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Response of Diabetic Macular Edema to Anti-VEGF Medications Correlates with Improvement in Macular Vessel Architecture Measured with OCT Angiography

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Purpose: Improvements in best-corrected visual acuity (BCVA) and central subfield thickness (CST) have been well documented after intravitreal injection of anti-VEGF medications in diabetic macular edema (DME); however, their effect on the vasculature of the macula in diabetic retinopathy (DR) remains poorly understood. Our aim was to explore the effect of intravitreal injection of anti-VEGF on parameters of retinal vascular microstructure in DR with OCT angiography (OCTA).

Design: Retrospective study of adult patients with DME that were treated with anti-VEGF intravitreal injections at the University of Illinois at Chicago between 2017 and 2022.

Participants: Forty-one eyes from 30 patients with nonproliferative or proliferative DR with a mean age of 58.83 ± 11.71 years, mean number of intravitreal injections of 2.8 ± 1.4, and mean follow-up of 6.5 ± 1.7 months.

Methods: ImageJ was employed to measure parameters of retinal vascular microstructure in OCTA images, which included perfusion density, vessel-length density (VLD), vessel diameter, and foveal avascular zone (FAZ) characteristics (area, perimeter, and circularity). Student *t* tests and analysis of variance were used to determine statistical significance.

Main Outcome Measures: A primary analysis was performed comparing the mean of each parameter of all patients as a single group at the beginning and end of the study period. A subgroup analysis was then performed after stratifying patients based on visual improvement, change in CST, prior injection history, and number of injections.

Results: Eyes demonstrated statistical improvement in BCVA logarithm of the minimum angle of resolution score and CST after anti-VEGF treatment. Primary analysis showed a reduction in the vessel diameter of the superficial and deep retinal vasculature, as well as an increase in the circularity of the FAZ within the superficial retinal vasculature after anti-VEGF treatment. Subgroup analysis revealed that eyes with improvement in BCVA exhibited reduced vessel diameter in the superficial retinal vasculature and that eyes with the largest decrease in CST displayed increased perfusion density and VLD in the deep retinal vasculature.

Conclusions: Intravitreal injection of anti-VEGF agents to treat DME improved parameters of retinal vascular microstructure on OCTA over a period of 3 to 9 months, and this effect was most pronounced in eyes that experienced improvement in BCVA and CST.

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Diabetic retinopathy (DR) is the most common complication of diabetes mellitus (DM) and a major cause of vision loss in the United States and worldwide.[1] In the United States, 40.3% and 8.2% of the individuals aged > 40 years with DM are estimated to be afflicted with DR and vision-threatening DR, respectively.[2] Furthermore, the number of individuals worldwide who have DM is expected to increase from 415 million in 2015 to 642 million by the year 2040[3,4]; thus, a similar trend for DR is expected. Based on these epidemiologic data, there is a need for continued advancement in methodologies available for screening and directing early treatment of DR to meet the growing needs of society.

Damage to the retinal vasculature underlies DR pathogenesis, ultimately manifesting as microaneurysms (MAs), capillary occlusion and collapse, vascular dilation and shunting, neovascularization (NV), and enlargement of the foveal avascular zone (FAZ). The pathogenesis of DR is

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owed, in part, to the deleterious effects of overexpression of VEGFs in response to retinal ischemia. Indeed, upregulation of VEGF via activation of hypoxia-inducible factor 1 drives vascular permeability and proliferation that underlies diabetic macular edema (DME) and NV in proliferative DR (PDR), respectively.[5] Intravitreal injection of anti-VEGF agents has been demonstrated to improve DR severity on photography as well as DME as measured by best-corrected visual acuity (BCVA) and central subfield thickness (CST) on spectral-domain OCT compared with conventional treatment[6,7]; however, their effect on the vasculature microstructure in vivo remains an area of interest.[8]

Fluorescein angiography (FA) has traditionally been employed to visualize areas of capillary nonperfusion, vascular leakage, NV, and derangements to the FAZ in DR; however, this technology is cumbersome in the clinic and has poor resolution of deeper vascular layers. OCT angiography (OCTA) is a newer technology that utilizes low-coherence infrared light to capture 3-dimensional images of the retinal vasculature based on image contrast between sequential b scans at the same location over time, and perfused vessels are detected as erythrocyte flow against a static background.[9] In addition to detecting MAs, intraretinal microvascular abnormality, and NV in parallel with the clinical examination, OCTA can detect more subtle and microscopic changes to the retinal vasculature that manifest earlier in the disease course, including perfusion density and vessel caliber as well as the shape and size of the FAZ.[9] Literature evidence has yielded contrasting results when examining the effect of intravitreal injection of anti-VEGF on the vasculature of the macula, as visualized with FA and OCTA, in DR. For example, some studies demonstrate stabilization or improvement of macular ischemia, whereas others indicate worsening of ischemia.[8]

In light of this lack of consensus, we performed a 5-year retrospective study at a major urban center to explore the effect of intravitreal injection of anti-VEGF medications on the vascular microstructure of the macula, visualized by OCTA, in nonproliferative DR (NPDR) and PDR with DME after $\geq$ 1 intravitreal injection of anti-VEGF medication over a period of 3 to 9 months. Our data suggest that anti-VEGFs ameliorate the damage to the retinal vasculature of the macula during DR pathogenesis, and this effect may be most pronounced in patients who respond to treatment with clinical improvement in BCVA and CST.

