

## REVIEW ARTICLE

# Review on Recent Trials Evaluating the Effect of Intravitreal Injections of Anti-VEGF Agents on the Macular Perfusion of Diabetic Patients with Diabetic Macular Edema



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**Abstract: Background:** Diabetic macular edema (DME) is a major cause of vision loss in diabetics worldwide. Anti-vascular endothelial growth factor (anti-VEGF) agents have become the mainstay of treatment of vision loss due to DME. Long-term effects of these agents on the macular perfusion (MP) are a current concern.

**Objective:** To review recently published studies that evaluated the effect of intravitreal injection of anti-VEGF agents on the MP of diabetics with DME.

**Methods:** Different databases were searched including PubMed, Medline, Ovid, Science Direct, and Google Scholar for relevant studies published between 2010 and 2019. All studies found were compared regarding methodology and results and included in this review. Some studies relating to retinal perfusion in general and not strictly MP were also included for comprehensiveness.

**Results:** Several studies utilizing different anti-VEGF agents were identified. All the large randomized controlled clinical trials identified utilized primarily fluorescein angiography (FA) and human graders and found generally no worsening of MP associated with anti-VEGF agents use in diabetic patients with DME. Some of these studies, however, depended on post-hoc analysis. Several more recent, but smaller case series, have utilized the relatively new and non-invasive optical coherence tomography angiography (OCTA) in this evaluation and found more conflicting results.

**Conclusion:** The large clinical trials recently performed depended mainly on FA in the analysis of MP changes following injections and generally found no worsening of MP. More recently, smaller case series have utilized OCTA in this analysis, yielding more conflicting results. Large randomized controlled trials using OCTA are thus needed.

**Keyword:** Anti-VEGF agents, diabetic macular edema, fluorescein angiography, macular perfusion, optical coherence tomography angiography, clinical trials.

## 1. INTRODUCTION

The prevalence of diabetes mellitus (DM) has progressively increased recently worldwide and 430 million people around the globe are predicted to have DM by the year 2030 [1]. In the United States, an estimated 29% of adult patients with DM have diabetic retinopathy (DR) and 3% have diabetic macular edema (DME) [2], with DM being the leading cause of blindness in people between the ages of 20 and 74 years [3].

DME is an increase in the thickness of the central macular region of the retina due to fluid accumulation that is frequently associated with loss of vision [4]. In fact, it is the

commonest cause of vision loss in diabetics and affects an estimated 21 million people worldwide [5]. Treatment of macular edema due to various retinal conditions, including DME, currently depends on the repeated intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents [6-9]. This may be due to the fact that VEGF is central to the pathogenesis of many of these retinal conditions including DR and DME [10].

VEGF-A is a member of the VEGF family that is the most implicated in the pathogenesis of various retinal diseases due to its pro-angiogenic and vaso-permeability effects that result in disruption of the blood-retinal barriers [6, 11]. It has five different isoforms, and most of the currently available anti-VEGF agents block all these isoforms [6]. VEGF also has several physiologic functions including regulation of normal vasculogenesis and angiogenesis [12], acting as a survival factor for retinal vessels during their development [11], maintenance of the choriocapillaris and consequently

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**Table 1. Studies analyzing macular perfusion changes using fluorescein angiography in DME.**

Study	Number of Eyes	Study Design	Imaging Modality	Agent Used	Outcome Assessed	Results	References
BOLT study	40 in anti-VEGF group	Prospective controlled	FA using human graders	Bevacizumab (2 injections) vs laser	Change in FAZ and perifoveal capillaries	No worsening of ischemia	[20-22]
Chung <i>et al.</i>	59	Retrospective study	FA using human graders	Bevacizumab (3 injections)	Effect of severity of baseline ischemia on final VA	50% of ischemic group lost > 1 ETDRS line	[23]
Campochiaro <i>et al.</i>	666 in all groups	Retrospective controlled	FA using human graders	Ranibizumab 0.2 and 0.5 mg vs sham	Change of posterior retinal nonperfusion	Less worsening with anti-VEGF	[24]
Wyckoff <i>et al.</i>	466 in all groups	Retrospective controlled	FA using human graders	Aflibercept (2 dosing regimens) vs laser	Change of retinal non-perfusion	Less worsening with anti-VEGF	[26]
Feucht <i>et al.</i>	28	Retrospective case series	FA using human	Bevacizumab (1 injection)	Change of FAZ area	FAZ area increased by 19.7%	[27]
Erol <i>et al.</i>	29	Prospective case series	FA	Bevacizumab (3 injections)	Change of FAZ area	FAZ increased by 13%	[28]

FA, fluorescein angiography; FAZ, foveal avascular zone; VEGF, vascular endothelial growth factor.

the photoreceptors [13], and possessing a neuroprotective role that leads to the reduction of neuronal apoptosis [14]. This raises concerns regarding the long-term suppression of intraocular VEGF levels using intravitreally injected anti-VEGF agents that is frequently needed for the long-term treatment of retinal diseases [15, 16].

