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ORIGINAL PAPER

Optical coherence tomography angiography analysis of macular vessel density before and after anti-VEGF therapy in eyes with diabetic retinopathy

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Abstract

Purpose To evaluate changes in macular vessel density following intravitreal anti-VEGF injection in patients with diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR).

Methods In this retrospective case series, optical coherence tomography angiography (OCTA) images from 55 eyes of 35 patients with either DME (46 eyes) or PDR (9 eyes) were included. Macular capillary vessel density at the level of the superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP) before and after anti-VEGF treatment was calculated. Longitudinal changes in vessel density following serial anti-VEGF treatment were analyzed in a subset of eyes.

Results Vessel density in the SCP, DCP or TCP was not found to be significantly different after one, two or three intravitreal injections ($p > 0.05$ for all time points). Subgroup analysis revealed no significant change in the DME and PDR subgroups (all $p > 0.05$). Multivariate analysis revealed no effect of type of injected anti-VEGF agent or presence of previous treatment on VD measurements (all $p > 0.05$). There was no correlation between the anatomic response of DME to treatment and VD measurements.

Conclusions In this study, macular vessel density remained statistically unchanged following up to three intravitreal injections of any anti-VEGF agent. This indicates that there may not be an early effect of anti-VEGF treatment on macular vessel density and its effect on macular perfusion may not be a direct change in microvascular flow.

Keywords Aflibercept · Anti-VEGF · Bevacizumab, diabetic retinopathy · Macular edema · Optical coherence tomography angiography · Proliferative diabetic retinopathy · Ranibizumab · Vessel density

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Introduction

Diabetic retinopathy (DR) is characterized by two distinct types: non-proliferative DR (NPDR) and

proliferative DR (PDR). The progression of microvascular damage and loss of capillary perfusion corresponds with progression of diabetic retinopathy severity [1]. Complications of DR include vitreous hemorrhage and diabetic macular edema (DME), the main causes of deterioration of vision in patients with DR [2]. Vascular endothelial growth factor (VEGF) plays a significant role in this ongoing microvascular insufficiency and thus also in the development of PDR and DME [3].

Fluorescein angiography (FA) remains invaluable in identifying leaking vessels in both DME and PDR. However, FA is time-consuming, invasive and carries the risk of complications, including severe allergic reactions. Optical coherence tomography angiography (OCTA), an extension of optical coherence tomography (OCT), is a noninvasive, depth resolved imaging technique that allows visualization of the superficial and deep retinal capillary plexus, which is not possible on FA [4]. The application of OCTA to studying microvascular changes in diabetic patients may help in understanding the pathogenesis of DR and the changes occurring in response to various treatments [5, 6].

Although the use of anti-VEGF treatment is now the standard treatment in cases of DME, its effect on retinal perfusion is still controversial. Some studies have shown slowing or even improvement in macular non-perfusion following anti-VEGF treatment, with a growing body of evidence for its use in PDR as well [7–9]. Fewer case report studies, however, considered increased VEGF as a compensatory mechanism in restoring macular perfusion; in addition, anti-VEGF treatment may increase the severity of non-perfusion with subsequent visual deterioration [10, 11]. However, all the previous studies have relied on FA to assess changes in macular perfusion, which has lower resolution and limited sensitivity. In this study, OCTA was used to quantify vessel density (VD) for the evaluation of macular perfusion following anti-VEGF treatment.

Patients and methods

This study protocol was approved by the Institutional Review Board at the Tufts Medical Center. The research adhered to the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

Diabetic patients above the ages of 18 who received anti-VEGF injections for either DME and/or PDR and had high-quality OCTA images at New England Eye Center, Boston between January 2015 and December 2017 were retrospectively identified. Data collected from medical record review included baseline demographics (gender, duration of diabetes, medical history and ocular history (type of intravitreal medication). The most recent ophthalmologic examination findings such as best-corrected visual acuity (BCVA), slit-lamp biomicroscopy and fundus examination were recorded. Exclusion criteria were myopia greater than 4 diopters, confounding ocular conditions such as uveitis, uncontrolled glaucoma, history of endophthalmitis, vitreomacular traction, panretinal photocoagulation (PRP) focal laser treatment within the last 3 months, intravitreal injection of corticosteroid or any ocular surgery within the last 6 months. Eyes with media opacity degrading the quality of OCTA image were excluded. Treatment with previous anti-VEGF injections did not exclude patients from this study.

