseline characteristics of patients with DME.

Number of patients, ***n***	39
Number of eyes, ***n***	39
Age ***(years)***	68.4 ± 7.5
Gender ***(M/F)***	22/17
Duration of diabetes	10.9 ± 6.4
Smoking status, ***n***	12
Systemic hypertension, ***n***	29
Phakic eyes at baseline, ***n***	16
Phakic eyes at "study visit" ***n***	10
Initial BCVA ***(logMAR)***	0.22 ± 0.56
Initial CMT ***(μm)***	327.8 ± 42.1
Type of diabetes ***(1/2)***	4/35
HbA1c values at baseline (%)	7.1 ± 2.1
HbA1c values at study visit (%)	7.4 ± 3.6
Intraretinal Fluid at baseline, ***n***	39
Subretinal Fluid at baseline, ***n***	10

Quantitative values are expressed in mean±SD (standard deviation).
n number; ***DME*** diabetic macular edema; ***BCVA*** best-corrected visual acuity; ***logMAR*** logarithm of the minimum angle of resolution; ***CMT*** central macular thickness

3.3. Quantitative OCT analysis

According to the quantitative OCT analysis, the mean foveal inner retinal thickness was 265.42±75.2 μm, in the parafoveal area, the mean inner retinal thickness was 236.4±41.2 μm and in the perifoveal area, the mean inner retinal thickness was 199.6±28.5 μm, at the "study visit".

Concerning the outer retinal thickness, the foveal outer retinal thickness measured 91.7±13.9 μm during the ""study visit"". In the parafoveal region, the outer retinal thickness was recorded at 123.8 ±16.8 μm. In the perifoveal area, the outer retinal thickness was determined to be 112.6±16.3 μm during the ""study visit"".

3.4. Regression analysis

Concerning qualitative parameters, the strongest association with

Table 2
Characteristics of diabetic patients at "study visit".

Previous treatment with periphery laser, ***n. of eyes***	17 (43%)
Total follow-up after anti-VEGF therapy initiation, ***(years)***	2.2 ± 0.6
Final BCVA ***(logMAR)***	0.15±0.69
Final CMT ***(μm)***	273.8 ± 38.5
Pseudophakia, ***n. of eyes***	29 (74%)
Number of intravitreal treatments (anti-VEGF + DEX implant)	8.36± 5.17
ELM integrity, ***n. of eyes***	
• Absent	1
• Disrupted	11
• Intact	27
EZ integrity, ***n. of eyes***	
• Absent	1
• Disrupted	17
• Intact	21
RPE integrity, ***n. of eyes***	
• Absent	2
• Disrupted	3
• Intact	34
DRIL, ***n. of eyes***	
• Absent	16
• Presence	23

Quantitative values are expressed in mean±SD (standard deviation). ***DME*** diabetic macular edema; ***BCVA*** best-corrected visual acuity; ***logMAR*** logarithm of the minimum angle of resolution; ***CMT*** central macular thickness
n number; ***VEGF*** vascular endothelial growth factor; ***PDR*** proliferative diabetic retinopathy; ***DEX*** dexamethasone; ***ELM*** external limiting membrane; ***EZ*** ellipsoid zone; ***RPE*** retinal pigment epithelium; ***DRIL*** Disorganization of the Retinal Inner Layers **.**

the visual acuity at ""study visit"" was found in relation to the presence of DRIL (r = 0.488, P=0.043) and the ELM alteration, (r = 0.324, P=0.045). However, no statistically significant associations were identified with the EZ status (p=0.076) and the RPE status (p=0.061) in relation to BCVA at ""study visit"" (Table 3).

Regarding quantitative parameters, a strong correlation was noted between the visual acuity during the "study visit" and the foveal and parafoveal thicknesses of both the inner and outer retina ($p < 0.05$) (Table 3). Additionally, these visual outcomes were not significantly impacted by factors such as age, the number of anti-VEGF injections,

Table 3
Results of stepwise multiple regression analysis of the association between visual acuity at "study visit" and other variables.

