

Foveal Microvascular Integrity Association With Anti-VEGF Treatment Response for Diabetic Macular Edema

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Received: February 15, 2021

Accepted: July 1, 2021

Published: July 29, 2021

Citation: Huang WH, Lai CC, Chuang LH, et al. Foveal microvascular integrity association with anti-VEGF treatment response for diabetic macular edema. *Invest Ophthalmol Vis Sci.* 2021;62(9):41. <https://doi.org/10.1167/iovs.62.9.41>

PURPOSE. To investigate the association between foveal microvascular integrity and anti-vascular endothelial growth factor (VEGF) treatment response for diabetic macular edema (DME).

METHODS. This retrospective study enrolled 58 eyes (from 45 patients) with DME. Treatment strategy was three to five monthly anti-VEGF injections followed by a PRN protocol. Treatment with an intravitreal corticosteroid would be considered for persistent DME after five consecutive anti-VEGF injections. Eyes achieving a treatment-free interval \geq four months within two years were classified into the good clinical course group (group 1). Eyes with frequent recurrent edema (treatment-free interval $<$ four months) or requiring an intravitreal corticosteroid within two years were classified into the suboptimal clinical course group (group 2). Foveal microvascular integrity was evaluated by two continuous variables, that is, vessel density (%) within a width of 300 μ m around the foveal avascular zone (FD-300) on optical coherence tomography angiography (OCTA) and perifoveal leakage (area %) on fluorescein angiography (FA).

RESULTS. There were 37 eyes in group 1 and 21 eyes in group 2. FD-300 (odds ratio 0.733, 95% CI 0.620–0.867, $P < 0.001$) and perifoveal leakage (odds ratio 1.064, 95% CI 1.007–1.124, $P = 0.027$) were significantly associated with suboptimal clinical course. Area under curve (AUC) was 0.820 for FD-300 and 0.723 for perifoveal leakage in predicting clinical course. FD-300 was negatively correlated with perifoveal leakage (coefficient = -0.325 , $P = 0.014$).

CONCLUSIONS. Compromised foveal microvascular integrity, represented by lower FD-300 and more severe perifoveal fluorescein leakage, was associated with suboptimal clinical course in anti-VEGF treatment for DME. A negative correlation between FD-300 and perifoveal leakage existed.

Keywords: diabetic macular edema, antivasular endothelial growth factor, fluorescein angiogram, optical coherence tomography, optical coherence tomography angiography, foveal vessel density

Diabetic macular edema (DME) may affect up to 7% of diabetic people and is one of the most important causes of visual loss in these patients.¹ Anti-vascular endothelial growth factor (VEGF) provided a paradigm shift in the treatment of DME and significantly improves the visual outcome.² However, the treatment response differs among individuals, with the required number of injections varying from a few injections per year to almost monthly injections.^{2,3} Macular edema could persist in 32% to 66% of patients after 24 weeks of anti-VEGF treatment, and 44% to 68% of these patients may develop chronic persistent DME through two years.⁴ It is important to identify factors associated with treatment response for DME. In patients with persistent or frequently recurrent DME, an early switch to intravitreal corticosteroids may lead to better functional and anatomical outcomes.^{5,6} It

could also reduce the treatment burden and improve patient compliance.^{7,8}

Multimodal images are useful tools for the diagnosis and the monitoring of DME. Fluorescein angiography (FA) has been regarded as the gold standard for evaluating microvascular structural and functional changes in retinal vascular diseases.⁹ Optical coherence tomography (OCT) and OCT angiography (OCTA) are rapid and noninvasive technologies that enable the quantification of the structural and microvascular changes in retinal diseases.⁹ Many new qualitative and quantitative parameters have been explored. For instance, intraretinal hyperreflective foci, disorganization of retinal inner layers (DRIL), disruption of the ellipsoid zone line, parafoveal vessel density, and morphological change of foveal avascular zone (FAZ) have been found to be

associated with the anatomical or the functional outcomes of DME.^{10–14} However, whether these imaging parameters could predict the anti-VEGF injection intervals and the number of required injections in patients with DME have remained largely unknown.

We hypothesized that pathological changes of microvasculature around the fovea might associate with recalcitrant DME. This study evaluated the association between foveal microvascular integrity and the clinical course under anti-VEGF treatment for DME. We aimed to identify the patients with good versus suboptimal clinical course—defined by whether they have achieved a treatment-free interval \geq four months within two years. This may help customize treatment strategies in DME.

