Long-Term Impact of Diabetic Retinopathy on Response to Anti-VEGF Treatment in Neovascular AMD

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Purpose. To explore the long-term effect of diabetic retinopathy on response to anti-vascular endothelial growth factor (VEGF) treatment in age-related macular degeneration–associated type 1 macular neovascularization (MNV) using optical coherence tomography angiography (OCTA).

Methods. A total of 45 eyes with exudative neovascular age-related macular degeneration (nAMD) with type 1 MNV were included in the analysis. Among them, 24 eyes of 24 patients had no history of diabetes mellitus (DM) in their anamnesis and were assigned to the Not Diabetic group; 21 eyes of 21 patients had mild diabetic retinopathy and were included in the Diabetic group. We considered the following outcome measures: (1) best-corrected visual acuity changes; (2) central macular thickness; (3) MNV lesion area; and (4) MNV flow area. The OCTA acquisitions were performed at the following time points: (1) baseline visit, which corresponded to the day before the first injection; (2) post-loading phase (LP), which was scheduled at 1 month after the last LP injection; and (3) 12-month follow-up visit.

Results. All morphofunctional parameters showed a significant improvement after the LP and at the 12-month follow-up visit. Specifically, both the Diabetic group and the Not Diabetic group displayed a significant reduction of MNV lesion areas at both the post-LP assessment ($P = 0.026$ and $P = 0.016$, respectively) and the 12-month follow-up ($P = 0.039$ and $P = 0.025$, respectively). Similarly, the MNV flow area was significantly decreased in both the Diabetic group and the Not Diabetic group at the post-LP assessment ($P < 0.001$ and $P = 0.012$, respectively) and at the 12-month follow-up ($P = 0.01$ and $P = 0.035$, respectively) compared to baseline. A smaller reduction in the MNV lesion area was observed in the Diabetic group at both the post-LP evaluation ($P = 0.015$) and the 12-month follow-up ($P = 0.032$). No other significant differences were found between the groups for the other parameters ($P > 0.05$).

Conclusions. Our results indicated that the Diabetic group exhibited a smaller reduction in MNV lesion area after 12 months of anti-VEGF treatment. This highlights the importance of considering diabetic retinopathy as a potential modifier of treatment outcomes in nAMD management, with DM serving as a crucial risk factor during anti-angiogenic treatment.

Keywords: nAMD, diabetic retinopathy, OCTA, macular neovascularization

Age-related macular degeneration (AMD) represents a leading cause of vision loss, as it affects over 6 million people globally.[1] Advanced stages of AMD consist of geographic atrophy and neovascular AMD (nAMD). The latter form, also known as wet or exudative AMD, results in a severe vision impairment due to the development of exudative macular neovascularization (MNV).[2,3] However, the pathogenesis of AMD is multifaceted, involving a range of environmental and genetic factors. These factors include advanced age, smoking, cardiovascular disease, and diabetic angiopathy. The intricate interaction of these elements contributes to the development and progression of AMD.[4–9] The relationship between diabetes mellitus (DM) and nAMD remains a topic of debate within the scientific community. Whereas some studies have reported a correlation between DM and nAMD, suggesting that DM may increase the risk of developing nAMD, other reports propose that diabetes could actually act as a protective factor against nAMD. Additionally,

some studies have found no significant association between these two conditions. This conflicting evidence highlights the complexity of understanding the interplay between DM and nAMD, warranting further research to elucidate the true nature of their relationship.[10–13]

Diabetic retinopathy (DR), characterized by damage to the blood vessels in the retina, stands as one of the most prevalent retinal vascular diseases. It exhibits a relatively high prevalence among individuals with DM.[14–16] Whereas AMD is traditionally viewed as primarily affecting the outer retinal layers, specifically the complex comprised of the retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris (CC), recent evidence suggests that this complex (RPE–CC) is impaired in individuals with DM, even in the absence of DR. These findings disrupt the traditional paradigm of these conditions and emphasize the interconnectedness of retinal pathology spanning various layers and diseases.[17,18]

The recent implementation of novel techniques of retinal imaging, such as optical coherence tomography angiography (OCTA), has allowed researchers to deeply explore microvascular alterations in different retinal disorders. In particular, OCTA has been widely used in MNV assessment, at the beginning and in response to anti-vascular endothelial growth factor (anti-VEGF) therapy.[19]

Previous research focused on evaluating the role of DR on nAMD through the use of OCTA.[11,20] In our previous study, which investigated the impact of DR on morphological changes in type 1 MNV associated with AMD, we observed a lesser reduction in MNV area following the loading dose of anti-VEGF therapy in eyes with DR.[11] However, a significant limitation of our findings was the short follow-up duration, limited to only 1 month after the loading phase (LP) of antiangiogenic therapy. Therefore, given the current dearth of long-term evidence regarding the effects of DR on morphological changes of MNV in nAMD, the objective of this study was to conduct a thorough long-term analysis, spanning 1 year, of DR-related morphological alterations in type 1 MNV during anti-VEGF therapy as evaluated by OCTA.