Methods, Intervention, or Testing

Study Design

This was a retrospective study which assessed the impact of intravitreal injection of anti-VEGF on the microstructure of the retinal vasculature as captured by OCTA imaging over a period of 3 to 9 months. The IRB/Ethics Committee at the University of Illinois Office for the Protection of Research Subjects determined that patient informed consent was not required for this study and granted an IRB exemption. The research here adhered to the tenets of the Declaration of Helsinki. Eligible patient charts were reviewed in the electronic medical record. Patients seen and treated between December 31, 2017, and August 8, 2022, by a single provider at the University of Illinois at Chicago Retina Service were included in the study, provided they met each of the following inclusion critera: (1) aged $\geq$ 18 years; (2) had DME associated with NPDR or PDR; (3) received $\geq$ 1 intravitreal anti-VEGF injection during a 3 to 9 month period; and (4) OCTA imaging at the beginning and end of the treatment period. Patients were excluded if they met any of the following exclusion criteria: (1) poor quality imaging, which rendered measurement suboptimal (e.g., poor signal strength with high background, poor segmentation); (2) surgical intervention for DR before intravitreal injection (since pars plana vitrectomy may affect OCTA indices)[10]; or (3) other significant retinal or choroidal vascular pathology(ies) that could confound analysis (e.g., neovascular age-related macular degeneration, neovascular central serous chorioretinopathy, retinal vein occlusion, retinal artery occlusion, and/or ocular ischemic syndrome). OCTA images with signal strength of $\geq$ 6 were included for analysis. Those images with signal strength of $<$ 6 (17 images of the total of 82 images in the study) were reviewed manually and included if the vessels were successfully segmented from background after autothresholding. Conversion of Snellen BCVA to logarithm of the minimum angle of resolution BCVA was based on previously published guidelines.[11]

Data Analysis

OCT angiography images measuring 3 $\times$ 3 mm were captured with the AngioVue Retina imaging system (Optovue Inc). Per the manufacturer's output, the superficial retinal vasculature and deep retinal vasculature corresponded to vasculature between the inner limiting membrane and inner plexiform layer (IPL) vs. the IPL and outer plexiform layer, respectively. Image processing was performed with Adobe Illustrator (Adobe) and ImageJ Fiji suite (Fiji; https://imagej.net/software/fiji/). Importantly, the output for the OCTA images contained colored crosshairs, which were deleted to leave an empty space such that they were not included as blood vessels during analysis. The perfusion density was determined by first applying an autothreshold step using the Huang2 and IsoData protocols for the superficial and deep retinal vascular layers, respectively. Next, the histogram function was employed to determine the total number of pixels that correspond to blood vessels. Perfusion density was calculated as the number of pixels corresponding to blood vessels divided by the total number of pixels within the image and then multiplied by 100 to be expressed as a percentage. A similar ImageJ strategy has been discussed by Kumawat et al.[12] The vessel-length density (VLD) was determined with the same autothreshold step as described above. The skeletonize plugin was then applied to reduce blood vessels to a width of a single pixel.[13] Next, the histogram function was employed as above, and the VLD was calculated as the number of pixels corresponding to skeletonized blood vessels divided by the total number of pixels within the image and expressed as micrometers^{-1}. The average vessel diameter was determined by executing the vessel analysis plugin (https://imagej.net/plugins/vessel-analysis).[14,15] Here, the vessel caliber was measured in 4 predetermined areas (each 3.7 mm^2 in size): superotemporal, superonasal, inferotemporal, and inferonasal to the FAZ per eye. The measurements for each of the aforementioned areas in a single eye were averaged, and the average was then used as a single value for analysis. The FAZ was measured using the previously described (https://imagej.net/plugins/level-sets) and validated level sets plugin in ImageJ. As descried in Lin et al,[16] images were converted to an 8-bit type and the program was executed using the Active Contours method with curvature equaling 1.00 and convergence equaling 0.0100 parameters. One important difference is that because of deletion of the crosshairs during

image processing as above, the boundary of the FAZ measurement extended into the area of the vacated crosshairs. Therefore, before executing the program, we also smoothened the images 3 times and 5 times for the inner and deep retinal vasculature, respectively, to avoid this incorrect propagation. If the boundary of the FAZ continued to track in the area of the vacated crosshairs, noise was added to the corresponding area and vessels that were crossed by the crosshairs (and thus deleted) were manually filled in. This additional step was always performed for paired eyes. FAZ area, perimeter, and circularity (circularity = 4π[area/perimeter^2]) were then obtained with the measure and set measurements tools from the analyze menu (https://imagej.nih.gov/ij/docs/menus/analyze.html; see Fig S1, available at www.ophthalmologyscience.org).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism software (GraphPad) using paired and unpaired 2-tailed Student *t* tests, multiple paired *t* tests with correction for multiple comparisons using the Holm−Sidak method, and 2-way analysis of variance followed by the test for linear trend and Tukey's multiple comparisons test. A P value < 0.05 was considered significant.