METHODS

Patients

This retrospective study enrolled patients with DME receiving anti-VEGF treatment at Keelung Chang Gung Memorial Hospital between August 2016 and August 2019. The study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital (IRB no. 202001542B0) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was waived by the Chang Gung Memorial Hospital Institutional Review Board (IRB no. 202001542B0). The inclusion criteria were type 2 diabetic patients with any stage of diabetic retinopathy and central-involving DME. Both focal and diffuse edema were eligible. The exclusion criteria were (1) macular edema caused by other ocular diseases (such as Irvine-Gass syndrome, retinal vein occlusion, age-related macular degeneration, and so on); (2) presence of epiretinal membrane or tractional retinal detachment; (3) prior anti-VEGF treatment; (4) prior intravitreal or periocular corticosteroid injection; (5) retinal photocoagulation within three months prior to enrollment; (6) prior vitrectomy surgery; (7) vitreous hemorrhage obscuring details in retinal images; (8) poor OCTA image quality (scan quality index $< 6/10$), (9) timing of OCTA capture > 24 months from baseline; and (10) follow-up duration < 1 year or inadequate for determining the clinical course.

Each patient underwent best-corrected visual acuity (BCVA), color fundus photography, FA, OCT, and OCTA at baseline. BCVA, OCT, and OCTA were repeated at each follow-up visit. The anatomical and functional changes of foveal microvasculature were evaluated by OCTA and FA, respectively.

The treatment protocol of DME in our hospital was compliant with the reimbursement criteria of the National Health Insurance of Taiwan and the recommendations from experts' consensus in Taiwan.¹⁵ Anti-VEGF treatment (aflibercept, ranibizumab, or bevacizumab) for DME was initiated when central retinal thickness (CRT) was ≥ 300 μm . The loading phase consisted of three to five monthly anti-VEGF injections. During the maintenance phase, further injections could be performed in a pro re nata (PRN) protocol if persistent or recurrent macular edema was found. Persistent and recurrent macular edema was defined by the presence of sub-/intraretinal fluid and CRT ≥ 300 μm . If the macular edema persisted after five consecutive anti-VEGF injections, treatment could be switched to intravitreal corticosteroid [0.7 mg dexamethasone intravitreal implant (Ozurdex) or triamcinolone acetonide 2 mg/0.05 ml] at the physician's discretion. Focal retinal photocoagulation

could be used after six months. Patients were examined monthly in the first six months. For those patients with stable macular condition, the follow-up interval could be gradually extended to up to three months. In this study, the treatment-free interval was defined as the period absent of recurrent macular edema and with no anti-VEGF injection administered. Eyes were classified according to their clinical course during anti-VEGF treatment. Eyes would be classified into the good clinical course group (group 1) if they could achieve a treatment-free interval ≥ 4 months within two years. They would be put into the suboptimal clinical course group (group 2) if they had frequent recurrent macular edema and were unable to achieve a treatment-free interval ≥ 4 months within two years. Eyes that required intravitreal corticosteroid for persistent macular edema after the loading phase were also classified into group 2. Eyes with complete resolution of the DME after injection but with frequent recurrence (interval < 4 months) were classified as group 2 because of the frequent injections required.

OCT and OCTA Parameters

OCT and OCTA images were obtained using an AngioVue (Optovue RTVue XR Avanti; Optovue Inc., Fremont, CA, USA) machine. The CRT on OCT was the average thickness over the 1 mm diameter central subfield in the ETDRS circle.

OCTA scans of 3×3 mm² centered on the fovea were used. OCTA images at macular edema remission (or minimized) stage were analyzed. The built-in AngioAnalytics software (version 2017.1.0.151) was employed to make all OCTA measurements. The quantitative analysis of FAZ was conducted using OCTA images of the whole inner retinal layer. FAZ was defined as the area encompassing the central fovea where there are no vessels. The foveal vessel density within a width of 300 μm around the FAZ (FD-300) (Fig. 1), FAZ size, FAZ perimeter, and a-circularity index were all automatically calculated using the machine software.

Parafoveal vessel densities for both superficial vascular plexus (SVP) and deep vascular plexus (DVP) were collected. The autosegmentation default for the SVP slab includes vasculature between the internal limiting membrane (ILM) and 10 μm above the inner plexiform layer (IPL). The default for the DVP slab includes the vasculature between 10 μm above IPL and 10 μm below the outer plexiform layer. (Fig. 1) Projection artifact removal algorithm is available in this version of the software. Manual correction for the segmentation error was required in about half of the eyes. The correction was done by a senior ophthalmologic resident (WHH) and confirmed by a retinal specialist (LY).