Several animal studies have shown possible harmful cellular effects following VEGF inhibition. In neonatal mice for example, anti-VEGF injections were found to inhibit developing retinal capillary plexuses [17], while in adult mice, anti-VEGF injections resulted in regression of normal airway blood vessels [18]. In another study of adult transgenic mice with RPE-specific VEGF-A inactivation, there was complete ablation of the choriocapillaris with progressive dysfunction of the cone photoreceptors [13]. This may suggest possible harmful effects on human eyes following repeated intravitreal injections of anti-VEGF agents that may be more exaggerated in diabetic patients with already structurally and functionally compromised vasculature [15, 16].

Because of these concerns, the effect of repeated intravitreal injections of different anti-VEGF agents on the macular perfusion of diabetic patients with DME has been assessed in several recent studies using various imaging modalities. The aim of this review is to summarize the results of these studies.

## 2. METHODS

A systematic literature search was performed using PubMed, Medline, Ovid, Science Direct, and Google Scholar for articles published between January 2010 and December 2019. We also searched Clinicaltrials.gov for relevant and recently completed but unpublished studies with posted results. Keywords searched included: anti-VEGF, VEGF inhibition, bevacizumab, ranibizumab, aflibercept, macular perfusion, retinal ischemia, macular ischemia, vascular density, retinal perfusion, foveal avascular zone, capillary reperfusion, capillary non-perfusion, diabetic retinopathy, optical coherence tomography angiography, fluorescein angiography, and diabetic macular edema. The reference lists of iden-

tified papers were examined to find additional relevant articles. Only peer-reviewed articles published in English language were included with no restrictions applied to study type. All studies found were compared regarding methodology and results and included in this review. Some studies relating to retinal perfusion in general and not strictly macular perfusion were also included for comprehensiveness.

## 3. RESULTS

### 3.1. Analysis of Macular Perfusion Changes Following Intravitreal Anti-VEGF Injections using Fluorescein Angiography (Table 1)

Several prospective studies assessed the effect of intravitreal injections of anti-VEGF agents on the macular perfusion of diabetic patients with DME using fluorescein angiography (FA), however, some of these studies depended on a post-hoc analysis. Fluorescein angiography imaging depends on intravenous injection of a fluorescent dye followed by serial imaging of the retinal vasculature using a blue light. This blue light is absorbed by the fluorescent dye that is present in the retinal vessels and is re-emitted as another light of longer wavelength that allows visualization of the retinal vasculature and areas of capillary loss on a background of diffuse choroidal fluorescence (Fig. 1A and B). The extent of this capillary loss can be graded and forms the basis of fluorescein angiography use in the assessment of the macular perfusion status of diabetics in a qualitative manner [19].

The BOLT study was a prospective study that compared the efficacy of 6 weekly intravitreal bevacizumab injections to 4 monthly modified Early Treatment Diabetic Retinopathy Study (ETDRS) macular laser treatment and showed more improvement of vision with bevacizumab injections compared to macular laser at 2 years with no evidence of worsening of macular ischemia in either group at 4 months after treatment initiation. A total of 40 eyes were evaluated in the bevacizumab group. The study utilized FA to analyze changes in the macular perfusion and evaluated changes only related to the fovea as well as the status of perifoveal

**Table 2. Studies analyzing retinal perfusion changes using ultra-widefield fluorescein angiography.**

Study	Number of Eyes	Study Design	Imaging Modality	Agent Used	Outcome Assessed	Results	References
RECOVERY study	20	Prospective randomized controlled	UWFA using human graders	Aflibercept (monthly vs quarterly for 12 months)	Change in retinal nonperfusion in PDR	More ischemia worsening with quarterly	[29]
Bonnin <i>et al.</i>	18	Retrospective case series	UWFA	Ranibizumab (13 eyes) or Aflibercept (5 eyes)	Retinal reperfusion after 3 injections	No reperfusion of either arterioles or venules	[30]
Couturier <i>et al.</i>	10	Prospective case series	WF-OCTA and UWFA	Ranibizumab or Aflibercept	Retinal reperfusion after 3 injections	No reperfusion	[31]
PERMEATE study	14 with DME	Prospective case series	UWFA	Aflibercept (12 months)	Change in panretinal ischemic index	Improved from 5.0±4.1% to 4.7±3.5%	[32]

DME, diabetic macular edema; PDR, proliferative diabetic retinopathy; UWFA, ultra-widefield fluorescein angiography; WF-OCTA, widefield optical coherence tomography angiography.

capillaries qualitatively using human graders [20-22]. Patients with severe macular ischemia at baseline, however, defined as a foveal avascular zone  $\geq 1000 \mu\text{m}$  in greatest linear dimension or severe perifoveal capillary network disruption on FA were excluded from the study. In another retrospective study evaluating the effect of macular ischemia diagnosed by FA on the visual acuity following bevacizumab injections for DME in 59 eyes, there was a negative effect from the macular ischemia on the short term visual acuity following injections, where 50% of the patients in the ischemic group experienced visual losses of  $\geq 1$  ETDRS line [23]. This may indicate a possible aggravation of macular ischemia following anti-VEGF injections in cases with more baseline macular ischemia on FA.