Results

Study Population

A total of 740 eyes belonging to 386 patients were reviewed with a diagnosis of NPDR or PDR, of which 210 received $\geq$ 1 injection during the study period for DME. Of these, 20 eyes had received pars plana vitrectomy and 17 eyes had additional confounding pathology(ies) and thus were excluded from analysis. Ninety-six eyes had no available imaging because OCTA was only obtained if clinically warranted. Of the 77 eyes with available OCTA imaging, 13 had imaging with improper formatting (e.g., wrong imaging dimensions), 20 had poor signal strength with high background, and 3 had errors in segmentation. Importantly, patients were not excluded if they had already received laser photocoagulation or intravitreal anti-VEGFs; thus, several patients were not treatment naïve. Ultimately, there were 41 eyes of 30 patients (56% females, 40/41 were in type 2 diabetic patients), with a mean age of 58.83 ± 11.71 years that had a mean number of 2.8 ± 1.4 intravitreal injections (range 1−6) during the study period. The mean follow-up was 6.5 ± 1.7 months (range 3−9 months). Anti-VEGFs employed during the study included ranibizumab (58.8%), bevacizumab (29.8%), and aflibercept (11.4%). Of note, our study population included a large proportion of patients of Hispanic ethnicity (50.0% of patients corresponding to 56.0% of eyes). Other patient demographic and medical history data are available in Tables S1 and S2 (available at www.ophthalmologyscience.org).

Effect of Anti-VEGFs on Parameters of Retinal Vascular Architecture and Characteristics of the FAZ

We first performed a primary, pooled analysis comparing the mean of each OCTA parameter of all patients as a single group at the beginning and end of the study period. Eyes demonstrated statistical improvement in mean BCVA logarithm of the minimum angle of resolution score (00.27 ± 0.20 vs. 0.22 ± 0.20; $P = 0.04$) and CST (308.95 ± 73.10 μm vs. 289.54 ± 62.80 μm; $P = 0.02$) when comparing before with after anti-VEGF treatment data during the study period (Fig 2A, B). Primary OCTA analyses showed a reduction in the mean vessel diameter of the superficial (35.09 ± 0.96 μm vs. 34.70 ± 1.06 μm; $P = 0.02$) and deep retinal vasculatures (33.96 ± 0.86 μm vs. 33.70 ± 0.77 μm; $P = 0.03$) when comparing baseline data at the beginning of the study period to that from the end of the study period (Fig 2C). Mean perfusion density of the superficial (39.59 ± 4.70% vs. 39.75 ± 4.19%; $P = 0.76$) and deep (29.49 ± 4.79% vs. 29.42±4.14%; $P = 0.94$) retinal vasculatures, as well as VLD of the superficial (5.45 ± 0.78 μm^{-1} vs. 5.50±0.72 μm^{-1}; $P = 0.62$) and deep (4.72 ± 0.83 μm^{-1} vs. 4.76 ± 0.78 μm^{-1}; $P = 0.78$) retinal vasculatures, were not significantly affected by treatment with anti-VEGFs (Fig S3A−C, available at www.ophthalmologyscience.org).

Primary OCTA analyses also demonstrated an increase in the mean circularity of the FAZ within the superficial retinal vasculature (0.35 ± 0.09 vs. 0.38 ± 0.11; $P = 0.03$) after anti-VEGF treatment (Fig 2D). The mean circularity of the FAZ within the deep retinal vasculature (0.51 ± 0.13 vs. 0.52 ± 0.12; $P = 0.58$), area of the FAZ of the superficial (0.62 ± 0.39 mm^2 vs. 0.60 ± 0.31 mm^2; $P = 0.59$) and deep retinal vasculatures (0.40 ± 0.22 mm^2 vs. 0.41 ± 0.21 mm^2; $P = 0.31$), as well as perimeter of the FAZ of the superficial (4.87 ± 2.32 mm vs. 4.59 ± 1.61 mm; $P = 0.24$) and deep retinal vasculatures (3.15 ± 1.03 vs. 3.18 ± 1.05 mm; $P = 0.41$), were not statistically different after treatment compared with before treatment (Fig S3D−F).

Subgroup Analysis to Explore the Effect of Anti-VEGFs on OCTA Indices

We hypothesized that inclusion of nonresponders or poor responders to anti-VEGFs in our primary analysis may have masked the positive vascular effects of anti-VEGFs in patients with a robust response to treatment. More specifically, we hypothesized that improvement in BCVA and CST would correlate with positive changes to the retinal vascular architecture and FAZ shape characteristics. To test this hypothesis, we first separated eyes into 2 groups corresponding to eyes that demonstrated either: (1) visual improvement (n = 18), defined as a gain of $\geq$ 1 Snellen lines; or (2) visual stability (n = 17) or worsening (n = 6; total n = 23), defined as either no gain in a Snellen line or a loss of $\geq$ 1 more Snellen lines. When comparing these indices of posttreatment to baseline within each group, we found a significant decrease in the mean vessel caliber of the superficial retinal vasculature in the improved subgroup only (35.23 ± 1.04 μm vs. 34.43 ± 0.76 μm; $P = 0.003$; Fig 4A, left panel); whereas all other parameters were not statistically different pretreatment vs. posttreatment (Fig 4B−F, left panels; Fig S5, left panels, available at www.ophthalmologyscience.org). However, comparing the mean change (Δ) in OCTA parameters across the treatment period between