Methods

Study Participants

This was a retrospective study involving 45 eyes of 45 patients with a diagnosis of exudative nAMD and naïve type 1 MNV undergoing anti-VEGF treatment with a follow-up extending to up to 1 year. All of the patients were enrolled at the Medical Retina Service of the Department of Translational Biomedicine Neuroscience, University of Bari Aldo Moro, Italy, between September 2021 and September 2023. The study adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of Department of Translational Biomedicine Neuroscience, University of Bari Aldo Moro. Informed consent was obtained from all subjects included in the study.

A total of 45 eyes presenting a diagnosis of exudative nAMD with type 1 MNV were included in the analysis.[21] Among them, 24 eyes of 24 patients had no history of DM in their anamnesis and were assigned to the Not Diabetic group; 21 eyes of 21 patients who had mild DR were included in the Diabetic group. The classification of mild non-proliferative diabetic retinopathy (NPDR) was based on the Early Treatment Diabetic Retinopathy Study (ETDRS) score. Specifically, we defined mild NPDR as the presence of at least one microaneurysm and/or mild hemorrhages, in accordance with the ETDRS criteria.[22] None of the eyes included in the study had undergone prior treatment and were subjected to a loading dose of anti-VEGF therapy (aflibercept). This initial treatment was comprised of three monthly injections of aflibercept, followed by a pro re nata (PRN) regimen for up to 1 year.

The following exclusion criteria were adopted: (1) presence of MNV types other than type 1 MNV; (2) infection or inflammation affecting one or both eyes; (3) advanced cataract; (4) presence of other macular and/or optic nerve (e.g., glaucoma) pathologic conditions; (5) history of previous anti-VEGF treatments or retinal laser therapy in the study eye; (6) history of myocardial infarction or cerebrovascular disease within the last 6 months; (7) myopia greater than 3.00 diopters; (8) evidence of complications associated with neovascular AMD, including massive subretinal hemorrhage, RPE tear, and vitreous hemorrhage; (9) previous vitreoretinal procedure in the study eye; or (10) neurodegenerative diseases such as Alzheimer disease or Parkinson disease. Moreover, images presenting a poor quality (<6/10), incorrect or incomplete segmentation, and/or motion artifacts were excluded.

Study Protocol

The OCTA assessment was performed using a XR Avanti AngioVue OCTA (Optovue, Fremont, CA, USA). The follow-up was based on (1) baseline visit corresponding to the day before the first injection; (2) post-LP, which was scheduled at 1 month after the last LP injection; and (3) 12-month follow-up, which was conducted 12 months after the initiation of treatment and was scheduled to occur 30 ± 7 days after the final anti-VEGF injection administered during the study period. At each visit, patients underwent a complete ophthalmological examination, including best-corrected visual acuity (BCVA), measured in Snellen and converted to logMar; intraocular pressure assessment; dilated fundus ophthalmoscopy; and OCT and OCTA assessment.

We considered the following outcome measures: (1) BCVA changes, (2) central macular thickness (CMT), (3) MNV flow area, and (4) MNV lesion area.

Imaging Processing

En Face. OCTA 3 × 3-mm scans and structural OCT and OCTA images were acquired with the RTVue XR Avanti (Optovue) device with an A-scan rate of 70,000 scans per second and with a light source length of 840 nm. The latter system combines the acquisition of both horizontal and vertical volume scans (containing 400 A-scans) in order to reduce motion artifacts.[23]

Central Macular Thickness. CMT was measured within the 1-mm-diameter innermost circle of the ETDRS grid of the Optovue software centered over the fovea.

Macular Neovascularization Analysis. To visualize the entire MNV, we employed the methodology outlined by de Carlo et al.[24] In brief, a manual segmentation was obtained with the slab having the RPE as the upper limit and Bruch's membrane as the lower limit to encompass the entire MNV type 1. Consequently, adjustments were made to the outer and inner boundary levels to include the entire MNV area, as seen in the corresponding OCT B-scan images (Fig. 1). Subsequently, a region of interest outlining the