OCTA imaging of diabetic eyes was performed with the RTVue XR 100 Avanti instrument (Optovue, Inc., Fremont, CA, USA). High-quality 3×3 mm and 6×6 mm images with strong signal–noise ratio and adequate centration on the fovea were selected. Images with 6/10 score and above were included. Automated segmentation was used to evaluate superficial and deep capillary retinal plexus projections using the AngioVue module. If errors in segmentation were detected, manual correction was performed using AngioVue proprietary software. Manual correction was done in 9 eyes due to inaccurate determination of the deep retinal capillary plexus boundaries in 6 eyes, and inaccurate determination of the outer boundary of the superficial capillary plexus in 3 eyes. The superficial retinal capillary plexus (SCP) was delineated with an inner boundary at the internal limiting membrane (ILM) and an outer boundary 10 μm inside the inner plexiform layer (IPL). The deep retinal capillary plexus (DCP) was segmented with an inner boundary 10 μm inside the IPL and an outer boundary at 10 μm beneath the outer plexiform layer (OPL). In addition, the total retinal capillary plexus (TCP) was manually customized by selecting the inner boundary at the ILM and the outer boundary set at 40 μm above the retinal pigment epithelium (RPE) to avoid any flow signals from choriocapillaries. This segmentation was

performed because segmentation in the presence of diabetic macular edema can be challenging (Fig. 1).

Quantitative analysis of vessel density was performed on the OCTA enface images using the AngioVue software. VD analysis computes the percentage of area occupied by OCTA detected vasculature in a measured area. The vascular density of the SCP, DCP and TCP in the whole ETDRS grid (composed of concentric rings at 1, 3, 6 mm from the foveal center) and its subsets were automatically generated by the software as the proportion of the measured area occupied by blood vessels with flow, defined as pixels having decorrelation values above the threshold level [12, 13]. We included VD measurement in the whole ETDRS grid before and after treatment, which corresponds to 3 mm circle in 3 × 3 mm OCTA scan and 6 mm circle in 6 × 6 mm OCTA scan.

Statistical analysis was performed using SPSS software (version 25; SPSS, Inc., Chicago, IL, USA). Paired *t* test and repeated measures ANOVA analysis were used for quantitative data analysis before and after injections. Multivariate analysis was done to analyze the effect of previous treatment and type of anti-VEGF used on the VD measurements change. Independent sample *t*-test was done between baseline VD measurements in treatment-responsive and treatment-resistant DME patients. Binominal logistic regression and linear regression analysis was done between the DME response and VD measurement values before and after the first anti-VEGF injection, respectively. A *p*-value less than 0.05 was considered significant.

Results

Fifty-five eyes of 35 diabetic patients including 15 females (43%) and 20 males (57%) with a mean age of 61.0 years (range: 38–86 years) were included. The