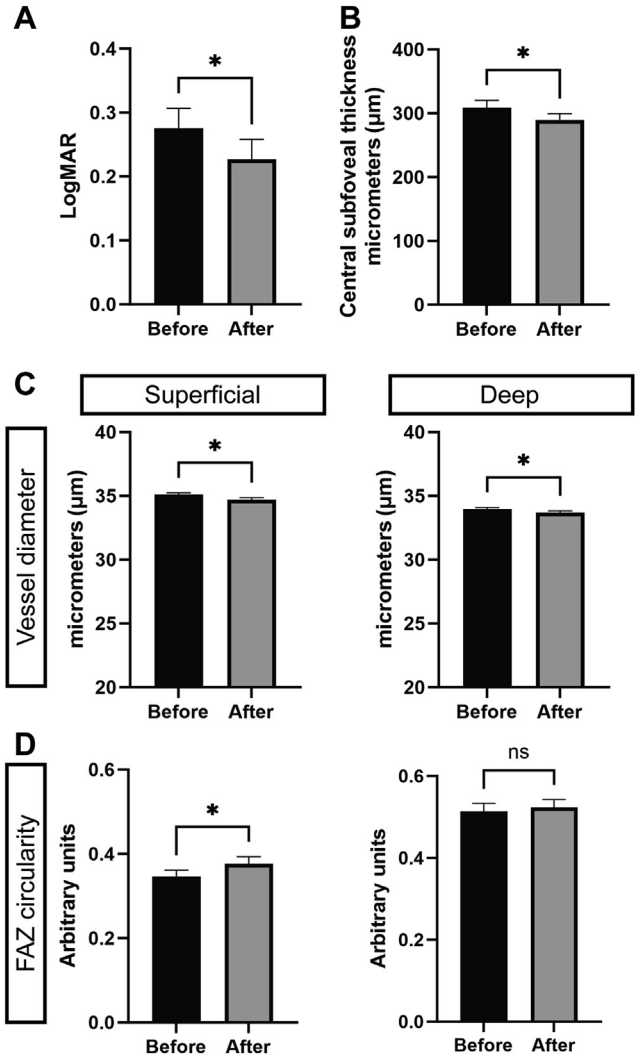

Figure 2. Treatment with anti-VEGFs resulted in reduced vessel diameters and increased foveal avascular zone (FAZ) circularity. Treatment with anti-VEGFs resulted in improved (**A**) best-corrected visual acuity (logarithm of the minimum angle of resolution [logMAR]) and (**B**) central subfield thickness (micrometers [µm]) in the cohort (n = 41). **C, D,** The left and right columns of graphs correspond to the mean of OCT angiography parameters of the superficial and deep retinal vasculatures, respectively. **C,** Mean vessel diameters, measured in µm and (**D**) FAZ circularity, measured in arbitrary units, were obtained with ImageJ. Vessel diameters in the superficial and deep retinal vasculatures were significantly reduced and FAZ circularity in the superficial retinal vasculature was significantly increased after treatment with anti-VEGFs. Black bar: before treatment. Gray bar: after treatment. Error bars: standard error of the mean. Paired 2-tailed *t* tests were employed to test statistical significance. *$P \leq 0.05$, ns = not significant.

groups demonstrated differential responses in a favorable direction for the improved group for vessel diameter, perfusion density, and VLD (Fig 4, right panels), but not for FAZ characteristics (Fig S5, right panels). Although significance was not reached, eyes belonging to the improved subgroup, as expected, showed the largest decrease in mean CST (331.33 ± 80.25 µm versus 295.28 ± 62.33 µm; $P = 0.07$; Fig S6A, available at www.ophthalmologyscience.org). Importantly, the observed effect was not due to differences in the number of injections received (Fig S6B).

We next created 4 subgroups (n = 10−11 per subgroup) based on the quartiles (Qs) of responses of the CST to anti-VEGF treatment (Q1 [high responders] through Q4 [non-responders or poor responders]) to explore the correlation between CST improvement and parameters of retinal vascular architecture and characteristics of the FAZ (Fig 7A). Although significance was not achieved, quartiles corresponding to eyes with decreasing CST (Q1−Q3) had a trend toward improved BCVA (Fig S6C). When comparing within each group, the highest responding group (Q1) achieved a significant increase in mean perfusion density (29.66 ± 4.37% vs. 32.82 ± 3.32%; $P = 0.007$) and VLD (4.88 ± 0.83 μm^{-1} vs. 5.45 ± 0.61 μm^{-1}; $P = 0.02$) of the deep retinal vasculature (Fig 7B, C; Fig S8, available at www.ophthalmologyscience.org). More interestingly, comparing the mean change (Δ) in OCTA parameters across the treatment period of each quartile using 1-way analysis of variance, followed by the test for linear trend and multiple comparisons tests, found a graded response for perfusion density of the deep retinal vasculature in a favorable direction for the high-responder (Q1) group (Fig 9A). Likewise, Δ FAZ area of the deep retinal vasculature and Δ FAZ circularity of the superficial retinal vasculature showed statistical improvement for the upper quartiles when compared with the lower quartiles (Fig 9B, D; Fig S10, available at www.ophthalmologyscience.org). Because quartiles did not differ significantly in the number of injections (although Q1 was noted to have on average more injections in our sample), the observed effect was again likely not due to differences in the number of injections received (Fig S6D).

Additional analysis demonstrated that eyes that were either naïve to intravitreal injection (Fig S11, available at www.ophthalmologyscience.org) or had received ≥ 3 intravitreal injections (Fig S12, available at www.ophthalmologyscience.org) had no significant difference for any of the measured parameters when comparing pretreatment and posttreatment. Taken together, eyes with a more robust response to anti-VEGFs in terms of an improvement in BCVA and a greater reduction in CST showed evidence for improved retinal vasculature architecture, whereas eyes with a neutral response to anti-VEGFs or progression of disease did not.