In an unplanned retrospective analysis of the RIDE and RISE studies, 2 parallel prospective multicenter trials comparing 2 doses of ranibizumab (0.3 and 0.5 mg) to sham injections, there was worsening of posterior retinal nonperfusion in all groups, but more in the sham injected group. A total of 666 eyes were analyzed in these studies and there was more improvement in the visual acuity using both ranibizumab doses compared to sham injections. The conclusion was that monthly injections of ranibizumab can thus slow, but not completely prevent retinal capillary closure in patients with DME. The study also depended on FA for the pre and post-treatment analyses and used trained human graders [24]. The authors suggested that worsening of retinal nonperfusion in patients with DME could be the result of VEGF-induced leukostasis that was reversed partially by intravitreal ranibizumab treatment [24, 25].

In a post-hoc analysis of the effect of aflibercept injections for DME on the macular perfusion of diabetic patients in the VISTA study, a double-masked randomized controlled study that involved 466 diabetic patients with DME and compared laser treatment to 2 different aflibercept dosing regimens, there was both more improvement in retinal nonperfusion and slowing of worsening retinal nonperfusion with aflibercept injections compared to the laser treatment [26]. The study also used fluorescein angiography and human graders for this assessment.

An increase in the size of the FAZ following intravitreal injections of bevacizumab for the treatment of DME has

however, been previously reported using FA in several non-comparative studies [27, 28]. In a retrospective study which included 28 eyes of 28 patients with DR, the FAZ area increased by 19.7% 6-8 weeks following a single bevacizumab injection for DME [27], while in a prospective study which included 29 eyes of 29 patients with chronic DME there was a 13% increase in the area of the FAZ following 3 monthly intravitreal bevacizumab injections, which was greater in patients with milder DR [28]. This could be due to the progression of ischemia due to the underlying DR or due to VEGF inhibition or both [28]. Controlled studies are needed to further explain these results.

### 3.2. Studies Utilizing Ultra-widefield Imaging in the Evaluation of Retinal Nonperfusion Following Anti-VEGF Injections (Table 2)

Several studies utilized ultra-widefield imaging, imaging modalities that can image the posterior pole together with the peripheral retina in a single image capture, to evaluate the effect of intravitreal injections of anti-VEGF agents on the retinal nonperfusion of patients with diabetic retinopathy. In the RECOVERY trial, a prospective randomized clinical trial which evaluated the effect of monthly versus quarterly aflibercept injections on the retinal nonperfusion in patients with proliferative diabetic retinopathy, there was more worsening of the retinal nonperfusion and the ischemic index in the quarterly versus the monthly group but the mean baseline value of retinal nonperfusion area was higher in the monthly group. The study included 20 eyes in each group and utilized human graders that manually mapped areas of retinal nonperfusion on ultra-widefield fluorescein angiography images at baseline and after 1 year [29].

In another retrospective study using ultrawide field imaging in 18 eyes, Bonnin *et al.* found that anti-VEGF injections were associated with improved DR severity score (DRSS) on ultrawide field color fundus photographs, however, they did not find evidence of reperfusion of either arterioles or venules in or around non-perfusion areas on ultrawide field FA following 3 intravitreal monthly injections [30]. Thirteen eyes were treated with ranibizumab while 5 eyes were treated with aflibercept in that study. After the last injection, 83% of studied eyes showed occlusion of few