Automated Quantification of Perifoveal Leakage on FA

Pretreatment FA images obtained using Heidelberg Retina Angiograph 2 (HRA 2; Heidelberg Engineering, Heidelberg, Germany) were analyzed. Two images of 768×768 pixels obtained at one and five minutes after dye injection (Figs. 2A, B), were selected from each eye. The size of the field of view was 30×30 degrees. The images were processed using ImageJ software (Fiji, National Institutes of Health, Bethesda, MD, USA. <https://imagej.net/ImageJ>) in the following steps. (1) *Image alignment and crop*. The images were automatically aligned with each other in a stack (**Linear Stack Alignment with SIFT**). Images of 384×384 pixels centered at

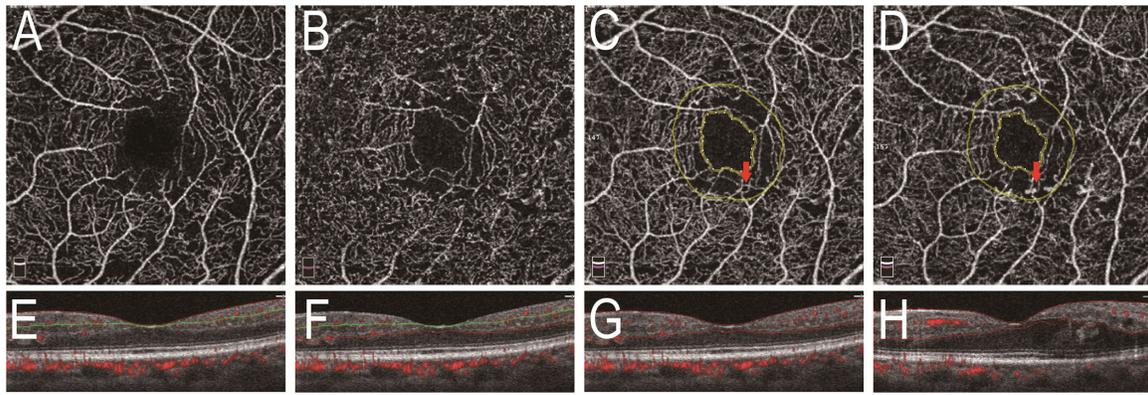


FIGURE 1. Optical coherence tomography angiography (OCTA) images of an eye from suboptimal clinical course group (group 2). (A) Superficial vascular plexus (SVP) slab. (B) Deep vascular plexus (DVP) slab. (C) Full inner retinal layer slab in macular edema remission stage. *Yellow lines* demarcate boundaries of FD-300. (D) Full inner retinal layer slab in macular edema recurrent stage. When comparing to the edema remission stage (C), a missing vessel (*red arrow*) and a few prominent microaneurysms could be found at the recurrent macular edema stage (D). However, the FD-300 values were similar [39.4% in (C) versus 40.2% in (D)] in the two timings. (E–H) B-scans showing corresponding location of segmentation of slabs in (A–D).