Discussion

The effect of anti-VEGFs in improving BCVA and CST in DME is well established[6,7]; however, their effect on reversing the characteristic vascular changes in DR remains an area of research that needs further attention. OCTA may be useful in detecting subclinical DR as well as stigmata of NPDR and PDR.[9] Other research groups have examined the response of OCTA indices to anti-VEGFs in hopes to inspire novel therapeutic endpoints and treatment paradigms; however, a consensus as to their overall net

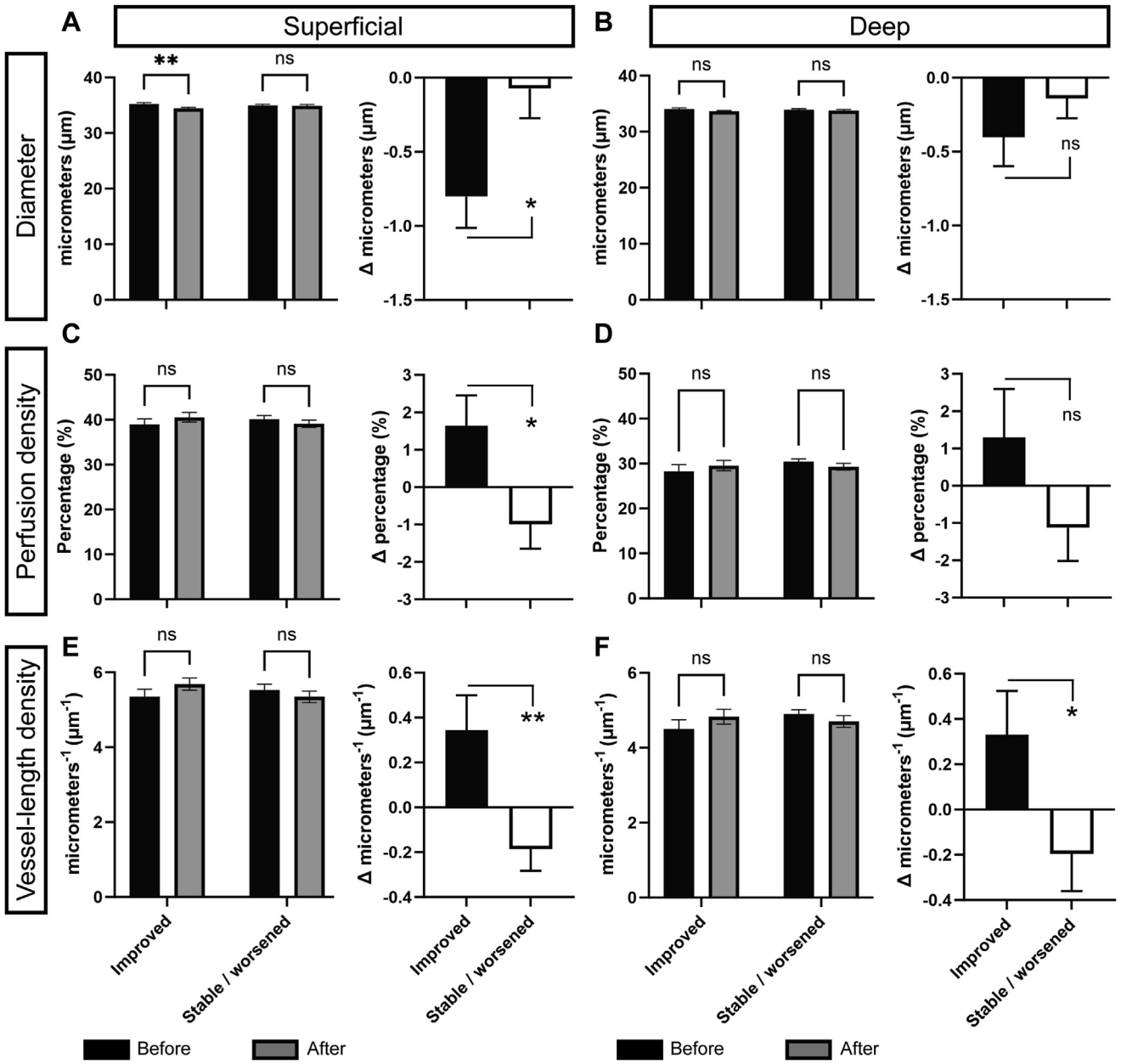

Figure 4. Eyes with best-corrected visual acuity gain exhibited improvement in OCT angiography (OCTA) parameters after anti-VEGF injection. Eyes were separated into 2 groups corresponding to eyes that demonstrated either: (1) visual improvement (n = 18); or (2) visual stability (n = 17) or worsening (n = 6; total n = 23). Each subfigure consists of 2 panels showing: (left) mean OCTA values before and after treatment for each group, and (right) mean change (Δ) in OCTA parameters across the treatment period (parameter after treatment − parameter before treatment) within each group. The 2 left and 2 right columns of graphs correspond to the superficial and deep retinal vasculatures, respectively. **A, B,** Average vessel diameters, measured in micrometers (μm). **C, D,** Perfusion density, measured in percentage (%)**. E, F,** Vessel-length density, measured in micrometers^{-1} (μm^{-1}). Error bars: standard error of the mean. Multiple paired *t* tests with correction for multiple comparisons using the Holm−Sidak method (left panels) and unpaired 1-tailed *t* tests (right panels) were employed to test statistical significance. *$P \leq 0.05$, **$P \leq 0.01$, ns = not significant.