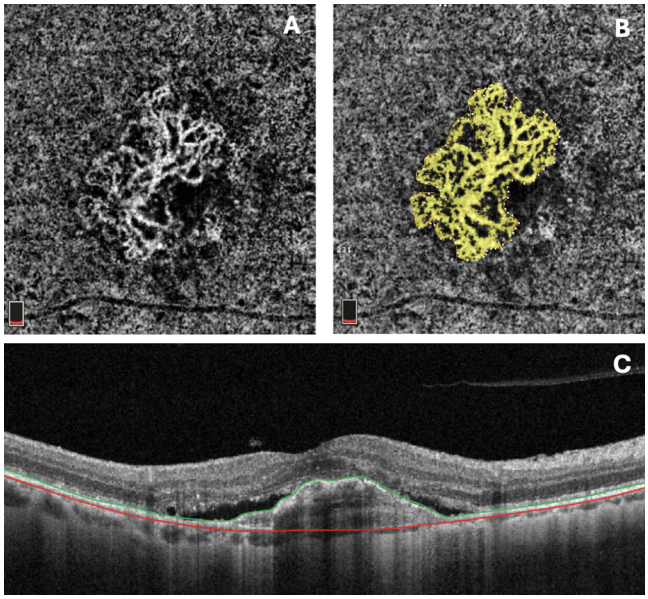

Figure 1. (**A**) OCTA en face image of a patient affected by AMD-associated MNV type 1 at baseline visit. (**B**) A region of interest outlining the perimeter of the MNV was delineated on the CC en face image. The boundaries of the MNV lesion were manually defined in each CC en face image. (**C**) Spectral-domain OCT scan of a patient affected by AMD-associated MNV type 1 at the baseline visit. The manual segmentation was obtained with the slab having the RPE as the upper limit and Bruch's membrane as the lower limit to encompass the entire MNV type 1. Consequently, adjustments were made to the outer and inner boundary levels to include the entire MNV area, as seen in the corresponding OCT B-scan images.

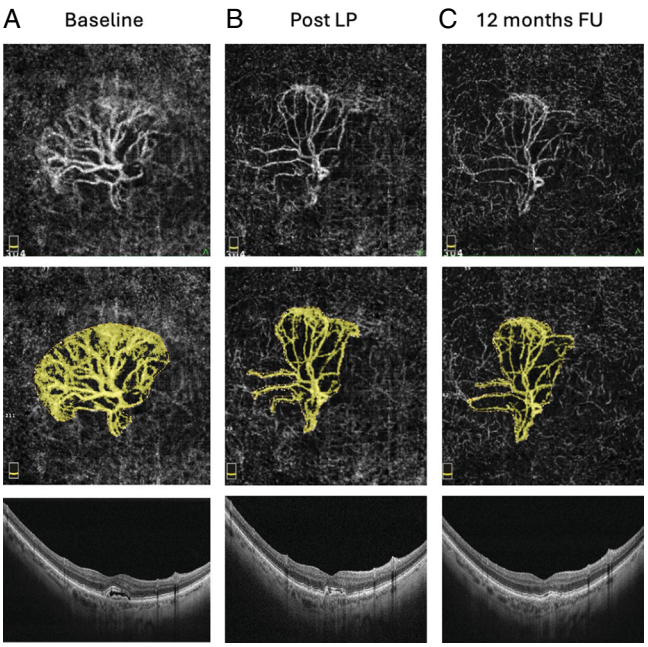

Figure 2. OCT and OCTA scans from an enrolled non-diabetic patient affected by type 1 MNV in AMD at the different time points. Scans were acquired at the baseline visit, post-LP visit, and 12-month follow-up. The figure shows the MNV changes throughout the follow-up. (**A**) baseline visit; (**B**): post-LP visit; (**C**) 12-month follow-up.

perimeter of the MNV was delineated on the CC en face image (Fig. 1). The boundaries of the MNV lesion were manually defined by two masked retinal experts (GB, PV) in each CC en face image. The interobserver agreement, on average, was determined to be excellent in the assessment of MNV borders, with a coefficient of 0.92 (confidence interval, 0.88–0.94). Subsequently, the automatic software of the device calculated the MNV area (comprised of the entire area within the drawn region of interest) and the MNV flow area (representing the flow area within the user-defined MNV lesion area) at the baseline, post-LP, and 12-month follow-up visits in both groups (Fig. 2).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 20.0 (IBM, Chicago, IL, USA). Normality of the data was assessed by using the Shapiro–Wilk test. A paired *t*-test was used to compare quantitative metrics among the different time points. To compare delta changes between the groups, Friedman nonparametric tests were performed. A chosen level of statistical significance was set at $P < 0.05$.

Results

Demographics

A total of 45 eyes of 45 patients with naïve nAMD-related type 1 MNV were included in the analysis. Among them, 20 were women and 25 were men. In the Not Diabetic group, the mean ± SD age was 78.29 ± 5.27 years; the mean age of the Diabetic group was 80.25 ± 7.52 years. The comparison between the groups was not statistically significant ($P = 0.177$). The mean number of injections at the 12-month follow-up was 7.88 ± 0.91 in the Diabetic group and 7.42 ± 0.87 in the Not Diabetic group ($P = 0.339$). None of the patients in the Diabetic group presented with diabetic macular edema (DME) at baseline or during the follow-up period. In the Diabetic group, 35% of patients were phakic; in the Not Diabetic group, 42% were phakic ($P = 0.622$). Table 1 reports the characteristics of patients included in the analysis.

BCVA and Central Macular Thickness

The mean BCVA at the baseline visit was 0.54 ± 0.34 logMAR in the Diabetic group, and it was 0.50 ± 0.33 logMAR in the Not Diabetic group ($P = 0.307$). Compared with baseline, BCVA significantly improved at post-LP and the 12-month follow-up (T1: 0.42 ± 0.29 logMAR, $P < 0.001$; T2: 0.41 ± 0.20 logMAR, $P = 0.002$, respectively) in the Diabetic group (Table 2). However, no significant difference was observed between the BCVA at post-LP and that at the 12-month follow-up ($P = 0.916$). Conversely, in the Not Diabetic group, BCVA showed significant improvement from baseline both at the post-LP assessment (0.39 ± 0.37 logMAR, $P = 0.002$) and at the 12-month follow-up (0.32 ± 0.35 logMAR, $P = 0.006$) (Table 2). Furthermore, no statistically significant difference in BCVA was observed between these subsequent time points ($P = 0.511$).