	Δ VA as dependent variable	
	Standardized ß Coefficient (SE)	P value
Age	0.028 (0.022)	0.118
Previous treatment with periphery laser	0.678 (0.068)	0.326
Total follow-up after anti-VEGF therapy initiation	0.255 (0.01)	0.396
Diagnosis of PDR	0.289 (0.11)	0.148
Pseudophakia	-0.449 (0.002)	0.232
Number of intravitreal treatments (anti-VEGF + DEX implant)	-1.268 (0.023)	0.361
ELM integrity	0.324 (0.005)	0.045*
EZ integrity	0.146 (0.233)	0.076
RPE integrity	- 0.131 (0.026)	0.061
Presence of DRIL	0.488 (0.001)	0.043*
Foveal inner retinal thickness	0.421 (0.77)	0.006*
Foveal outer retinal thickness	0.326 (0.049)	0.003*
Parafoveal inner retinal thickness	0.428 (0.222)	0.018*
Parafoveal outer retinal thickness	0.452 (0.122)	0.020*
Perifoveal inner retinal thickness	0.148 (0.318)	0.678
Perifoveal outer retinal thickness	0.140 (0.135)	0.856

Quantitative values are expressed in mean±SD (standard deviation). ***n*** number; ***VEGF*** vascular endothelial growth factor; ***PDR*** proliferative diabetic retinopathy; ***DEX*** dexamethasone; ***ELM*** external limiting membrane; ***EZ*** ellipsoid zone; ***RPE*** retinal pigment epithelium; ***DRIL*** retinal inner layer disorganization. Significative P values are marked with an asterisk sign.

switch to intravitreal dexamethasone therapy, the time interval between the initiation of therapy and the "study visit", or prior macular laser treatment (Table 3).

3.5. Repeatability

The intergrader repeatability, as indicated by the unweighted Kappa (k) values, were as follows: 0.94 for ELM appearance, 0.89 for EZ appearance, 0.94 for RPE appearance, and 0.92 for the presence of DRIL. After a process of adjudication between the graders, agreement was achieved for all discrepancies.

4. Discussion

This study explores the enduring visual results of patients with resolved DME who underwent treatment with anti-VEGF therapy for a minimum of 2 years. Notably, the study's findings establish a significant association between OCT-identified morphological alterations and the long-term visual outcomes. Specifically, we observed a robust correlation between final visual acuity and the appearance of the ELM, the presence of DRIL, as well as the foveal and parafoveal thicknesses of both the inner and outer retina.

Recent studies using OCT have shown that the integrity of the foveal photoreceptor layer is closely linked to visual acuity in various eye conditions such as neovascular AMD [18], macular hole [19], central serous chorioretinopathy [20] and retinal vein occlusion [21,22]. The photoreceptor layer is known to be susceptible to ischemic or inflammatory damage; thus, significant photoreceptor cell loss may occur, potentially leading to the disruption of the EZ, ELM, and RPE in eyes affected by DME.

In the present study, we observed that the status of the ELM exhibited a stronger correlation with the final BCVA compared to the status of the EZ and the RPE. The ELM is not a conventional membrane; instead, it comprises a series of tangentially oriented adhesions called *zonulae adherens*. These adhesions connect the apical processes of Müller cells with the inner segments of photoreceptors. This unique layer plays a role in maintaining the alignment and orientation of photoreceptors [23]. Additionally, the ELM serves as a barrier, preventing the passage of proteins from the subretinal space to the inner retina. Consequently, the ELM serves as a crucial landmark in structural OCT for assessing the integrity of photoreceptor inner segments. Our study has demonstrated that the preservation of this structure is the most reliable predictor of visual improvement in DME eyes undergoing anti-VEGF for a long time.

However, the initial EZ and RPE status had no significant correlation with the final BCVA. The difference in the status of ELM, EZ and RPE between neovascular AMD and DME may be due to the different process of retinal structural damage. The BRB is a crucial physiological barrier responsible for regulating the movement of ions, proteins, and water into and out of the retina [24,25]. It consists of both inner and outer components. In the case of diabetic retinopathy, the initial changes occur in the inner BRB, while neovascular AMD is characterized by alterations in the outer BRB [26,27].

We hypothesize that the damage to the external limiting membrane seen in DME could be a consequence of mechanical compression caused by intracellular fluid [28]. Consequently, the sudden appearance of intraretinal fluid in DME eyes might lead to early damage to the photoreceptor layer, starting with the ELM. It's important to note that damage to Müller cells might play a role in both ELM disruption and the alteration of barrier properties within the ELM. This disruption in barrier properties could lead to disturbances in fluid dynamics, allowing blood components to migrate into the outer retinal layers, potentially exacerbating the damage to photoreceptors [29,30]. Therefore, the evaluation of the ELM may prove valuable in diseases that cause significant photoreceptor damage, including damage to inner segments or cell bodies.