**Table 3. Studies analyzing macular perfusion changes using optical coherence tomography angiography.**

Study	Number of eyes	Study design	Imaging Modality	Agent Used	Outcome Assessed	Results	References
Ghasemi Falavarjani <i>et al.</i>	13 with DME	Prospective case series	OCTA using VD from machine software	Bevacizumab, Ranibizumab, Aflibercept (1 injection by any)	Change in FAZ area and vascular density	FAZ-A increased, and VD of foveal area decreased ( $p>0.05$ )	[46]
Sorour <i>et al.</i>	46 with DME or PDR	Retrospective case series	OCTA 3X3 and 6X6 scans and VD machine	45.7% Bevacizumab, 42.4% Aflibercept, and 11.9% Ranibizumab	Change in vascular density after 3 injections	No change in vascular density	[47]
Couturier <i>et al.</i>	10 eyes of 9 patients	Prospective case series	OCTA 3X3 scan using machine software for VD	Ranibizumab or Aflibercept (3 injections)	Change in vascular density after 3 injections	SCP drop from $39.5\pm 6.9\%$ to $36.6\pm 4.3\%$ and DCP from $44.7\pm 6.2\%$ to $42.5\pm 3.8\%$ ( $p>0.05$ )	[31]
Busch <i>et al.</i>	23	Retrospective case series	OCTA 3X3 scan	Aflibercept	FAZ area and vascular density changes	SCP FAZ increased by $0.07\text{mm}^2$ and DCP FAZ decreased by $0.04\text{mm}^2$ ( $p>0.05$ )	[48]
Hsieh <i>et al.</i>	50 with DME and 22 healthy controls	Retrospective controlled	OCTA with custom developed Matlab software for image processing and analysis	Ranibizumab (3 injections)	Change in FAZ-A, FAZ-CI, AVC, vessel tortuosity, and VD	Improved FAZ-A (-31%), AVC (-4.3%), and inner (+5.9%) and outer (+8.8%) PF-VD in the SCP, and FAZ-A (-31%), FAZ-CI (-4.2%), and inner (+9.1%) and outer (+9.4%) PF-VD in DCP ( $p\leq 0.05$ )	[49]
Dastiridou <i>et al.</i>	20	Prospective case series	Swept Source OCTA	Aflibercept (3 injections)	Change in FAZ area and VD	FAZ of DCP and VD of central area decreased ( $p\leq 0.05$ )	[50]
Pereira <i>et al.</i>	5 with DME and DMI	Prospective case series	FA, OCTA, and MP	Bevacizumab (6 injections)	Change in FAZ area on FA and OCTA (manually measured in both)	FA FAZ from $1.35\pm 1.44\text{mm}^2$ to $1.02\pm 1.02\text{mm}^2$ ( $p=0.19$ ) and OCTA FAZ from $0.82\pm 0.55\text{mm}^2$ to $0.92\pm 0.57\text{mm}^2$ ( $p=0.02$ )	[51]
Elnahry <i>et al.</i> (IMPACT Study)	40 eyes	Prospective case series	OCTA 3X3 and 6X6 scans with image processing and alignment	Bevacizumab (3 injections)	Change in FAZ area, FD, VD, and skeleton VD	Increase in FAZ area and decrease in FD, VD, and skeleton VD of Full, SCP, and DCP	[52]
Michalska and Heinke	3 eyes	Retrospective	OCTA 3X3 scans	Aflibercept (3-5 injections)	Change in VD	Insignificant change in VD ( $p>0.05$ )	[53]
Elnahry <i>et al.</i>	2 eyes of 1 patient	Prospective	OCTA 6X6 scan with machine software	Bevacizumab (repeated 3 monthly injections)	Change in built-in machine VD	Reversible worsening of VD with injections	[15]
Barash <i>et al.</i>	9 with PDR and 5 with DME	Retrospective case series	OCTA macular and peri-papillary scans	Bevacizumab or Aflibercept (immediately after injections)	Macular and peri-papillary VD changes	SCP dropped by 7.8% while the DCP VD dropped by 3.5% immediately after injection	[54]

AVC, average vessel caliber; DCP, deep capillary plexus; DME, diabetic macular edema; FA, fluorescein angiography; FAZ, foveal avascular zone; FAZ-A, foveal avascular zone area; FAZ-CI, foveal avascular zone circulatory index; FD, fractal dimension; Full, full retinal thickness; MP, micropertimetry; OCTA, optical coherence tomography angiography; PDR, proliferative diabetic retinopathy; PF, parafoveal; SCP, superficial capillary plexus; VD, vascular density.

vessel segments (mean:  $6 \pm 11$  per eye) passing through the non-perfusion areas. The study used i2k Align retina software (DualAlign, Clifton Park, NY) to align pre-treatment and post-treatment images, which allowed only areas common to both images to be compared. In another study by the same group in which ultrawide field imaging was performed using both swept-source widefield optical coherence tomography angiography (OCTA) and ultra-widefield FA, there was no improvement in retinal nonperfusion or evidence of arteriolar or venular reperfusion using either modality following 3 intravitreal monthly anti-VEGF injections despite

the improvement of the DRSS. Following injections, however, there was occlusion of some distal vessel segments, a mean of 2.33 and 3.7 newly occluded vessels per eye on ultra-widefield FA and widefield OCTA, respectively. The study included 10 eyes of 9 patients and used both ranibizumab and aflibercept [31].

In the PERMEATE study, a prospective study that included 29 treatment naïve eyes with macular edema due to DM (14 eyes) or retinal vein occlusion (15 eyes), patients received aflibercept injections every 4 weeks for the first 6

months followed by every 8 weeks for the next 6 months with specific rescue criteria to return to every 4 weeks dosing and were followed up with ultra-widefield fluorescein angiography. At 12 months, the mean panretinal leakage index decreased from 3.4% to 0.4% (-88.7%), which was statistically significant, while the mean panretinal ischemic index increased from 5.5% to 8.7% (+59.2%), which however was not statistically significant. When eyes with DME were analyzed separately, however, the mean panretinal ischemic index showed a slight improvement from  $5.0 \pm 4.1\%$  at baseline to  $4.7 \pm 3.5\%$  at 12 months, which was also not statistically significant [32].

### 3.3. Studies Utilizing Macular OCTA in the Evaluation of Macular Perfusion Changes Following Anti-VEGF Injections (Table 3)

Optical coherence tomography angiography (OCTA), a relatively new extension of optical coherence tomography (OCT), is a non-invasive, dye-free imaging modality that can obtain high resolution 3D images of all retinal vascular layers separately as well as the choroidal vasculature [33, 34]. The technology depends on comparing the decorrelation signal between consecutive, repeated OCT B-scans that are acquired in rapid succession at the same retinal location, allowing motion contrast generated by the flow of red blood cells in blood vessels to be detected. These changing contrasts between scans are translated to blood vessels in the final OCTA image with non-perfused areas appearing as flow void areas (Fig. 1C and D) [34, 35]. OCTA can therefore precisely and reliably delineate areas of capillary non-perfusion and image the foveal avascular zone without obscuration of these areas by dye leakage compared to FA [36-38]. Furthermore, it allows quantitative automatic measurements of the vascular density and fractal dimension reliably and reproducibly in the macular area in an objective manner that does not require human graders [39-43]. Because OCTA does not require intravenous dye injection, which has been associated with side effects including nausea, vomiting, and anaphylaxis, this allows OCTA to be repeated frequently with no risks or discomfort to the patient [44]. These advantages may allow OCTA to be more suited for the analysis of changes in the macular perfusion following DME treatment [45].