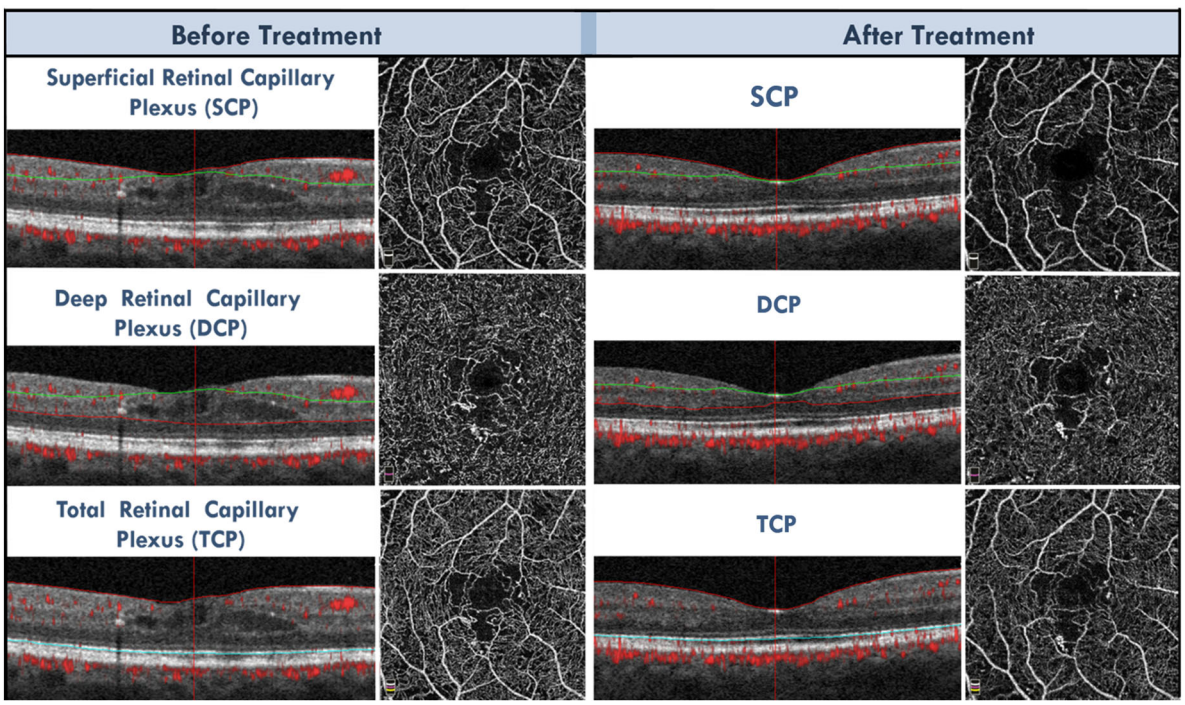

Fig. 1 Enface and B-scan optical coherence angiograms of one patient before and after anti-VEGF treatment, illustrating the 3 retinal segmentations used in the study. Upper row represents superficial retinal capillary plexus (SCP) segmentation which is delineated by internal limiting membrane, and 10 µm inside the inner plexiform layer (IPL). The middle row represents the deep retinal capillary plexus (DCP), segmented with an inner boundary 10 µm inside the IPL and an outer boundary at 10 µm beneath the outer plexiform layer (OPL). The lower row represents the total retinal capillary plexus (TCP), which was manually customized with an inner boundary at the ILM and the outer boundary set at 40 µm above the retinal pigment epithelium (RPE)

indication for intravitreal anti-VEGF injection was DME in 46 eyes and PDR in 9 eyes (PDR without DME in 6 eyes or PDR with mild DME in 3 eyes). The study eyes had a total of 92 injections, of which 42 were bevacizumab, 39 were aflibercept, and 11 were ranibizumab. Baseline OCTA was obtained within an average of 8.5 days before injection (range 0–97 days) and 50.1 days after anti-VEGF injection (range: 21–90 days). Forty-six eyes received OCTA after the first injection, 28 eyes received an additional OCTA after a second injection and 26 eyes received an additional OCTA following a third injection. Length of time between successive anti-VEGF injections ranged from 29 to 62 days (Table 1). In the whole series of patients, 30 eyes were treatment naïve, and 25 eyes had received previous anti-VEGF treatment. Among the 46 eyes with available OCTA after the first anti-VEGF injection, 23 eyes were treatment naïve and 23 eyes were not. In the last group of previously treated patients, 13 eyes were included after 3 months of last anti-VEGF injection (after washout), and the remaining 10 eyes were on regular anti-VEGF treatment with no washout period.

Change in central retinal thickness (CRT) was statistically significant after single anti-VEGF injection in the whole series of patients ($-$ 26.08 $\pm$ 38.01 μm) and DME subgroup ($-$ 29.76 $\pm$ 40.74 μm), while the change in CRT was not significant in the PDR subgroup ($-$ 6.0 $\pm$ 8.31 μm) (Table 2).