Regarding the CMT, no difference was found between the two groups at baseline ($P = 0.550$). In the Diabetic group, there was a significant reduction compared to baseline (286.58 ± 56.93 μm) at both the post-LP assessment (239.18 ± 50.29 μm, $P = 0.002$) and the 12-month follow-

Table 1. Demographic Characteristics of Patients Included in the Analysis

	Diabetic Group	Not Diabetic Group	*P*
n	21	24	0.177
Age (y), mean ± SD	80.25 ± 7.52	78.29 ± 5.27	0.366
Female, *n*	12	8	0.113
Cardiovascular disease,* *n* (%)	5 (23.8)	4 (16.66)	0.564
Number of anti-VEGF injections, mean ± SD	7.88 ± 0.91	7.42 ± 0.87	0.339
DME presence, *n* (%)	0 (0)	—	—
Phakic status, *n* (%)	7 (35)	10 (42)	0.622

The 12-month follow-up OCT was analyzed at 12 months from treatment initiation, corresponding to 30 ± 7 days after the last intravitreal injection for both groups.

* History of heart attack or stroke.

Table 2. Morphofunctional Results at Different Time Points

	Diabetic Group, Mean ± SD				Not Diabetic Group, Mean ± SD			
	Baseline	Post-LP	12-Month Follow-Up	*P*	Baseline	Post-LP	12-Month Follow-Up	*P*
BCVA (logMAR)	0.54 ± 0.34	0.42 ± 0.29	0.41 ± 0.20	0.001* 0.002† 0.916‡	0.50 ± 0.33	0.39 ± 0.37	0.32 ± 0.35	0.002* 0.006† 0.511‡
CMT (μm)	286.58 ± 56.93	239.18 ± 50.29	256.88 ± 71.31	0.002* 0.044† 0.208‡	290.08 ± 62.11	233.17 ± 41.23	234.23 ± 39.82	0.002* 0.001† 0.939‡
MNV lesion area (mm^2)	1.52 ± 2.01	1.21 ± 1.9	1.23 ± 2.2	0.026* 0.039† 0.468‡	1.47 ± 2.38	1.01 ± 1.79	0.78 ± 1.09	0.016* 0.025† 0.008‡
MNV flow area (mm^2)	0.97 ± 1.16	0.67 ± 1.06	0.72 ± 1.19	0.001* 0.01† 0.401‡	0.78 ± 1.34	0.55 ± 0.95	0.41 ± 0.43	0.012* 0.035† 0.037‡

* *P* value of the paired *t*-test comparing quantitative metrics between the baseline visit and post-LP.

† *P* value of the paired *t*-test comparing quantitative metrics between the baseline visit and 12-month follow-up.

‡ *P* value of the paired *t*-test comparing quantitative metrics between post-LP and 12-month follow-up.

up (256.88 ± 71.31 μm, $P = 0.044$). However, no differences were observed at the 12-month follow-up when compared to the post-LP measurements ($P = 0.208$) (Table 2). Similarly, in the Not Diabetic group, the CMT exhibited a significant reduction from baseline (290.08 ± 62.11 μm) at both the post-LP assessment (233.17 ± 41.23 μm, $P = 0.002$) and the 12-month follow-up (234.23 ± 39.82 μm, $P < 0.001$) (Table 2). However, no significant difference was observed between the CMT at post-LP and that at the 12-month follow-up ($P = 0.939$).

MNV Analysis

In the Diabetic group, there was a significant reduction in the lesion area of MNV when compared to the baseline visit (1.52 ± 2.01 mm^2) at both the post-LP assessment (1.21 ± 1.9 mm^2, $P = 0.026$) and the 12-month follow-up (1.23 ± 2.2 mm^2, $P = 0.039$). However, no significant difference was observed between these subsequent time points ($P = 0.468$). Conversely, in the Not Diabetic group, there was a significant reduction in the MNV lesion area from baseline (1.47 ± 2.38 mm^2) both at post-LP (1.01 ± 1.79 mm^2, $P = 0.016$) and at the 12-month follow-up (0.78 ± 1.09 mm^2, $P = 0.025$). This decreasing trend in MNV area remained significant between the post-LP and 12-month follow-up assessments ($P = 0.008$) (Table 2). Furthermore, no difference was found in the MNV area between the two groups at baseline ($P = 0.470$).