Ghasemi Falavarjani *et al.* used OCTA prospectively to evaluate the change in macular perfusion following a single intravitreal anti-VEGF injection in patients with macular edema secondary to DR or central retinal vein occlusion (CRVO) in a non-comparative case series [46]. The study found that the FAZ area and the foveal and parafoveal vessel density of the superficial and deep capillary plexuses did not significantly change after the single injection. Further analysis of data from the study, however, showed an increase in the FAZ area and a decrease in the vascular density of the foveal area following the single injection, which was not, however, statistically significant. This may have been due to the relatively small number of eyes enrolled in the study (18 eyes), the variability of the anti-VEGF agents used (beva-

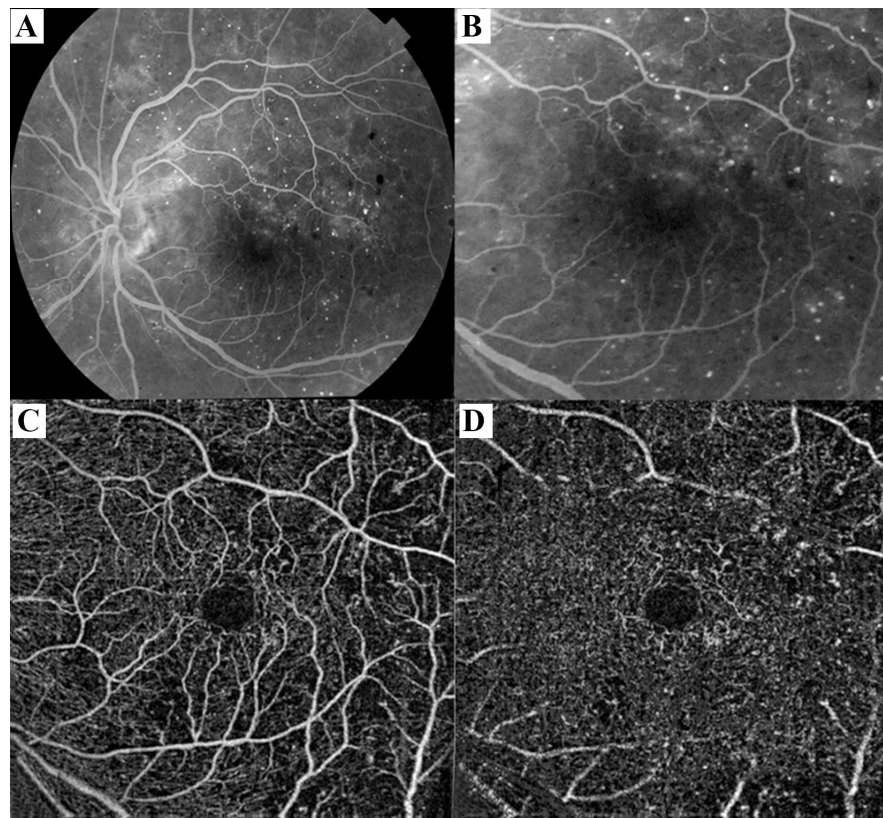
cizumab in 14 eyes, aflibercept in 3 eyes, and ranibizumab in 1 eye), the inclusion of 2 different etiologies for the macular edema (DME in 13 eyes and CRVO in 5 eyes), the short duration of the study (1 month), and the use of vascular density measurements from the machine software without further processing. The study also did not exclude patients with a history of previous anti-VEGF injections, which too may have influenced their results.

In a study by Sorour *et al.* that retrospectively evaluated the effect of repeated intravitreal anti-VEGF injections for the treatment of DME or proliferative diabetic retinopathy on the macular perfusion using both OCTA 3 X 3 mm and 6 X 6 mm scan protocols, there was no statistically significant difference in the macular perfusion after 1, 2, and 3 injections using either scanning protocols [47]. Although the study involved 46 eyes, which performed OCTA after the first injection, only 28 and 26 eyes performed OCTA after the 2nd and 3rd injections, respectively. The type of anti-VEGF agent used was variable (45.7% bevacizumab, 42.4% aflibercept, and 11.9% ranibizumab) and the mean interval between injections was 47 days. Half of the eyes (23 eyes) that performed OCTA after the 1st injection were not treatment naïve, where 13 eyes were included after 3 months of the last anti-VEGF injection (after washout), and the other 10 eyes were on regular anti-VEGF treatment with no washout period which could have influenced the results. Quantification of the vascular density in that study was also performed automatically using the built-in software of the machine (AngioVue software) with the ETDRS grid.