Despite its simplicity, the classification system employed in this

study is practical and user-friendly for clinical applications. It can be applied effectively to patients undergoing various therapies, including intravitreal injections of anti-VEGF and steroids and laser photocoagulation.

Importantly, the present study highlights the distinctive relationship between DRIL presence and visual acuity in resolved DME eyes. Prior research has indicated that DRIL represents a novel noninvasive parameter highly linked with visual acuity in eyes affected by ongoing or resolved DME after one year of treatment [13]. Plausibly, the strong correlation between foveal DRIL and visual acuity in DME-affected eyes can be attributed to the fact that the inner plexiform, inner nuclear, and outer plexiform layers house crucial anatomical structures essential for the transmission of visual signal from photoreceptors to retinal ganglion cells [27,31]. Notably, the failure to discern clear boundaries between these layers on high-resolution SD-OCT imaging may indicate the destruction or disarray of some axons and nuclei of amacrine, bipolar, and/or horizontal cells situated in these regions. Pelosini et al. [32] proposed that when edema elevates retinal thickness beyond a critical limit, bipolar axons might break, leading to the loss of visual information transmission from photoreceptors to ganglion cells. This destruction of bipolar cells might not be entirely reversible, potentially explaining cases where visual acuity does not recover following the resolution of DME.

Additionally, we performed a quantitative topographic assessment of both the inner and outer retina. Our findings suggest that a decrease in foveal and parafoveal thickness following anti-edema treatment is associated with visual improvement, probably due to the resolution of DME. However, despite the complete resolution of DME, certain patients demonstrate only marginal visual improvement [22,33]. Hence, relying solely on the reduction in macular thickness may not suffice in predicting favorable visual recovery [34]. It remains critical to evaluate OCT qualitative parameters in conjunction with quantitative data.

The present study has certain limitations that should be taken into account when interpreting our findings. Firstly, it was a retrospective analysis, which could make it vulnerable to selection and ascertainment bias. Additionally, our study cohort was not part of a larger multicenter trial, potentially limiting the generalizability of the results. Secondly, as mentioned earlier, our cohort consisted of patients who had at least one visit demonstrating resolved DME after a 2-year follow-up period. This selection criterion may introduce bias into the analysis.Additionally, we did not assess the qualitative OCT parameters at baseline, making it impossible to explore any potential correlation between the alteration in visual acuity and changes in qualitative OCT parameters. We also excluded patients with evidence of vitreomacular disorders or a history of vitreoretinal surgery, as these factors can affect qualitative and quantitative OCT analysis. Moreover, some patients in our study switched to dexamethasone therapy during their follow-up. However, our multivariate analysis did not find any significant impact of this treatment change on long-term outcomes. Furthermore, our study was not large enough to account for potential confounding factors such as the presence of other systemic disorders, the level of blood glucose control or the duration of diabetes.

In summary, our study has successfully identified specific OCT biomarkers that exhibit a strong correlation with the long-term visual outcomes in eyes with resolved DME treated using anti-VEGF therapy. Particularly, the absence of an intact ELM and the presence of DRIL were identified as qualitative parameters associated with poorer long-term visual outcomes in these patients. Additionally, a decrease in foveal and parafoveal thickness subsequent to anti-edema treatment has been observed to correspond with visual improvement. The integration of high-resolution OCT plays a critical role in the effective management of DME-affected eyes, enabling a comprehensive assessment of both inner and outer retinal structures, which serve as crucial indicators for predicting prognosis. Ultimately, our findings could serve as a valuable reference for determining the most optimal anatomical target for patients affected by this condition.

Statements

Funding

No funding was received for this research.

Written informed consent

We reached out to all enrolled individuals to secure their written informed consent for the retrospective utilization of their clinical information.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Pasquale Viggiano: Conceptualization, Methodology, Software, Validation, Writing – original draft. **Stela Vujosevic:** Conceptualization, Methodology, Software, Writing – original draft. **Francesca Palumbo:** Conceptualization, Methodology, Software, Writing – original draft. **Maria Oliva Grassi:** Methodology, Data curation. **Giacomo Boscia:** Data curation. **Enrico Borrelli:** Data curation. **Michele Reibaldi:** Data curation. **Luigi Sborgia:** Visualization, Investigation. **Teresa Molfetta:** Visualization, Investigation. **Federica Evangelista:** Visualization, Investigation. **Giovanni Alessio:** Visualization, Investigation. **Francesco Boscia:** Visualization, Investigation, Supervision, Writing – review & editing.

Declarations of competing interest

None.

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None.

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