Measurements of macular vessel density (VD) in the SCP, DCP and TCP were not statistically different before and after anti-VEGF injections. This was true for VD measurements following one, two or three injections as compared with baseline, for both 3 $\times$ 3 and 6 $\times$ 6 mm scans (Tables 3, 4) and (Fig. 2). With the exception of DCP in 3 $\times$ 3 mm scans that showed slight statistically insignificant increases in VD after the first injection, there was no trend for increase or decrease in VD measurements with increasing number of anti-VEGF injections (Fig. 2). Subgroup analysis of VD changes in patients with DME or PDR showed no statistically significant difference in each group pre- and post-injection (Table 3). The effect of previous treatment and type of anti-VEGF used on vessel density measurement changes after a single injection

Table 1 Baseline characteristics of patients included in the study

Number of patients	One eye enrolled from 15 patients				
	Two eyes enrolled from 20 patients				
	Total = 55 eyes from 35 patients				
Sex	Female	15 (42.15%)			
	Male	20 (57.85)			
Mean age (years)	61.0 ($\pm$ 11.2)				
Type of anti-VEGF and number of injections	Avastin	42 (45.7%)			
	Eylea	39 (42.4%)			
	Lucentis	11 (11.9%)			
Interval between injections (days)		Mean = 47, Range = (29–62)			
Timing of baseline OCTA (days)	Mean	8.54 $\pm$ 22.4			
	Median	Day 0			
	Range	(97–0)			
Timing of after injection OCTA (days)	Mean	50.1 $\pm$ 21.0			
	Median	49.0			
	Range	(21–90)			
Distribution of available OCTA follow-up for the patients		At base line	After 1st injection	After 2nd injection	After 3rd injection
	DME	46	38	23	23
	PDR	9	8	5	3
	total	55	46	28	26

Table 2 Analysis of changes in central retinal thickness (CRT) after one anti-VEGF injection, in diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) subgroups, and total patients' series

	Number	Baseline CRT Mean (SD) (μm)	Change in CRT Mean (SD) (μm)	*p*-value
Total series	46	348. ± 85.2	− 26.08 ± (38.01)	**< 0.000***
DME patients	38	367.42 ± 81.1	− 29.76 ± (40.74)	**< 0.000***
PDR patients	8	258.37 ± 26.4	− 6.0 ± (8.31)	0.08

*CRT signifcantly decreased after one anti-VEGF injection in the whole series of the patients and in the DME subgroup

of anti-VEGF were studied using multivariate analysis. Findings revealed that neither the type of anti-VEGF used nor being treatment naïve affected the findings on vessel density, and this result held for SCP, DCP, TCP in 3 × 3 and 6 × 6 mm scans. Presence of washout period or not before enrollment in study demonstrated no effect on VD measurement with anti-VEGF treatment (Table 5 and Fig. 3).

Finally, we looked for correlation between the degree of response of DME to anti-VEGF injection and baseline VD measurements in different retinal layers: SCP, DCP and TCP segmented from 3 × 3 and 6 × 6 mm scan size using binominal logistic regression model, to detect whether VD measurement will predict DME response to treatment or not, also VD measurement following injection of anti-VEGF was tested for correlation with DME response using linear regression model. Macular edema was considered responsive, if the central retinal thickness was decreased by 10% or more from the base line, and 24 eyes were treatment-resistant while 22 eyes were treatment-responsive. There was no statistically significant difference in VD measurements at baseline between them using independent sample t-test. And there was no correlation between DME response and VD measurements at baseline and after a single anti-VEGF injection (Tables 6 and 7).

Discussion

In this study, OCTA data of macular vessel densities in patients with DME and PDR were analyzed at baseline and after intravitreal anti-VEGF injection. Mean macular vessel density at the level of the SCP, DCP and TCP, as measured by OCTA in 3 × 3 and 6 × 6 mm scans, remained unchanged after one, two and three intravitreal anti-VEGF injections.