In the observational study by Couturier *et al.* that involved 10 eyes of 9 patients with DME and severe non-proliferative or proliferative DR injected with ranibizumab or aflibercept for 3 months, there was a decrease in the superficial capillary density from  $39.5 \pm 6.9\%$  to  $36.6 \pm 4.3\%$  and in the deep capillary density from  $44.7 \pm 6.2\%$  to  $42.5 \pm 3.8\%$  in the 3X3 mm macular area imaged by OCTA [31]. This, however, was not statistically significant, possibly due to the small number of included patients.

In another retrospective study that analyzed the FAZ area and vascular changes in the 3 X 3 mm macular area following aflibercept injections (mean:  $2.6 \pm 1.3$  injections) for DME using OCTA, there was enlargement of the FAZ area in the superficial capillary plexus from  $0.41 \pm 0.2 \text{ mm}^2$  to  $0.48 \pm 0.24 \text{ mm}^2$  but a decrease in the FAZ area in the deep capillary plexus from  $0.75 \pm 0.34 \text{ mm}^2$  to  $0.71 \pm 0.33 \text{ mm}^2$ . Both changes, however, were not statistically significant and the retinal vascular density was unchanged following the injections [48]. The study included a total of 23 eyes following the exclusion of ineligible eyes.

In a retrospective study by Hsieh *et al.* that used a custom-developed Matlab (Mathworks, Natick, MA) software for OCTA image processing and analysis and analyzed 5 OCTA biomarkers including FAZ area, FAZ circulatory index, average vessel caliber, vessel tortuosity, and vessel density and correlated them with visual acuity and central retinal thickness before and after 3 monthly ranibizumab injection



**Fig. (1).** FA and OCTA images of a patient from our research group are shown. Fluorescein angiography imaging uses an intravenously injected fluorescent dye which is detected inside retinal vessels by a blue light that is absorbed by the fluorescent dye and is re-emitted as another light of longer wavelength allowing visualization of the retinal vasculature and areas of capillary loss on a background of diffuse choroidal fluorescence (A and B). OCTA is a non-invasive, dye-free imaging modality that can obtain images of the superficial (C) and deep (D) retinal vascular layers separately by comparing the decorrelation signal between consecutive, repeated OCT B-scans that are acquired in rapid succession at the same retinal location, allowing motion contrast generated by the flow of red blood cells in blood vessels to be detected leading to vascular imaging.

tions for DME, there was a statistically significant improvement in the FAZ area (-31%), average vessel caliber (-4.3%), and inner (+5.9%) and outer (+8.8%) parafovea vessel density in the superficial capillary plexus, and a statistically significant improvement in the FAZ area (-31%), FAZ circulatory index (-4.2%), and inner (+9.1%) and outer (+9.4%) parafovea vessel density in the deep capillary plexus following the injections [49]. These measurements, however, did not return to their normal value as compared to measurements done on a healthy control group. There were no significant correlations between OCTA biomarkers and anatomical improvement, but the inner parafoveal vascular density of the superficial capillary layer was the biomarker that most significantly correlated with visual improvement following treatment. The study included 50 eyes of 50 diabetic patients and 22 eyes of 22 healthy persons.

In a prospective study of 20 eyes by Dastiridou *et al.* that evaluated the FAZ and vascular density following 3 aflibercept injections for treatment naïve patients with DME using swept source OCTA, there was a statistically significant decrease in the FAZ size in the deep capillary plexus but not in the superficial capillary plexus following injections with a high intergrader and intra-grader agreement for

manual FAZ measurements [50]. Vascular density of the central area showed an 8% decrease following the injections, which was also statistically significant. The authors suggested that the displacement of capillaries by the macular edema and segmentation errors, which are subsequently restored following treatment may play a role in the effects seen in the study.

In a prospective study of 5 eyes of 5 patients with DME associated with moderate to severe diabetic macular ischemia by Pereira *et al.*, functional and anatomical effects of monthly bevacizumab injections were assessed using FA, OCTA, and microperimetry [51]. Following 6 injections, the mean FAZ area on FA decreased from  $1.35 \pm 1.44 \text{ mm}^2$  to  $1.02 \pm 1.02 \text{ mm}^2$  ( $p=0.19$ ), however, 3 eyes had an increase in FAZ area. The mean FAZ area on OCTA (3 eyes analyzed) increased from  $0.82 \pm 0.55 \text{ mm}^2$  to  $0.92 \pm 0.57 \text{ mm}^2$  ( $p=0.02$ ), and microperimetry showed improvement from  $11.66 \pm 0.77 \text{ dB}$  to  $16.26 \pm 3.29 \text{ dB}$  ( $p=0.007$ ), which better correlated with retinal thickness improvement than with ischemic areas on FA or OCTA.