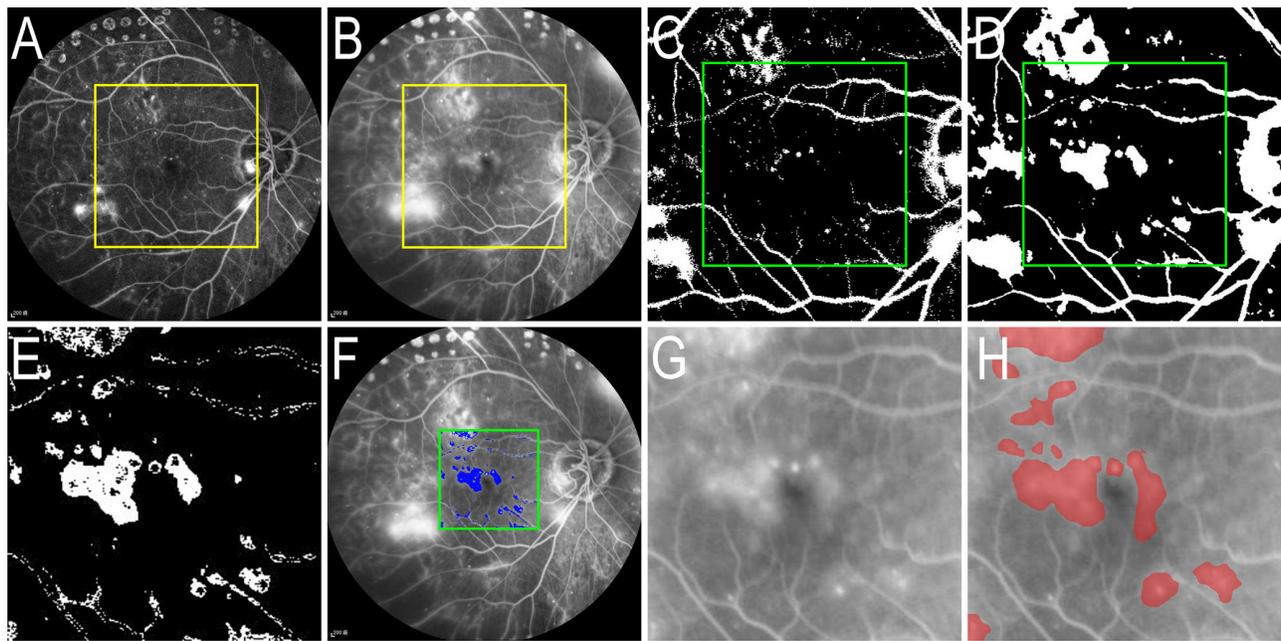


FIGURE 2. Fluorescein angiography (FA) images of an eye from the good clinical course group (group 1). (A) Image at 1 min. (B) Image at 5 min. (C, D) Images after automatic alignment and binarization of areas within *yellow squares* in (A, B). (E) Resultant image obtained by subtracting 1-min image from 5-min image within *green squares* in (C, D). Percentage area of perifoveal leakage (*whitish area*) was 21.4% in this image. (F) Superimpose the perifoveal leakage map superimposed on the original 5-min FA. (G) A perifoveal 5-min FA image used for validation process. (H) The perifoveal leakage area was manually annotated with *red color*.

fovea were cropped in the stack. (2) *Image thresholding and calculation.* The background brightness gradient (**Subtract Background**, rolling = 50 pixels) was removed, followed by binarization (**Auto Threshold**, method = default) of each image (Figs. 2C, D). The fluorescein leakage map at five min was created by subtracting the 1-min images from the 5-min images (**Image Calculator**). (3) *Feature extraction.* The images were cropped into 240 × 240 pixels, that is, the length of this square equals approximately the diameter of the perifoveal area in the ETDRS circle. The area percentage of perifoveal leakage was defined by the percentage of

whitish area in the final image (**Area fraction**) (Fig. 2E). The above steps could be automatically executed by ImageJ using Macros, except that a trained grader was required to select the location of the foveal center in Step (1).

The validation process involved 20 eyes (10 DME and 10 branch retinal vein occlusion) randomly selected from our FA image database. The perifoveal leakage in the 5-min images were manually annotated by a masked grader (YTL) using Photoshop CS6, version 13.0 × 64 (Adobe Systems Inc., San Jose, CA, USA) (Figs. 2G, H). The manually annotated leakage area was compared with the leakage area

calculated from the above image processing method. The intraclass correlation coefficient (ICC) was 0.764.

Statistical Analysis

The demographic and clinical characteristic differences between group 1 and group 2 were compared using generalized estimating equations (GEE). GEE models were also employed to determine the association between foveal microvascular imaging parameters and clinical course. This approach is particularly useful for ophthalmology studies, as it can account for the correlation between fellow eyes.¹⁶ Receiver operating characteristic (ROC) curves and the area under curve (AUC) were used for evaluating the predictive power of imaging parameters. Partial correlation was used to evaluate the relationship between FD-300 and perifoveal leakage after adjusting for the FAZ perimeter. All statistical analyses were conducted using IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA). A *P* value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 58 eyes with DME from 45 diabetic patients were analyzed in this study. Seven eyes had received panretinal photocoagulation and three eyes had received focal retinal photocoagulation before enrollment. The mean age of patients was 59.6 ± 9.4 years and 25 (56%) of the patients were male. Among eyes with DME, 37 (64%) were classified into the good clinical course group (group 1) and 21 (36%) were classified into the suboptimal clinical course group (group 2). Representative cases were illustrated in Fig. 3 and Fig. 4. Table 1 lists the demographic data and clinical characteristics of both groups. The mean posttreatment