Regarding the MNV flow area, in the Diabetic group there was a significant reduction compared to baseline (0.97 ± 1.16 mm^2) both at post-LP (0.67 ± 1.06 mm^2, $P < 0.001$) and at the 12-month follow-up (0.72 ± 1.19 mm^2, $P = 0.01$), whereas no significant difference was observed between these subsequent time points ($P = 0.401$). Conversely, in the Not Diabetic group, there was a significant reduction in the MNV flow area from baseline (0.78 ± 1.34 mm^2) both at post-LP (0.55 ± 0.95 mm^2, $P = 0.012$) and at the 12-month follow-up (0.41 ± 0.43 $P = 0.035$). This decreasing trend in MNV flow area remained significant between the post-LP and 12-month follow-up assessments ($P = 0.037$) (Table 2). Furthermore, no difference was found in the MNV flow between the two groups at baseline ($P = 0.326$).

Comparison Between Groups

The comparative analysis conducted between the Diabetic and Not Diabetic groups did not reveal significant differences concerning functional parameters (BCVA) and structural OCT parameters (CMT) assessed at various time points. However, it is essential to emphasize the significance between the two groups regarding anatomical parameters evaluated with OCTA. Specifically, a smaller reduction in the area of the MNV was observed in the Diabetic group both at the post-LP evaluation ($P = 0.015$) and at the 12-month follow-up ($P = 0.012$) (Fig. 3). Additionally, no differences were observed in terms of flow area between the two groups at all time points (Table 3).

Discussion

In this retrospective study employing OCT and OCTA, we investigated the impact of DR on the longitudinal morphological and functional alterations in type 1 nAMD-associated

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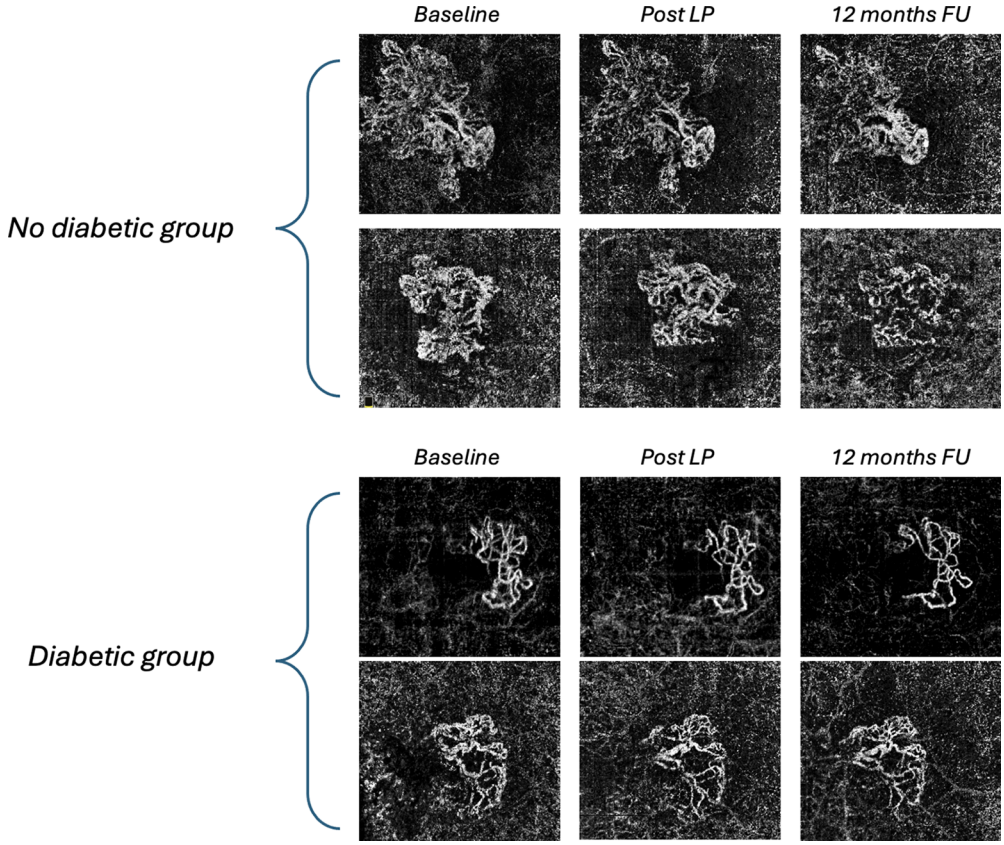

Figure 3. Representative OCTA images showing the evolution of MNV in the Not Diabetic and Diabetic groups over time. The figure presents two cases from each group at three time points: baseline, post-LP, and 12-month follow-up. In the Not Diabetic group (*top two rows*), a reduction in MNV size and complexity can be seen over time. In contrast, the Diabetic group (*bottom two rows*) showed less pronounced changes in MNV structure and size, particularly between post-LP and 12-month follow-up.

MNV following the LP and 12-month follow-up of anti-VEGF treatment. Our findings indicate that eyes with mild NPDR exhibited distinct responses to treatment for nAMD. Specifically, the Diabetic group demonstrated a limited reduction in the MNV lesion area after 1 year of intravitreal therapy. This observation underscores the importance of considering such differential responses, particularly because OCTA-based metrics are currently utilized in the management of both nAMD and DR.