effect remains controversial, because some studies found a positive effect whereas others found a neutral or negative effect.[8] We therefore performed a retrospective study assessing the impact of intravitreal injection of anti-VEGFs on the microstructure of the retinal vasculature as captured by OCTA imaging in our DME patients who received ≥ 1 anti-VEGF injection over a period of 3 to 9 months. In our primary, pooled analysis we found that the mean vessel diameter decreased in both the superficial and deep retinal vasculature after exposure to anti-VEGFs. Furthermore, our data suggested that the circularity of the FAZ in the superficial retinal vasculature increases after treatment with anti-VEGF agents. It is posited that loss of circularity of the FAZ corresponds with capillary drop out at

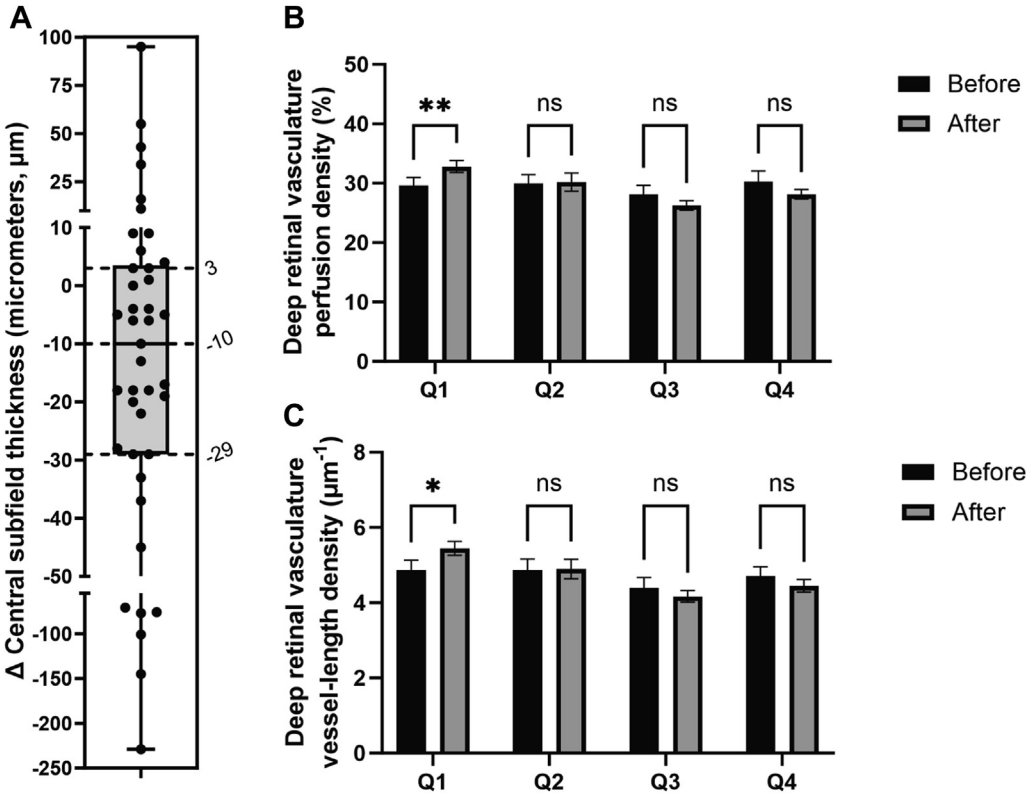

Figure 7. OCT angiography measurements differed based on the response of the central subfield thickness (CST) to treatment with anti-VEGFs. **A,** Box-and-whisker plot of the change in CST (posttreatment and pretreatment) for each patient. Eyes were separated into 4 groups based on the change (**Δ**) in CST (CST after and CST before) after administration of anti-VEGF medications. The groups were designated based on the quartiles of responses, where −29, −10 (median), and 3 µm define the quartiles. 11, 10, 10, and 10 eyes belonged to Q1 (high responders), Q2, Q3 and Q4 (nonresponders or poor responders), respectively. **B,** Perfusion density, measured in percentage (%) and (**C**) vessel-length density, measured in μm^{-1}, of the deep retinal vasculature were obtained with ImageJ and were significantly increased in Q1 only after treatment with anti-VEGFs. Black bar: before treatment. Gray bar: after treatment. Error bars: standard error of the mean. Multiple paired *t* tests with correction for multiple comparisons using the Holm−Sidak was employed to test statistical significance. $*P \leq 0.05$, $**P \leq 0.01$, ns = not significant. Q = quartile.

the margin of the FAZ.[9] Thus, an increase in circularity may correspond with recovery of perfusion at the edges of the fovea, suggesting a positive effect for anti-VEGFs on retinal vascular health.

Retinal vessel caliber has been discussed in the literature as a biomarker for DR disease burden.[17] Literature evidence suggests that increased vessel caliber, both of arterioles and venules, may predict the incidence or progression of DR in both type 1 DM and type 2 DM.[17–22] Moreover, Kim et al[23] observed a positive correlation between vessel diameter measured with OCTA and severity of DR. This dilation has been hypothesized to be: (1) a response within the retina to maintain capillary perfusion and; and (2) a manifestation of impaired vessel autoregulation that ultimately renders the retinal vasculature more susceptible to damage mediated by blood pressure fluctuations and less capable of titrating perfusion to metabolic demand.[24] In addition, it has previously been observed that laser photocoagulation reduces the diameter of larger vessels in DR[24,25] and focal/grid laser reduces the diameter of macular arterioles and venules posttreatment.[26,27] The findings in Hsieh et al[28] agree with our study in that vessel caliber in the macula is increased in the setting of DME when compared with controls and decreases in response to ranibizumab injection. To this end, a decrease in the vessel diameter in a 3 × 3 mm grid centered in the macula after treatment with anti-VEGFs in our study suggests that this class of medications may also improve vascular health in addition to BCVA and CST.

