Optical Coherence Tomography Angiography Analysis of the Foveal Avascular Zone and Macular Vessel Density After Anti-VEGF Therapy in Eyes With Diabetic Macular Edema and Retinal Vein Occlusion

Khalil Ghasemi Falavarjani,1,2,3 Nicholas A. Iafe,1,4 Jean-Pierre Hubschman,1,4 Irena Tsui,1,4 Srinivas R. Sadda,1,2 and David Sarraf1,4,5

1Department of Ophthalmology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States
2Doheny Eye Institute, University of California Los Angeles, Los Angeles, California, United States
3Eye Research Center, Rassoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
4Stein Eye Institute, University of California Los Angeles, Los Angeles, California, United States
5Greater Los Angeles VA Healthcare Center, Los Angeles, California, United States

Correspondence: David Sarraf, Stein Eye Institute, UCLA, 100 Stein Plaza, Los Angeles, CA 90095, USA; sarraf@jsei.ucla.edu.
Submitted: August 22, 2016
Accepted: October 24, 2016

PURPOSE. To evaluate the changes in foveal avascular zone (FAZ) area and the retinal capillary density after a single intravitreal anti-VEGF injection for macular edema secondary to diabetic retinopathy or retinal vein occlusion.

METHODS. In this prospective noncomparative case series, 18 eyes of 15 patients with diabetic macular edema (13 eyes) or macular edema secondary to central retinal vein occlusion (5 eyes) were included. Optical coherence tomography angiography (OCTA) images were obtained, and retinal capillary vessel density and FAZ area were measured in the foveal and parafoveal regions at the level of the superficial (SCP) and deep retinal capillary plexus (DCP) before and at the first visit after intravitreal injection.

RESULTS. The mean interval between baseline and follow up OCTA was 32.5 ± 9.4 (range, 21-50) days. Foveal and parafoveal vessel density in the SCP and DCP were not significantly different before and after intravitreal injection (all P > 0.1), nor was FAZ area (P = 0.48 and P = 0.42, respectively). No significant difference was found between eyes with diabetic macular edema and those with retinal vein occlusion with respect to the mean change of vessel density and FAZ area (all P > 0.05).

CONCLUSIONS. In this pilot study, retinal capillary density and FAZ area remained statistically unchanged in the short-term after a single intravitreal injection of an anti-VEGF agent.

Keywords: aflibercept, anti-VEGF, bevacizumab, diabetic retinopathy, foveal avascular zone, macular edema, optical coherence tomography angiography, retinal vein occlusion, ranibizumab, vessel density

Intravitreal anti-VEGF therapy is the standard of care for diabetic macular edema (DME) and cystoid macular edema (CME) associated with retinal vein occlusion (RVO). Several studies have shown that anti-VEGF injections significantly improve vision and reduce fluid in DME and RVO.1,2 The macular capillary system in eyes with diabetic retinopathy (DR) and RVO are susceptible to closure, and VEGF inhibition has been shown to induce retinal arteriolar vasoconstriction.3-5 The results of studies reporting the effect of anti-VEGF therapy on retinal capillaries are conflicting. While some studies have reported that frequent intravitreal anti-VEGF injections can slow the progression of retinal capillary closure in patients with DME and CME secondary to RVO, others have noted progressive capillary closure.6-10 The most important drawback of these studies is the use of fluorescein angiography (FA) to quantify ischemia. The assessment of nonperfusion by FA can be limited due to masking by leakage and hemorrhage and the inability to accurately assess the deep retinal capillary plexus.11

Optical coherence tomography angiography (OCTA) is a noninvasive modality for vascular mapping without dye injection and has the advantage of providing high speed, 3-dimensional imaging of the retinal and choroidal vasculature. Several studies have illustrated the proficiency of OCTA to identify and quantify retinal capillary density in different ocular diseases including AMD, DR, and RVO.12-14 The aim of this study was to evaluate the short-term changes in the fovea avascular zone (FAZ) and macular vessel density in eyes with macular edema due to DR or RVO after a single intravitreal injection of anti-VEGF therapy.

METHODS

This study was a prospective noncomparative case series. Institutional review board approval from the University of California at Los Angeles (Los Angeles, CA, USA) was obtained and informed consents were obtained from each patient. The

I 489.7

ISSN: 1552-5783

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.
study adhered to the tenets of the declaration of Helsinki and followed the Health Insurance Portability and Accountability Act. From February to May 2016, consecutive patients with macular edema secondary to diabetic retinopathy or retinal vein occlusion who were candidates for intravitreal anti-VEGF injection therapy were identified. Patents with uveitis, uncontrolled glaucoma, vitreous hemorrhage, vitreomacular traction, and any evidence of fibrovascular proliferation in the macular area were excluded. Also, eyes with significant media opacity affecting the quality of the images were excluded. History of previous anti-VEGF injections was not a criterion for exclusion.