Vessel density, defined as the percentage of area occupied by vessels relative to the entire image, was one of the first generated metrics from OCT angiograms. An early demonstration by Hwang et al. showed that VD can be used as a marker for quantifying capillary perfusion [14]. Several studies have since demonstrated a reduction in parafoveal vessel density in the superficial and deep retinal vascular layers in diabetic eyes compared to normal controls [15, 16]. Moreover, VD has demonstrated a negative correlation with increasing severity of DR [17]. Measurements from the deep capillary plexus appear to show greater correlation in the context of disease stratification [18, 19]. Furthermore, VD measurements from the RTVue XR Avanti have been shown to have a high repeatability, validating their use in following retinal microvasculature changes over time [20].

Spaide et al. analyzed OCTA images of patients receiving anti-VEGF treatment for CME secondary to CRVO and concluded that, although there was resolution of edema, there was no qualitative change in flow patterns in either the superficial or deep layers. However, no quantitative data were published in this study [21].

Previous studies based on FA have illustrated that anti-VEGF therapy slows the progression, or at least does not worsen, macular capillary non-perfusion [8, 22, 23]. Quantitative analysis was performed in a recently published study and concluded that there is no statistically significant change in mean VD at SCP and DCP after a single intravitreal anti-VEGF injection for DME. These results are consistent with our findings. However, this study included only patients with DME who had received a single anti-VEGF injection and

Table 3 Analysis of vessel density (VD) changes after one anti-VEGF injection

	Mean difference	SD	*p*-value
Superficial retinal capillary plexus			
3 × 3 mm scan			
Total series	− 0.35	4.69	0.60
DME patients	− 0.14	4.70	0.85
PDR patients	− 1.38	4.82	0.44
6 × 6 mm scan			
Total series	− 0.04	5.01	0.94
DME patients	0.45	4.82	0.56
PDR patients	− 2.41	5.60	0.26
Deep retinal capillary plexus			
3 × 3 mm scan			
Total series	− 8.80	50.60	0.24
DME patients	− 1.76	8.12	0.19
PDR patients	− 42.25	120.68	0.35
6 × 6 mm scan			
Total series	− 0.22	6.02	0.79
DME patients	0.22	6.33	0.83
PDR patients	− 2.36	3.77	0.12
Total retinal capillary plexus			
3 × 3 mm scan			
Total series	− 0.65	3.66	0.22
DME patients	− 0.80	3.80	0.20
PDR patients	0.03	3.03	0.97
6 × 6 mm scan			
Total series	0.09	5.96	0.91
DME patients	0.79	5.97	0.41
PDR patients	− 3.22	4.97	0.10

Vessel density did not show significant change after anti-VEGF injection in superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP) both in 3 × 3 mm scans and 6 × 6 mm scans, in the whole series of patients (46 eyes) and in diabetic macular edema (DME, 38 eyes) and proliferative diabetic retinopathy (PDR, 8 eyes) subgroups

Table 4 Analysis of vessel density changes after two and three anti-VEGF injections

	Mean difference	SD	*p*-value
Superficial retinal capillary plexus			
3 × 3 mm scan			
2 Injections	1.16	4.69	0.19
3 Injections	1.43	4.02	0.08
6 × 6 mm scan			
2 Injections	0.41	4.17	0.60
3 Injections	1.02	4.01	0.22
Deep retinal capillary plexus			
3 × 3 mm scan			
2 Injections	− 0.02	7.20	0.98
3 Injections	− 0.33	7.07	0.81
6 × 6 mm scan			
2 Injections	0.21	5.26	0.83
3 Injections	1.10	4.83	0.27
Total retinal capillary plexus			
3 × 3 mm scan			
2 Injections	0.02	3.49	0.96
3 Injections	− 0.31	4.84	0.74
6 × 6 mm scan			
2 Injections	2.18	9.05	0.21
3 Injections	0.70	3.67	0.35

Vessel density did not show significant change after 2 (28 eyes) and 3 (26 eyes) successive anti-VEGF injections in the superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP), both in 3 × 3 mm scans and 6 × 6 mm scans

their small series did not allow for multivariate analysis for the effect of the presence of previous treatment on VD measurement [13]. Similar findings have been noted after intravitreal dexamethasone implant for DME, as the foveal and parafoveal VD in the superficial and deep capillary plexuses remained unchanged up to 120 days after injection [24]. Our results are also consistent with a case report of 3 patients who received intravitreal aflibercept for DME with no differences in VD for the whole macular area in 3 × 3 mm scans prior to status post-injections in all 3 cases [25].