In a prospective study by our group that evaluated the effect of 3 intravitreal monthly bevacizumab injections for DME on the macular perfusion of treatment naïve diabetic

patients using OCTA (IMPACT Study), the FAZ area increased by 8.1%, the fractal dimension (FD) of the full retinal thickness (Full) and the superficial capillary plexus (SCP) decreased by 1.3%, the FD of deep capillary plexus (DCP) decreased by 1.9%, vascular density (VD) of Full decreased by 8% decreased, VD of SCP decreased by 9.1%, VD of DCP decreased by 10.6%, skeleton VD of Full decreased by 13.3%, skeleton VD of SCP decreased by 12.5%, and skeleton VD of DCP decreased by 16.3% in the 6X6 mm macular area following the injections, which were all statistically significant ( $p < 0.05$ ) [52]. Findings in the 3X3 mm area mirrored those in the 6X6 mm area. The study included 40 eyes of 26 patients, used a semi-automated program for image processing, and used i2k Align retina software for automated image alignment.

Michalska-Małecka and Heinke Knudsen retrospectively reviewed 3 patients treated with 3-5 injections of aflibercept for DME and imaged before and after treatment with OCTA using the 3 X 3 mm scanning protocol [53]. There was little change in the vascular density of all patients following the injections using the built-in machine software, although the patients showed improvement in vision and central retinal thickness.

In a prospective long-term follow-up study of 2 eyes of 1 patient treated for DME using repeated intravitreal monthly bevacizumab injections over a course of 1 year by our group, OCTA revealed decreased vascular density of the superficial and deep capillary plexuses following the injections which subsequently returned to the baseline value when injections were withheld [15]. In instances where only one eye was injected, vascular density decreased only in the injected eye but remained stable in the noninjected eye. Vascular density measurements were performed using the built-in machine software.

In a retrospective study that evaluated macular and peripapillary vascular density changes immediately following an intravitreal anti-VEGF injection (bevacizumab or aflibercept) for various pathologies including DR (9 with PDR and 5 with DME), there was a statistically significant decrease in vascular perfusion density in most areas of the superficial and deep vasculature, with more affection of the superficial layer, and the optic nerve head and radial peripapillary capillary layer, especially temporally [54]. In the study, the mean pre-treatment intraocular pressure (IOP) was 17.15 mmHg while the mean immediate post-treatment IOP, taken approximately 15 seconds after the injection, was 46.35 mmHg as measured by Tonopen (Reichert, Depew, NY). The superficial macular density decreased by 7.8%, while the deep macular density decreased by 3.5% immediately (within 3 minutes) following the injection. This indicates a direct but transient effect from increased IOP following the injections on the vascular density as measured using OCTA.

In a study investigating the effect of aflibercept injections for treatment naïve wet age-related macular degeneration on the vascular density of the macular area using OCTA, there was a statistically significant decrease in the superficial foveal and parafoveal vascular density after 1, 2, and 3

injections compared to baseline (31% reduction in the vascular density of the foveal area and 6.6% reduction in parafoveal vascular density after 3 injections) [55]. This is of interest since patients with a history of retinal vascular disease were excluded from the study, which suggests that anti-VEGF agents may affect the vascular density in the absence of a retinal vascular pathology. The automated measurements of the AngioVue software were used in this study which included 15 eyes and lacked a control group.

#### 4. DISCUSSION

The effect of repeated intravitreal injections of different anti-VEGF agents on the macular perfusion of diabetic patients with DME has been evaluated in various controlled trials and case series. Originally, these studies have depended on FA in this evaluation [20-28], with several studies that followed that employed ultra-widefield FA imaging for the evaluation of the effect of these injections on the peripheral retinal perfusion as well [29-32]. Recently, studies evaluating macular perfusion changes have depended more on the use of the relatively newly introduced and promising OCTA technology [46-55], a dye-free imaging modality that depends on comparing the decorrelation signal between consecutive, repeated OCT B-scans acquired in rapid succession at the same retinal location, which allows motion contrast generated by the flow of red blood cells in vessels to be detected leading to imaging of perfused vessels and detection of flow-void areas [34-38].

Although the new OCTA technology is very promising and is rapidly being adopted by many researchers and clinicians in the field, it should be noted that it is an emerging technology that is still under development with several already acknowledged limitations that may influence the correct interpretation of its results including signal strength which may impair imaging of small vessels if low, localized signal losses due to localized media opacities, prolonged scan time, shadow artifacts, projection artifacts of superficial layers on deeper ones, movement of the patient's eye and head, and inability to image vessels with slow flow [56, 57]. OCTA imaging is, therefore, currently more prone to artifacts and interpretation errors compared to conventional FA, which warrants extreme caution when acquiring and interpreting OCTA images to avoid reaching wrong or inaccurate conclusions. This may partly explain the different and conflicting results obtained from studies performed by different research groups using OCTA as seen in the current review. As the technology gets better and faster, however, several of these limitations will be eliminated leading to more reliable and reproducible results with more solid conclusions.