follow-up duration was 33.2 ± 11.4 months. Forty-three (74%) eyes had a follow-up duration \geq two years. Twelve eyes in group 1 and three eyes in group 2 had follow-up duration < two years. Those three eyes in group 2 had follow-up durations of 15, 16, and 16 months, respectively. They started receiving intravitreal Ozurdex injections for persistent macular edema after seven, six, and eight consecutive aflibercept injections, respectively. There were no significant differences between the two groups in age, sex, DM duration, baseline glycosylated hemoglobin, incidence of hypertension, baseline BCVA, CRT at all time points, lens status, severity of diabetic retinopathy, all OCTA-derived parameters except FD-300, follow-up duration, panretinal photocoagulation treatment required, and focal retinal photocoagulation required.

Comparing with group 1, group 2 had worse BCVA at six and 12 months, more severe perifoveal leakage on FA, lower FD-300, and more eyes requiring intravitreal corticosteroid injections. Group 2 also had a significantly higher number of anti-VEGF injections in the first year (5.6 versus 4.9, *P* = 0.037) and in the entire study period (12.8 versus 6.3, *P* < 0.001).

In GEE models, FD-300 and perifoveal leakage were significantly associated with anti-VEGF treatment response after adjusting for age, sex, and severity of diabetic retinopathy (Table 2). The AUC was 0.820 for FD-300 and 0.723 for perifoveal leakage in predicting the suboptimal clinical course. A cutoff at FD-300 < 42.1 resulted in a sensitivity of 85.7% and a specificity of 73.0%. When using perifoveal leakage > 30.6 as the cutoff, the sensitivity was 71.4% and the specificity was 62.2%. FD-300 was negatively correlated with perifoveal leakage (partial correlation coefficient = -0.325 , *P* = 0.014) after adjusting for FAZ perimeter (Fig. 5).