Our multivariate analysis shows that the lack of changes in VD measurements following anti-VEGF treatment was not affected by previous treatment received. And even the group that was on regular treatment (without washout) did not turn up to show significant change in VD with anti-VEGF treatment, which may suggest that the absence of changes in VD measurements cannot be attributed to limited number of injections.

In our study, a statistically significant decrease in CRT in the total series and the DME subgroup was not accompanied by a change in VD. This suggests that anti-VEGF reduces leakage in the macula by acting on permeability factors, thus decreasing vessel hyperpermeability and preventing leakage. The previously

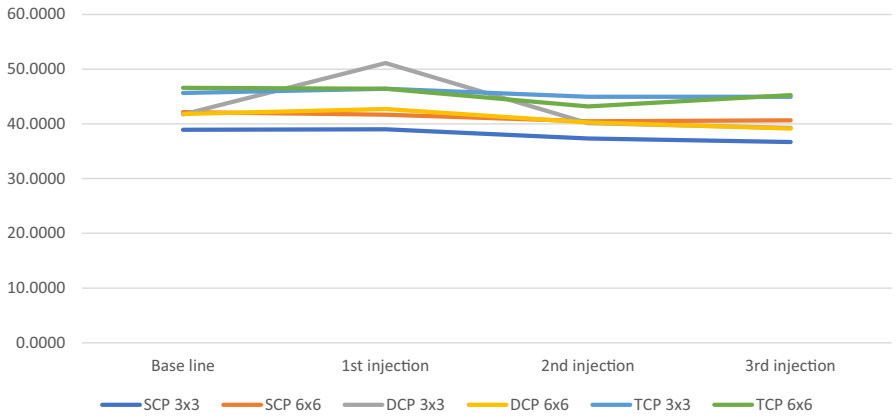

Fig. 2 Representation of mean vessel density (VD) measurements through all injections. Vessel density measurements remained statistically unchanged with increasing number of injections, in superficial retinal capillary plexus in either OCTA 3 × 3 mm scans and 6 × 6 mm scans (SCP 3 × 3) & (SCP 6 × 6), in deep retinal capillary plexus of 3 × 3 and 6 × 6 OCTA scans (DCP 3 × 3) & (DCP 6 × 6) and total retinal capillary plexus segmented from 3 × 3 and 6 × 6 OCTA scans (TCP 3 × 3) & (TCP 6 × 6), the graph remained nearly linear, with exception of DCP 3 × 3 which demonstrated the initial increase (statistically insignificant) before resuming nearly baseline measurement and remained otherwise flat afterward

Table 5 Multivariate analysis of effect of type of injected anti-VEGF and the presence of previous treatment on vessel density (VD) measurements in different OCAT segmentations

	F	*p*-value
Effects of type of injected anti-VEGF in all VD measurements		
Pillai's trace	1.106b	0.368
Between-subjects effects of type of injected anti-VEGF		
SCP		
3 × 3 mm scan	0.576	0.566
6 × 6 mm scan	2.359	0.107
DCP		
3 × 3 mm scan	0.906	0.412
6 × 6 mm scan	0.283	0.755
TCP		
3 × 3 mm scan	0.001	0.999
6 × 6 mm scan	0.185	0.832
Effects of the presence of previous treatment in all VD measurements		
Pillai's trace	1.334b	0.266
Between-subjects effects of the presence of previous anti-VEGF treatment		
SCP		
3 × 3 mm scan	0.086	0.917
6 × 6 mm scan	0.602	0.552
DCP		
3 × 3 mm scan	1.715	0.192
6 × 6 mm scan	0.009	0.991
TCP		
3 × 3 mm scan	0.084	0.920
6 × 6 mm scan	0.576	0.567