Of note, the association between vessel caliber and DR severity may modulate with the area in which the vessels are measured, as vessels within the macula and periphery are thought to be more susceptible to vasodilation vs. collapse and occlusion, respectively.[24,29–31] Further advancement and clinical adoption of widefield OCTA should enhance our understanding of the regional differences of vessel diameter in DR.[31] In addition, the susceptibility of the retinal vasculature to diabetic challenge may vary with the depth of the vascular plexus under study.[29,32] This was also observed in our study, and improvements in imaging resolution should facilitate exploration of this phenomenon.

Our subgroup analysis suggests that patients with improvement in BCVA and CST differentially experience beneficial changes in OCTA indices when compared with

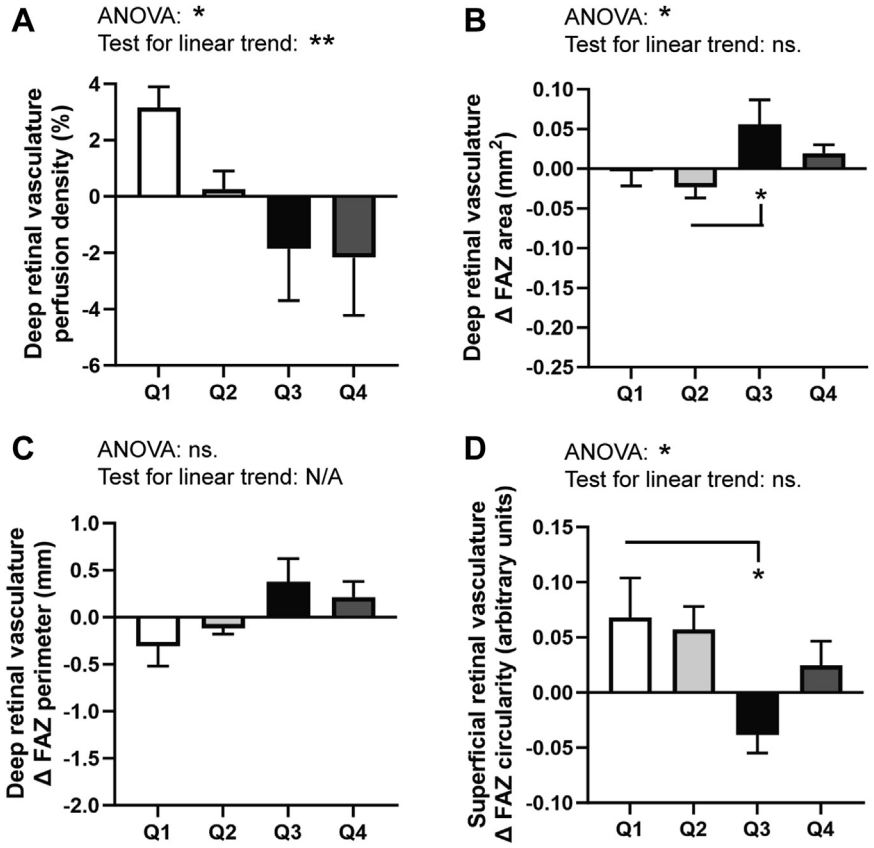

Figure 9. A favorable change in OCT angiography (OCTA) parameters occurred in eyes with a reduction in central subfield thickness (CST) after treatment with anti-VEGFs. Eyes were separated into 4 groups based on the change in CST (CST after vs. CST before) after administration of anti-VEGF medications. The groups were designated based on the quartiles of responses, where −29, −10 (median), and 3 μm define the quartiles (see Fig 7A). 11, 10, 10, and 10 eyes belonged to Q1 (high responders), Q2, Q3 and Q4 (nonresponders or poor responders), respectively. Each panel represents the mean change (Δ) in OCTA parameters across the treatment period (parameter after treatment − parameter before treatment) within individual quartiles. **A,** Perfusion density, measured in percentage (%)**. B,** Foveal avascular zone (FAZ) area, measured in mm^2 **C,** FAZ perimeter, measured in mm, of the deep retinal vasculature. **D,** FAZ circularity, measured in arbitrary units, of the superficial retinal vasculature. One-way analysis of variance (ANOVA) followed by the test for linear trend and Tukey's multiple comparisons test were performed for statistical analysis. *$P \leq 0.05$; **$P \leq 0.01$; ns = not significant; N/A = not applicable. Q = quartile.

poor responders or nonresponders Furthermore, we found that analysis of patients naïve to intravitreal injection or who have received ≥ 3 intravitreal injections was less correlated with improvement in OCTA measurements. Interestingly, Lee et al[33] also observed a differential effect of anti-VEGFs on OCTA indices when comparing responders vs. non-responders based on a reduction of > 50 μm of CST.[9] This observation is important since landmark clinical trials suggest that up to 40% of patients have chronic DME that is refractory to anti-VEGF monotherapy.[34,35] To this end, strategies to circumvent poor response to anti-VEGF therapy include intravitreal delivery of steroids,[36] trial of alternative anti-VEGFs,[35] and treatment with the angiopoietin-Tie2 pathway bispecific antibody faricimab.[37] Whether OCTA indices improve in refractory DME after a change in management remains yet to be elucidated.