It has been suggested that alterations in the structure and function of the RPE and the choroidal circulation, which are associated with diabetes, may elevate the risk of developing neovascular nAMD.[18,23] Indeed, previous histological reports have demonstrated basement membrane thickening of the CC and other small choroidal blood vessels associated with luminal narrowing and capillary dropout in diabetic eyes with prolonged disease duration.[23] Following OCTA analysis, it has been hypothesized that eyes with NPDR may exhibit macular hypoperfusion and photoreceptor damage as a consequence of CC impairment.[18]

However, there is limited evidence regarding the influence of DR on the management of patients with nAMD. Specifically, this subset of patients might exhibit divergent responses to anti-VEGF treatment over the long term, particularly concerning retinal anatomical changes. In our previous study examining the impact of DR on the response to the anti-VEGF loading phase treatment in patients with nAMD, we noted that the Diabetic group showed a smaller reduction in the size of the MNV compared to patients solely affected by nAMD.[10] Therefore, in this study, we chose to evaluate these preliminary results 1 year after the initiation of treatment by analyzing a similar group of patients. The results were encouraging, confirming the minor reduction in MNV after the LP in the Diabetic group. However, the most interesting finding pertained to the behavior of the neovascular lesion after 1 year of treatment initiation. Specifically, the Diabetic group did not exhibit a reduction in MNV after 12 months; instead, there was a lack of significant reduction of the area of the MNV. In contrast, the Not Diabetic group showed a continuous reduction in the size of the MNV. This last result appears to be highly significant, especially considering that the size of MNV evaluated with OCTA serves as a valuable biomarker in assessing the response or lack of response to treatment.[25,26]

The lack of significant reduction of the neovascular membrane area in the Diabetic group after 1 year of treatment suggests a distinct treatment response compared to the continuous reduction observed in the Not Diabetic group. This finding underscores the importance of considering DR as a potential modifier of treatment outcomes in neovascular AMD management.

Multiple mechanisms could be contributing to this result, and they may even coexist simultaneously; for example, it is plausible that the presence of DR alters the angiogenic pathways involved in MNV. DR is associated with dysregulated VEGF signaling and increased levels of inflamma-

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Table 3. Comparison Between the Two Groups at Different Time Points

	Δ Baseline, Post-LP (%), Mean ± SD			Δ Baseline, 12-Month Follow-Up (%), Mean ± SD			Δ Post-LP, 12-Month Follow-Up (%), Mean ± SD		
	Diabetic Group	Not Diabetic Group	*P*	Diabetic Group	Not Diabetic Group	*P*	Diabetic Group	Not Diabetic Group	*P*
BCVA (logMAR)	7.7 ± 17.7	9.3 ± 18.5	0.203	16.6 ± 14.5	20.3 ± 35.7	0.406	3.3 ± 18.8	12.8 ± 40.2	0.570
CMT (μm)	−14.7 ± 15.4	−14.9 ± 23.3	0.999	−7.2 ± 26.5	−15.8 ± 21.1	0.112	8.7 ± 22.4	1.1 ± 19.3	0.362
MNV lesion area (mm^2)	−19.6 ± 31.1	−30.9 ± 23.4	0.015	−12.3 ± 29.1	−26.6 ± 43.1	0.012	14.2 ± 31.1	−8.1 ± 49.1	0.145
MNV flow area (mm^2)	−23.1 ± 41.1	−32.6 ± 40.7	0.463	−9.2 ± 54.6	−17.5 ± 48.4	0.727	42.3 ± 88.1	16.2 ± 79.5	0.225

tory cytokines, which could potentially lead to a different response to anti-VEGF therapy. These altered pathways may result in a reduced responsiveness to anti-VEGF therapy, leading to a less pronounced reduction of neovascularization over time. Another possibility is that the microvascular changes associated with DR, such as capillary dropout and basement membrane thickening, create a less conducive environment for the resolution of neovascular complexes. The compromised vascular structure and function in diabetic eyes may impede the ability of anti-VEGF therapy to induce significant regression of the MNV, resulting in a lesser degree of reduction compared to non-diabetic eyes.

This latter observation is further supported by another of our previous studies, which found that the Diabetic group exhibited greater ischemia of the CC surrounding the MNV even before treatment initiation compared to the Not Diabetic group. Additionally, following the anti-VEGF loading phase, the Diabetic group did not experience significant changes, unlike the Not Diabetic group, which demonstrated a gradual reperfusion of the CC around the neovascular membrane.[19]

Additionally, we assessed changes in the MNV flow in both groups, revealing a decrease after the LP procedure and also after 1 year of anti-VEGF treatment. Our findings corroborate previous research, such as the study by Mastropasqua et al.[27] Importantly, we did not observe any significant differences in MNV flow between the two groups, although the Not Diabetic group exhibited a decreasing trend 12 months post-treatment. These results support the theory that anti-VEGF treatment leads to a reduction in the number and perfusion of smaller pathological vessels, but larger trunks remain adequately perfused, even in cases of nAMD complicated by initial stages of DR.[25]

The differential response to anti-VEGF therapy observed between the Diabetic and Non-Diabetic groups might be related to variations in vitreous VEGF concentrations. Although we did not directly measure VEGF levels in our study, previous research has shown that diabetic patients often have higher baseline VEGF concentrations. For example, Dell'Omo et al.[28] reported significantly elevated vitreous VEGF levels in diabetic patients compared to non-diabetic controls, with levels further increased in those with proliferative DR. In the context of AMD, Tong et al.[29] found that VEGF levels were significantly higher in the vitreous of patients with active choroidal neovascularization compared to those without. These findings suggest that our Diabetic group might have had higher baseline VEGF levels, potentially requiring more aggressive or prolonged anti-VEGF therapy to achieve reductions in MNV size similar to those observed in the Not Diabetic group.