Optical coherence tomography angiography was performed using an RTVue XR 100 Avanti instrument (Optovue, Inc., Fremont, CA, USA). For each eye, a 3 × 3-mm scan centered on the fovea was acquired. Automated OCT segmentation was performed using the Angio-Vue module. Optical coherence tomography angiography images were taken at baseline (1–7 days before injections) and at first visit after injection (21–50 days after injections). The superficial capillary plexus (SCP) en face image was segmented with an inner boundary at 3 μm beneath the internal limiting membrane and an outer boundary set at 15 μm beneath the inner plexiform layer, whereas the deep capillary plexus (DCP) en face image was segmented with an inner boundary 15 μm beneath the inner plexiform layer and an outer boundary at 70 μm beneath the inner plexiform layer. In addition, a thick "inner retinal slab" was manually customized by selecting an inner boundary at 3 μm beneath the internal limiting membrane and an outer boundary set at 70 μm or more beneath the inner plexiform layer. This "inner retinal slab" was performed to capture all flow signals displaced posteriorly by intraretinal cystoid spaces. The vascular density of the SCP, DCP, and the inner retinal slab in the fovea and parafovea, automatically generated by the instrument, was recorded. Vessel density was calculated as the proportion of the measured area occupied by blood vessels with flow, defined as pixels having decorrelation values above the threshold level. The fovea was defined as the area within the central 1-mm ring of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid (Fig. 1A). Parafovea was considered as the area between the central 1- and the 3-mm ring of the ETDRS grid. The FAZ area in the SCP and DCP was independently graded by two experienced investigators (KGF and NI, Figs. 1B, 1C). The graders manually outlined the inner border of foveal capillaries in the FAZ using ImageJ software (http://image.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The total number of pixels in the FAZ area was converted to millimeters squared for analysis. Eyes with significant image distortion or artifact preventing accurate measurement of the FAZ and vessel density were excluded.

Statistical analysis was performed using a SPSS software (version 16; SPSS, Inc., Chicago, IL, USA). Paired t-test was used for quantitative data analysis before and after injections. Mann-Whitney U test was used for comparison of the mean changes between the two groups (DME and RVO). Agreement between the graders for quantitative analysis was analyzed using intraclass correlation coefficient (ICC) statistics. A P value less than 0.05 was considered significant.

RESULTS

Eighteen eyes of 15 patients including 9 females and 6 males with a mean age of 69.2 ± 11.6 years were included. The indication for intravitreal injection was DME in 13 eyes and macular edema due to central retinal vein occlusion (CRVO) in five eyes. In five eyes, cystoid spaces were not present in the central subfield area. The injected anti-VEGF drug was bevacizumab in 14 eyes, aflibercept in three eyes, and ranibizumab in one eye. The mean interval between baseline and follow up OCTA was 32.5 ± 9.4 (range, 21–50) days.

TABLE. Vessel Density and FAZ Area Before and After Intravitreal Anti-VEGF Injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Injection</th>
<th>After Injection</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foveal vessel density in SCP (%)</td>
<td>25.8 ± 7.8</td>
<td>25.2 ± 5.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Foveal vessel density in DCP (%)</td>
<td>27.3 ± 5.6</td>
<td>25.2 ± 5.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Parafoveal vessel density in SCP (%)</td>
<td>45.6 ± 5.6</td>
<td>45.6 ± 6.08</td>
<td>0.95</td>
</tr>
<tr>
<td>Parafoveal vessel density in DCP (%)</td>
<td>50.4 ± 5.9</td>
<td>50.5 ± 5.3</td>
<td>0.96</td>
</tr>
<tr>
<td>FAZ area in SCP (mm²)</td>
<td>0.46 ± 0.38</td>
<td>0.48 ± 0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>FAZ area in DCP (mm²)</td>
<td>1.06 ± 0.51</td>
<td>1.09 ± 0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>Central subfield thickness (μm)</td>
<td>350.0 ± 127.3</td>
<td>280.6 ± 56.4</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Paired t-test.
FIGURE 2. Optical coherence tomography angiography of a patient with DME before (A–I) and 29 days after intravitreal injection of bevacizumab (J–R). Optical coherence tomography angiography images with segmentation to capture the superficial capillary plexus (A, J), deep capillary plexus (B, K) and all retinal capillary plexi (thick inner retinal slab) (C, L) with corresponding structural OCT (D–F, M–O) and vessel density maps (G–I, P–R) that show areas of decreased (white ellipse) and increased vessel density (white dashed ellipse) after injection. The changes in vessel density may represent alterations in the flow in the macular capillaries.
Central subfield thickness was 330.0 ± 127.3 μm before and 280.6 ± 56.4 μm after injections (P = 0.07).