Analysis revealed no statistically significant effect of type of injected anti-VEGF (Eylea, Lucentis, or Avastin) or the presence of previous treatment (naïve, previously treated with washout, and previously treated without washout period) on vessel density measurements after single injection nor between patients injected with different anti-VEGF. This was tested in superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP), which were segmented using both 3 × 3 and 6 × 6 mm scans

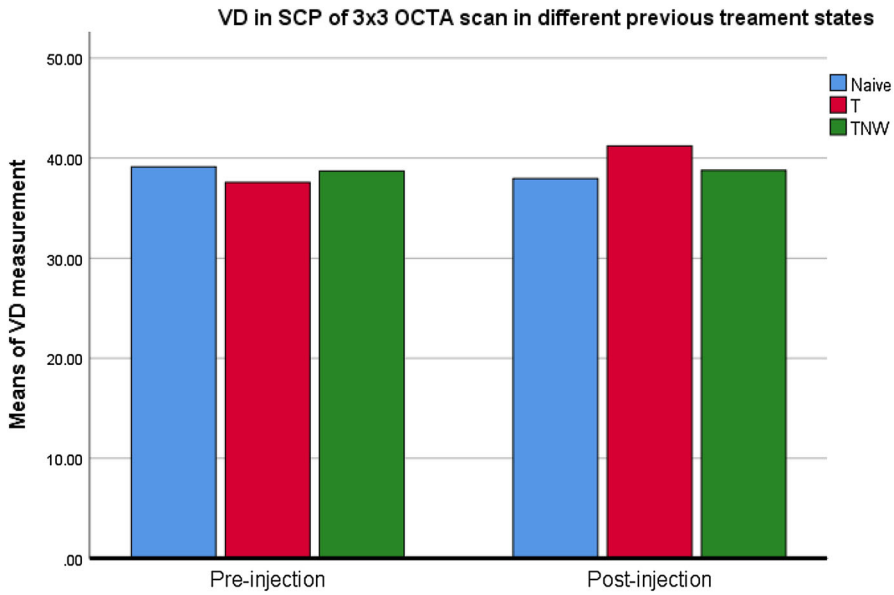

Fig. 3 VD measurements analysis of patients with different previous treatment states. This graph gives an example of similar behavior of VD measurements in superficial retinal capillary plexus (SCP) with anti-VEGF treatment, whether patient was treatment naïve before enrollment in study or was previously treated with enrollment after a washout period or not. (T means previously treated with washout period, TNW means on regular treatment without washout)

Table 6 Analysis of baseline vessel density (VD) measurements difference in treatment-responsive and treatment-resistant DME patients by independent sample *t*-test

	Mean difference between responders and non-responders	*p*-value
SCP		
3 × 3 mm scan	− 0.00379	0.998
6 × 6 mm scan	1.22159	0.411
DCP		
3 × 3 mm scan	0.14735	0.933
6 × 6 mm scan	− 1.70606	0.402
TCP		
3 × 3 mm scan	1.02576	0.541
6 × 6 mm scan	0.65227	0.729
CRT	− 60.37879	**0.015***

There was no statistically significant difference in the baseline VD measurements between DME eyes that subsequently developed good response to treatment, and those with poor response, this was tested in superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP), segmented from both 3 × 3 and 6 × 6 mm scans

*Baseline central retinal thickness (CRT) was statistically thicker in patients that achieved good response to treatment

reported slowing or improving of macular ischemia progression may be an indirect effect, resulting from improved tissue nutrition and not necessarily due to direct improvement in microvascular structure, as evidenced by our analysis of OCTA images.