It is crucial to note that, although all previously significant parameters assessed with OCTA showed notable changes, there were no significant differences between the two groups in terms of functional (BCVA) and anatomical parameters measured with structural OCT, specifically CMT. Indeed, both groups exhibited a notable improvement in BCVA and a significant reduction in CMT compared to baseline following the LP and 1 year after treatment initiation. However, no significant differences were observed in terms of CMT and BCVA between the LP assessment and measurements taken 1 year into treatment. This suggests that, although there might be variances in the underlying anatomical changes detected with OCTA, these differences do not substantially affect visual function. This conclusion

is supported by the similar improvements in visual acuity observed in both groups following treatment.

Our study has limitations that should be considered when interpreting our findings. First, our sample size was relatively small, potentially limiting the generalizability of our results. Additionally, the absence of a control group means that observed differences during the follow-up period could have occurred even without anti-angiogenic therapy, thus affecting the interpretation of treatment effects. Furthermore, the use of spectral-domain OCTA, which utilizes shorter wavelength light compared to swept-source OCTA, may result in reduced signal penetration through the RPE, potentially affecting the accuracy of our imaging data. Among the limitations of our study, we acknowledge that our analysis focused primarily on flow and density parameters of the MNV, in line with our previous research. Although this approach ensured consistency and comparability with our earlier findings, it may have limited our ability to provide a more comprehensive analysis of the vascular components affected by anti-VEGF therapy. A more detailed examination of additional OCTA parameters, such as the differentiation between trunk vessels and newly formed fine neovascularization or measurements of retinal vessel density in the macular area at baseline, could potentially offer deeper insights into the mechanisms underlying the differential responses observed between eyes with and without DR. Moreover, our study did not directly assess choroidal perfusion, which could potentially influence MNV changes, especially in diabetic eyes. Future studies incorporating choroidal perfusion measurements could provide valuable insights into the relationship between choroidal ischemia and MNV response to anti-VEGF therapy in eyes with DR. Finally, our analysis did not account for potential confounding factors related to systemic and ocular parameters, despite our efforts to ensure homogeneity in terms of age and axial length between the two patient samples. However, it is important to consider the strengths of our study, as well. Specifically, we exclusively enrolled patients with type 1 MNV who were treatment naïve, allowing for a focused investigation that leveraged the capabilities of OCTA, particularly in quantitative assessment. Additionally, to the best of our knowledge, our study is the first to analyze eyes with nAMD even in the presence of mild NPDR and to evaluate long-term morphovascular and morphofunctional changes following anti-VEGF treatment. Through OCTA imaging, we observed a distinct response to intravitreal therapy in terms of MNV lesion area in the Diabetic group, highlighting DM as a significant risk factor to consider in patients with nAMD who are undergoing anti-angiogenic therapy.

In conclusion, our study represents the first comprehensive investigation of the impact of DR on longitudinal morphological and functional changes in type 1 MNV associated with AMD in patients undergoing anti-VEGF therapy for 1 year. Utilizing OCTA, we unveiled a divergent response to intravitreal therapy in terms of MNV lesion area in the DR group. Specifically, the Diabetic group did not demonstrate a reduction in MNV area after 12 months; instead, the area of MNV remained largely unchanged. In contrast, the Not Diabetic group exhibited a continuous reduction in MNV size. The lack of significant reduction of the neovascular membrane area in the Diabetic group after 1 year of treatment indicates a distinct treatment response compared to the continuous reduction observed in the Not Diabetic group. This highlights the significance of considering DR as a potential modifier of treatment outcomes in nAMD management, with DM serving as a risk factor to consider during anti-angiogenic treatment. Future larger studies utilizing swept-source OCTA and longer follow-up durations are necessary to validate our preliminary findings.