An important strength of our study was that the population under analysis represents typical patients that may be encountered in a major city at a state funded hospital, in particular patients with more tenuous access to the healthcare system, poor blood glucose control, and imperfect follow-up. Of note, Hispanic or Latino patients constitute approximately 50% of research subjects; thus, our results should be carefully applied in the clinical setting. Although many studies have examined the effect of anti-VEGFs on OCTA indices,[8] we believe that our subgroup analyses showing a differential positive effect for anti-VEGF responders offer unique insights to the existing literature. To this end, we recommend that patients with a robust response to anti-VEGFs should be analyzed separately from those with a poor response to accurately understand the effects of anti-VEGFs on the retinal vasculature in DR. Finally, our study population was also characterized by a relatively balanced proportion of disease severity and included the main anti-VEGFs that are encountered in clinical practice: ranibizumab, bevacizumab, and aflibercept. Important considerations for data interpretation are that some study patients had already received anti-VEGFs and/or focal laser photocoagulation or panretinal photocoagulation before the study; however,

we did perform a subgroup analysis of those patients naïve to intravitreal injection.

A limitation of our study was its retrospective nature when compared with the robust, prospective nature of randomized controlled trials. Our relatively small sample size reflects the inherent disadvantages of OCTA as an imaging modality that is sensitive to artifacts, low quality signaling strength, and segmentation errors.[9] Indeed, our study excluded > 33% of patients because of this inherent disadvantage of OCTA, and, in our hands, measurements of FAZ area and perimeter in the superficial retinal vasculature were particularly sensitive to perturbations in image quality. The sensitivity of OCTA to artifacts is compounded by its difficulty in interpretation. To this point, we measured an increase in perfusion density and VLD in patients that exhibited a positive response to treatment with anti-VEGFs. Although this increase may reflect an actual improvement in retinal perfusion, alternative explanations may include: (1) unmasking (or improved signal strength) of poorly visualized vasculature as cystic fluid wanes or (2) closer vasculature packing by virtue of the disappearance of cystic fluid. Future studies would be required to elucidate the etiology of this observation. Another limitation of our study involves analysis of vessels en masse rather than differentiating the effect of anti-VEGFs on arterioles, capillaries, and venules separately. Artificial intelligence algorithms are now being created to generate artery−vein segmentation such that more detailed OCTA analysis can be executed in future studies by separating the differential effect of DR and its treatment on arterioles, venules, and capillaries.[38,39] Axial length discrepancies can affect OCTA parameters, and < 10% of our study eyes had high myopia or hyperopia.[40] Future studies should analyze these patient populations separately when larger study imaging databases are available. Of note, many OCTA manufacturers do not include advanced imaging analysis software, and, thus, research studies rely on diverse analysis algorithms. Our study involved a semiautomated approach based on already published ImageJ functions that are suitable for purposes of research but are cumbersome for clinical application. Furthermore, thresholding techniques for OCTA analysis should be carefully considered when applying the results of one study to another.[12] These shortcomings of OCTA as an imaging modality act as a barrier to its widespread inclusion into clinical practice.

Our data suggest that intravitreal injection of anti-VEGF agents to treat DME may reduce the diameter of the macular vessels of both the superficial and deep retinal vessels as well as increase the circularity of the FAZ within the superficial retinal vasculature over a period of 3 to 9 months. The changes we observed may represent a manifestation of improved vasculature health after treatment with anti-VEGF medications and were most apparent in patients with improvement in BCVA and a robust reduction in CST. Future studies should involve a long-term prospective study population that is naïve to treatment to better examine the effects of anti-VEGFs on retinal vascular structure, with careful attention paid to differences in responders vs. nonresponders. These studies would be further enhanced with inclusion of widefield OCTA[31] and artery−vein segmentation[38,39] such that the differential effects of retinal location and vessel type can be established, with the ultimate goal of establishing an efficient and reproducible analysis protocol for clinical application in the management of DR.

Footnotes and Disclosures

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Disclosures:

All authors have completed and submitted the ICMJE disclosures form.

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HUMAN SUBJECTS: Human subjects were included in this study. The institutional review board/ethics committee at the University of Illinois Office for the Protection of Research Subjects deemed patient informed consent was not required for this study and granted an institutional review board exemption. The research here adhered to the tenets of the Declaration of Helsinki.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Massengill, Lim

Data collection: Massengill, Cubillos, Sethi

Analysis and interpretation: Massengill, Cubillos, Sheth, Sethi, Lim

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Overall responsibility: Massengill, Lim

Abbreviations and Acronyms:
BCVA = best-corrected visual acuity; **CST** = central subfield thickness; **DM** = diabetes mellitus; **DME** = diabetic macular edema; **DR** = diabetic retinopathy; **FA** = fluorescein angiography; **FAZ** = foveal avascular zone; **IPL** = inner plexiform layer; **MA** = microaneurysm; **NPDR** = nonproliferative diabetic retinopathy; **NV** = neovascularization; **OCTA** = OCT angiography; **OPL** = outer plexiform layer; **PDR** = proliferative diabetic retinopathy; **Q** = quartile; **VLD** = vessel-length density.

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Diabetic macular edema, Diabetic retinopathy, Anti-VEGF, Optical coherence tomography angiography, Image analysis.

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