In our subgroup analysis, there was no difference in baseline measurements of VD at SCP, DCP and TCP between treatment responders and treatment-resistant cases. There was no significant correlation between response of DME to treatment and VD either at baseline or after a single anti-VEGF injection. This result may suggest that macular perfusion before or after treatment is not a predictor of treatment response, although the study does not have enough cases to elicit a definitive conclusion. Lee et al. demonstrated a similar lack of correlation between VD in the SCP and the response of DME to anti-VEGF treatment. Unlike in the present study, however, they did show that there

Table 7 Study of correlation between response of macular edema to anti-VEGF treatment and vessel density (VD) measurements

	Scan	*B*	*p*-value
Testing baseline VD measurement as predictor to DME response	SCP		
	3 × 3 mm	0.199	0.173
	6 × 6 mm	− 0.283	0.061
	DCP		
	3 × 3 mm	0.046	0.507
	6 × 6 mm	0.092	0.125
	TCP		
	3 × 3 mm	− 0.318	0.067
	6 × 6 mm	0.186	0.103
Testing DME response correlation with VD measurement following the first anti-VEGF injection	SCP		
	3 × 3 mm	− 2.504	0.213
	6 × 6 mm	− 2.320	0.196
	DCP		
	3 × 3 mm	− 0.604	0.801
	6 × 6 mm	− 2.394	0.296
	TCP		
	3 × 3 mm	− 1.372	0.400
	6 × 6 mm	− 1.659	0.407

Logistic regression analysis of DME response and vessel density measurement at baseline revealed that baseline VD measurements in superficial retinal capillary plexus (SCP), deep retinal capillary plexus (DCP) and total retinal capillary plexus (TCP) were not a good predictor for DME response following treatment. Also, DME response was not correlated to VD measurements after the first anti-VEGF injection. *B* is unstandardized coefficients

was significant correlation between the status of the deep retinal capillary plexus and the treatment response and suggested that the integrity of the deep capillary plexus may be a predictor for the effectiveness of the treatment, via its supposed role in removing excess fluid out of the retina [26]. This discrepancy may be the result of different definitions of persistent DME. We defined response as a reduction in CRT by 10% or more, which is consistent with most clinical trials [27–29]. However, Lee et al. defined a DME responder as having decrease of 50 μm from baseline in CRT. Thus, they may have selected a subgroup of responders that showed a very robust response to anti-VEGF treatment. Our results are consistent with the analyses from the RISE and RIDE studies for anatomic response in relation to the presence of macular non-perfusion at baseline, in which there were no significant differences in the efficacy of ranibizumab between patients with and without macular non-perfusion at baseline [8].

Our study had several limitations. As a retrospective analysis, it was subject to the limitations associated with this study design. In addition, the sample size may not have been large enough to detect small but significant changes. During our analysis, accurate segmentation was challenging in eyes with macular edema. We attempted to correct any erroneous segmentation manually and used a total retinal slab to account for any erroneous segmentation and/or projection artifacts in the SCP and DCP. This total retinal slab was bounded by the ILM as its upper boundary and the RPE, offset by 40 μm above the RPE, as its lower boundary. A similar study used an "inner retinal slab" with an inner boundary at 3 μm beneath the ILM and an outer boundary set at 70 μm beneath the inner plexiform layer (IPL) [13]. Although this segmentation can compensate for flow signals displaced by DME, however, it also depends on identification of retinal anatomical landmarks (IPL) which may be deceiving and difficult in the presence of DME. However, our customized total retinal slab

depends on recognition of the RPE, which is fairly robust despite the presence of inner retinal pathology. In addition, although we excluded images of obvious poor quality, the possibility of a measurement error due to the numerous artifacts associated with OCTA should be considered.

Strengths of the present study include that included patients had received up to three intravitreal anti-VEGF injections. To the best of our knowledge, this is the first study to analyze changes in VD in patients with PDR treated with anti-VEGF agents. Also, we compared VD measurements in patients' eyes injected with all major anti-VEGF agents, and treatment naïve versus previously treated patients.

In conclusion, our study showed no significant change in vessel density after one, two and three intravitreal anti-VEGF injections in both the DME and PDR subgroups. Neither the type of injected anti-VEGF agent nor previous treatment was associated with a change in VD. In addition, the response of DME to treatment was not correlated with changes in VD in the SCP, DCP or TCP. This further demonstrates that in the short-term, anti-VEGF injections do not impair foveal circulation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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