Results from this review were inconclusive regarding the effect of repeated intravitreal injections of anti-VEGF agents on the macular perfusion of diabetic patients with DME, however, we have identified a recent trend of shifting from conventional FA imaging for this evaluation to both ultra-widefield FA and OCTA imaging possibly because ultra-widefield FA allows better evaluation of the peripheral reti-

nal perfusion status while OCTA allows quantitative and objective evaluation of the macular perfusion status. Regarding studies using conventional FA, 3 studies that utilized bevacizumab, ranibizumab, and aflibercept for the treatment of DME did not find a negative effect from these anti-VEGF agents on the macular perfusion [22, 24, 26], while 3 other studies that have all utilized bevacizumab for DME treatment showed a negative effect from bevacizumab injections on the macular perfusion [23, 27, 28]. Using ultra-widefield FA, 2 studies showed a positive effect on peripheral retinal perfusion using aflibercept [29, 32], while 2 other studies that used ranibizumab and aflibercept showed no evidence of retinal reperfusion with further vascular occlusion following injections [30, 31]. All these studies utilized trained human graders in the evaluation of perfusion changes. A plethora of studies that evaluated the effect of anti-VEGF injections on the macular perfusion of diabetic patients using OCTA have recently been conducted by many research groups worldwide in a relatively short period of time. This probably reflects the current uncertainty surrounding the effect of these agents on the macular perfusion of diabetics, which prompted many researchers to attempt to resolve this uncertainty using the new and promising OCTA technology considering its perceived advantages in imaging the retinal microvasculature over conventional FA due to its ability to image capillary details, including the deep capillary plexus, in higher resolution without obscuration by dye leakage which allows better delineation and quantification of capillary non-perfusion areas [58, 59]. Regarding studies done using OCTA, 3 studies showed stable macular perfusion following injections [47, 49, 53], 6 studies showed worsening macular perfusion following injections [15, 31, 46, 51, 52, 54], and 2 studies showed conflicting results [48, 50]. This may have been due to differences in study design, patient inclusion criteria, or method of analysis.

Some factors associated with intravitreal injection of anti-VEGF agents may result in improvement of macular perfusion while other factors may result in its worsening, which could possibly explain why some patients experience improvement of perfusion following the injections while others experience worsening, likely depending on which factors predominate in each patient. Indeed, even in the single patient, it is apparent that some retinal areas become better perfused while others get worse on OCTA following the injections suggesting that several competing factors are in play [60]. Factors resulting in improvement of retinal perfusion following anti-VEGF injections include reversal of VEGF-induced leukostasis, which results in increased capillary occlusion [25], restoration of the normal retinal architecture [50], and inhibition of endothelial cell hypertrophy that is induced by local VEGF-A production and leads to progressive capillary lumen narrowing [61]. Factors explaining retinal perfusion worsening following anti-VEGF inhibition include induction of vasoconstriction of retinal blood vessels which was seen following bevacizumab and ranibizumab injections for DME and could be due to inhibition of nitric oxide which is known to occur with VEGF inhibition and also results in systemic hypertension in case of systemic VEGF in-

hibition [62, 63]. VEGF inhibition by bevacizumab was also found to decrease the mean blood flow velocity of the central retinal artery, temporal posterior ciliary arteries, and ophthalmic artery by 10%, 20%, and 20% respectively 4 weeks following a single injection in patients with wet age-related macular degeneration [64]. Another possible cause of decreased capillary density following VEGF inhibition in diabetic patients could be due to the loss of pericytes that normally surround mature capillaries and make them non-dependent on VEGF for survival [65, 66]. Loss of pericytes, which occurs early in DR, may render capillary endothelial cells again susceptible to VEGF inhibition, possibly leading to endothelial cell apoptosis and loss of capillaries [17, 67].

## CONCLUSION

In conclusion, various studies have been performed to evaluate the effect of repeated intravitreal injections of different anti-VEGF agents for DME on the macular and retinal perfusion status of diabetics with inconclusive results using conventional FA, ultra-widefield FA, and OCTA. There is a recent shift towards the use of widefield imaging and OCTA in this evaluation, but more prospective multicenter randomized controlled clinical trials using OCTA are needed to draw more solid conclusions. New anti-VEGF agents are continuously being developed and approved and will also require evaluation regarding their effect on retinal perfusion, preferably before their use becomes more widespread [68, 69]. Future advances in OCTA technology including increased scanning speed, decreased imaging artifacts, and wider scanning fields will further assist in this evaluation.

## LIST OF ABBREVIATIONS

AVC	= Average Vessel Caliber
CRVO	= Central Retinal Vein Occlusion
DCP	= Deep Capillary Plexus
DM	= Diabetes Mellitus
DME	= Diabetic Macular Edema
DR	= Diabetic Retinopathy
DRSS	= Diabetic Retinopathy Severity Score
ETDRS	= Early Treatment Diabetic Retinopathy Study
FA	= Fluorescein Angiography
FAZ	= Foveal Avascular Zone
FAZ-A	= Foveal Avascular Zone Area
FAZ-CI	= Foveal Avascular Zone Circulatory Index
FD	= Fractal Dimension
Full	= Full Retinal Thickness
IOP	= Intraocular Pressure
MP	= Microperimetry



OCT	= Optical Coherence Tomography
OCTA	= Optical Coherence Tomography Angiography
PDR	= Proliferative Diabetic Retinopathy
PF	= Parafoveal
SCP	= Superficial Capillary Plexus
UWFA	= Ultra-widefield Fluorescein Angiography
WF-OCTA	= Widefield Optical Coherence Tomography Angiography
VD	= Vascular Density
VEGF	= Vascular Endothelial Growth Factor

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### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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