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References

1. Vos T, Abajobir AA, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1211–1259.
2. Gheorghe A, Mahdi L, Musat O. Age-related macular degeneration. *Rom J Ophthalmol*. 2015;59(2):74–77.
3. Cheung LK, Eaton A. Age-related macular degeneration. *Pharmacotherapy*. 2013;33(8):838–855.
4. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: pooled findings from three continents. *Ophthalmology*. 2001;108(4):697–704.
5. Adams MKM, Chong EW, Williamson E, et al. 20/20—alcohol and age-related macular degeneration: the Melbourne Collaborative Cohort Study. *Am J Epidemiol*. 2012;176(4):289–298.
6. Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology*. 2013;120(11):2292–2299.
7. Katsi VK, Marketou ME, Vrachatis DA, et al. Essential hypertension in the pathogenesis of age-related macular degeneration: a review of the current evidence. *J Hypertens*. 2015;33(12):2382–2388.
8. Cougnard-Grégoire A, Delyfer MN, Korobelnik JF, et al. Long-term blood pressure and age-related macular degeneration: the ALIENOR study. *Invest Ophthalmol Vis Sci*. 2013;54(3):1905–1912.
9. Chen X, Rong SS, Xu Q, et al. Diabetes mellitus and risk of age-related macular degeneration: a systematic review and meta-analysis. *PLoS One*. 2014;9(9):e108196.
10. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL, 3rd. Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study (AREDS) AREDS report no. 19. *Ophthalmology*. 2005;112(4):533–539.
11. Viggiano P, Landini L, Grassi MO, et al. Effects of diabetic retinopathy on longitudinal morphological changes in AMD-associated type 1 macular neovascularization. *Sci Rep*. 2023;13(1):16337.
12. Tomany SC, Wang JJ, Van Leeuwen R, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. *Ophthalmology*. 2004;111(7):1280–1287.
13. Sahoo NK, Mehta MC, Rani PK, et al. Impact of age-related macular degeneration on diabetic retinopathy: an electronic health record based big data analysis from a tertiary eye centre in South India. *Indian J Ophthalmol*. 2021;69(11):3184–3188.
14. De Benedetto U, Sacconi R, Pierro L, Lattanzio R, Bandello F. Optical coherence tomographic hyperreflective foci in early stages of diabetic retinopathy. *Retina*. 2015;35(3):449–453.

15. Mastropasqua R, D'Aloisio R, Costantini E, et al. Serum microRNA levels in diabetes mellitus. *Diagnostics (Basel)*. 2021;11(2):284.
16. Rudraraju M, Narayanan SP, Somanath PR. Regulation of blood-retinal barrier cell-junctions in diabetic retinopathy. *Pharmacol Res*. 2020;161:105115.
17. Viggiano P, Costanzo E, Giannini D, et al. In vivo assessment of associations between photoreceptors structure and macular perfusion in type 1 diabetes. *Br J Ophthalmol*. 2023;107(11):1672–1679.
18. Borrelli E, Palmieri M, Viggiano P, Ferro G, Mastropasqua R. Photoreceptor damage in diabetic choroidopathy. *Retina*. 2020;40(6):1062–1069.
19. Viggiano P, Grassi MO, Pignataro M, et al. Topographical analysis of the choriocapillaris reperfusion after loading anti-VEGF therapy in neovascular AMD. *Transl Vis Sci Technol*. 2022;11(9):18.
20. Viggiano P, Miere A, Borrelli E, et al. The impact of diabetic retinopathy on the choriocapillaris in neovascular AMD. *Invest Ophthalmol Vis Sci*. 2023;64(14):32.
21. Viggiano P, Grassi MO, Boscia G, et al. Short-term morphofunctional changes in previously treated neovascular AMD eyes switched to brolucizumab. *J Clin Med*. 2022;11(19):5517.
22. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs - an extension of the modified Airlie House classification: ETDRS Report Number 10. *Ophthalmology*. 2020;127(4S):S99–S119.
23. Hidayat AA, Fine BS. Diabetic choroidopathy. Light and electron microscopic observations of seven cases. *Ophthalmology*. 1985;92(4):512–522.
24. de Carlo TE, Bonini Filho MA, Chin AT, et al. Spectral-domain optical coherence tomography angiography of choroidal neovascularization. *Ophthalmology*. 2015;122(6):1228–1238.
25. Coscas G, Lupidi M, Coscas F, Français C, Cagini C, Souied EH. Optical coherence tomography angiography during follow-up: qualitative and quantitative analysis of mixed type I and II choroidal neovascularization after vascular endothelial growth factor trap therapy. *Ophthalmic Res*. 2015;54(2):57–63.
26. Muakkassa NW, Chin AT, de Carlo T, et al. Characterizing the effect of anti-vascular endothelial growth factor therapy on treatment-naive choroidal neovascularization using optical coherence tomography angiography. *Retina*. 2015;35(11):2252–2259.
27. Mastropasqua L, Toto L, Borrelli E, Carpineto P, Di Antonio L, Mastropasqua R. Optical coherence tomography angiography assessment of vascular effects occurring after aflibercept intravitreal injections in treatment-naive patients with wet age-related macular degeneration. *Retina*. 2017;37(2):247–256.
28. Dell'Omo R, Cassetta M, Dell'Omo E, et al. Aqueous humor levels of vascular endothelial growth factor before and after intravitreal bevacizumab in type 3 versus type 1 and 2 neovascularization: a prospective, case-control study. *Am J Ophthalmol*. 2012;153(1):1009–1010.
29. Tong JP, Chan WM, Liu DTL, et al. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. *Am J Ophthalmol*. 2006;141(3):456–462.