The Table shows retinal capillary density and FAZ area and retinal thickness measurements before and after intravitreal injection. Foveal and parafoveal vessel density in the SCP, DCP, and inner retina were statistically unchanged before and after intravitreal injections (all P > 0.1). Mean change in foveal vessel density was −1.2 ± 5.7%, −2.5 ± 5.7%, and −2.1 ± 5.8% in SCP, DCP, and inner retinal slab, respectively. Mean change in parafoveal vessel density was −0.06 ± 5.5%, +0.05 ± 4.3%, and −1.7 ± 6.1% in the SCP, DCP, and inner retinal slab, respectively. FAZ area in SCP and DCP was not significantly different before and after intravitreal injections (P = 0.48 and P = 0.42, respectively). Figure 2 shows pre- and postinjection OCTA of a patient with DME. Mean change in FAZ area was +0.01 ± 0.08 and +0.02 ± 0.13 mm² in the SCP and DCP, respectively. No significant difference was found between the two groups (DME versus CRVO) in the mean change of vessel density and FAZ area (all P > 0.05).

Mean visual acuity was 0.32 ± 0.31 and 0.29 ± 0.31 LogMAR before and after intravitreal injection, respectively (P = 0.45).

Repeatability of the measurements between the graders for the pre- and post-FAZ area was excellent for the SCP (both ICC = 0.98), and good for DCP (preinjection ICC = 0.82, postinjection ICC = 0.83).

**Discussion**

In this study, mean retinal capillary density at the level of the SCP and DCP, as measured by OCTA, remained stable after a single intravitreal anti-VEGF injection. This is in accordance with previous studies that illustrated that anti-VEGF therapy did not worsen capillary nonperfusion. Campochiaro et al. measured the area of retinal capillary nonperfusion using FA analysis of patients with macular edema secondary to retinal vein occlusion after intravitreal ranibizumab injections. They demonstrated that the percentage of patients with no evidence of posterior retinal capillary nonperfusion was similar between the comparative groups at baseline, but at month 6, eyes treated with ranibizumab have less retinal nonperfusion. Retinal capillary nonperfusion was also evaluated in patients with DME after intravitreal ranibizumab injection. Although both ranibizumab and sham groups developed nonperfusion over the study period, it occurred at a faster rate in the sham group. The authors concluded that monthly intravitreal ranibizumab therapy can slow, but not completely prevent, retinal capillary closure in patients with DME.

In eyes with CME, the deep retinal capillary system may be displaced posteriorly and laterally by the physical effect of the cystoid spaces. Consequently, the improvement of vessel density after resolution of CME may simply reflect a resolution of segmentation artifact and not an improvement in the capillary flow. We selected a thick inner retinal slab to account for this artifact and illustrated that the capillary density changes in this slab reflect the changes in the SCP and DCP slabs. In a pilot study, Spaidé used volume-rendering to assess the retinal vasculature in CME secondary to CRVO. He showed that CME was associated with topographically colocalizing flow voids in the deep retinal capillary plexus and anti-VEGF treatment resulted in resolution of edema but no change in flow patterns in either the superficial or deep layers. However, he did not report quantitative measurements for comparison.

Previous studies have shown that FAZ area is an indicator of ischemia in diabetic retinopathy. Several studies have reported progressive enlargement of FAZ measured in FA images after anti-VEGF therapy in CME secondary to DR or RVO. Other studies however, could not confirm this finding. In this study, we measured FAZ area in the SCP and DCP and showed that the area remained statistically unchanged after intravitreal injection. Although cystoid spaces may affect the FAZ border at the DCP by displacing vessels, the FAZ in the SCP should not be affected. Therefore, the FAZ area in the SCP may be a more reliable index. Our findings are in line with those from Michaelides et al. who found that the FAZ area measured in FA images was statistically the same after intravitreal injection of bevacizumab. Alternatively, small sample size may account for the nonsignificant findings.

Several types of artifacts may affect the OCTA measurements, especially in eyes with ocular pathology. Unfortunately, many of these artifacts cannot be easily corrected with the current OCTA technology. Although we excluded images with severe distortion, the possibility of a measurement error due to other forms of artifacts including segmentation, motion, and projection artifact should be considered.

Our study had several limitations. The sample size was small and the number of eyes in each group was not enough to draw a definite conclusion. Previous reports have suggested that the effect of anti-VEGF treatment may differ based on the extent of capillary nonperfusion. Our study was underpowered to detect a correlation between the ischemic area and changes in vessel density after anti-VEGF therapy. Also, the capillary response to each anti-VEGF agent may differ; this study included eyes injected with one of the three major anti-VEGF agents. Flow reduction was only analyzed after one injection. This may not be enough to detect changes in the vascular density or FAZ that may develop with a greater number of injections or more chronic therapy. In addition, we evaluated mean retinal capillary density after intravitreal injections. This may not detect focal areas of increased or decreased vessel density. In conclusion, our study showed that vessel density and FAZ area remained stable after a single intravitreal anti-VEGF injection. Further studies with larger sample size, longer follow-up, and more injections are needed to confirm our results.

**Acknowledgments**

Disclosure: K. Ghasemi Falavarjani, None; N.A. Iafe, None; J.P. Hubschman, Alcon (C, F), Allergan (S), I. Tsui, None; S.R. Sadda, Optos (C, F), Genentech (C, F), Allergan (C, F), Carl Zeiss Meditec (F); D. Sarraf, Allergan (F), Genentech (C, F), Regeneron (F), Optovue (C, F, S)

**References**