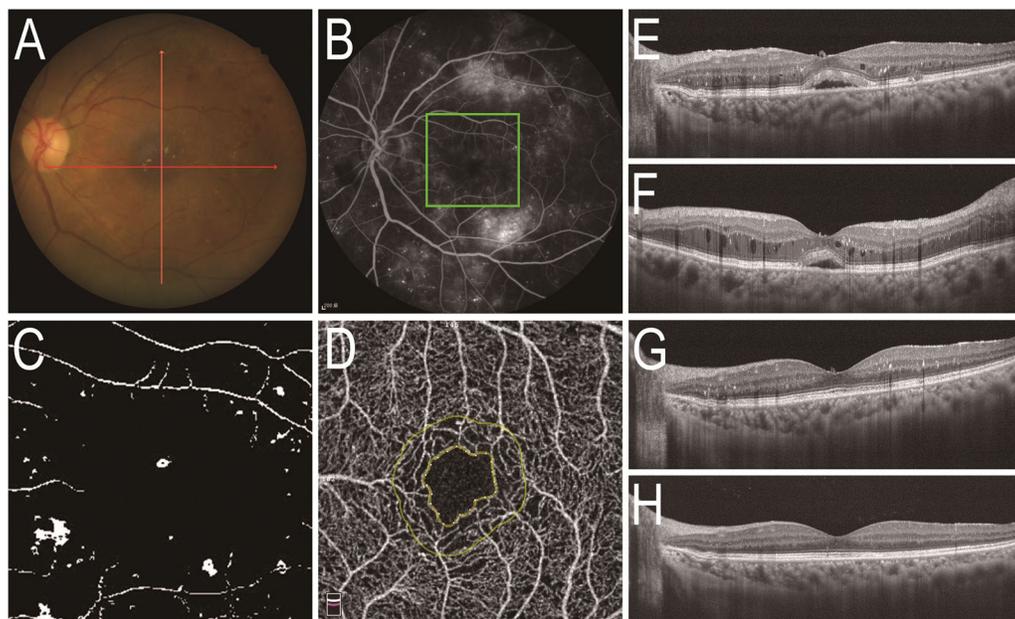


FIGURE 3. A representative patient from the good clinical course group (group 1). (A) Color fundus photo and (B) 5-min fluorescein angiography (FA) image at baseline. (C) Minimal perifoveal leakage could be found on the resultant image of automated FA leakage quantification. Percentage area of perifoveal leakage (whitish area) was 10.8%. (D) A full inner retinal layer slab of an OCTA image in the macular edema remission stage. Capillaries around the foveal avascular zone were generally intact. FD-300 was 45.7%. (E) The baseline horizontal optical coherence tomography (OCT) B-scan corresponding to the location of red arrow in (A). (F) The baseline vertical OCT B-scan corresponding to the location of orange arrow in (A). (G) Horizontal OCT at four months when three monthly anti-VEGF injections had been administered. (H) Horizontal OCT at 12 months when anti-VEGF injections had not been administered for eight months. No evidence of recurrent macular edema within this period.

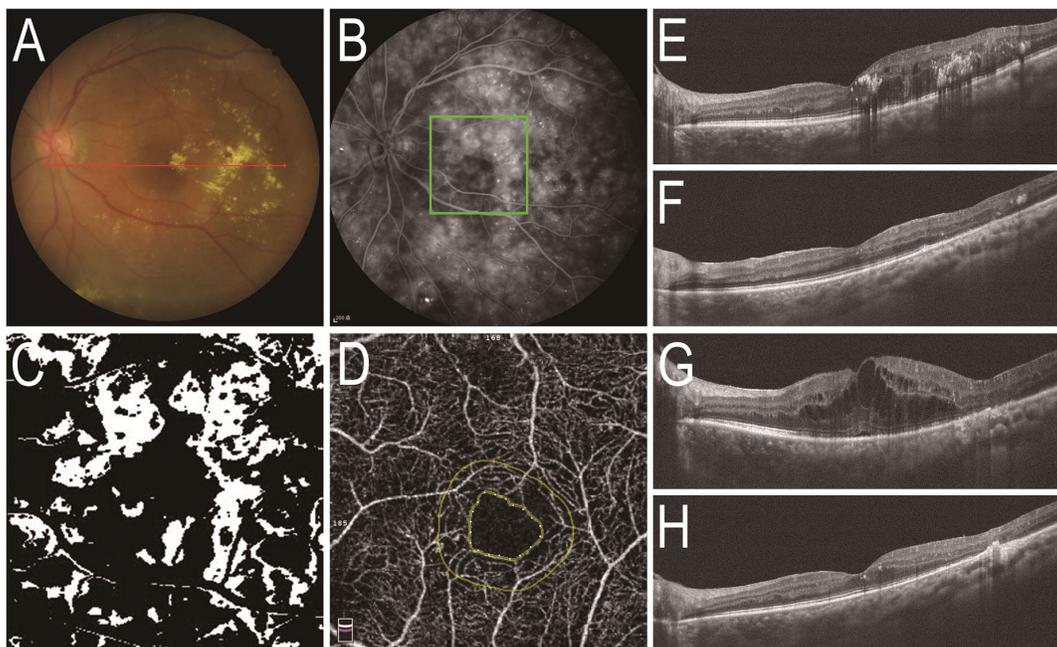


FIGURE 4. A representative patient from the suboptimal clinical course group (group 2). (A) Color fundus photo and (B) 5-min fluorescein angiography (FA) image at baseline. (C) Extensive perifoveal leakage could be found on the resultant image of automated FA leakage quantification. Percentage area of perifoveal leakage (whitish area) was 44.0%. (D) A full inner retinal layer slab of an OCTA image in the macular edema remission stage. Mild capillary loss could be found over temporal region. FD-300 was 40.1%. (E) Baseline horizontal optical coherence tomography (OCT) B-scan corresponding to the location of red arrow in (A). (F) Horizontal OCT at seven months when five monthly anti-VEGF injections had been administrated. (G) Horizontal OCT at 13 months when anti-VEGF injections had not been administrated for 3.5 months. Severe recurrent macular edema was found. (H) Horizontal OCT at two months after resuming anti-VEGF injections. Macular edema resolved completely.

TABLE 1. Demographic Data and Clinical Characteristics

	Group 1 (37 Eyes) ^a	Group 2 (21 Eyes) ^a	P Value ^b
Age	60.6 ± 9.3	56.6 ± 9.5	0.163
Sex, female:male, n (%)	15:22 (40:61)	11:10 (52:48)	0.453
Diabetes mellitus duration, years	12.9 ± 7.3	12.5 ± 9.9	0.892
Baseline HbA1c, %	7.8 ± 1.1	7.7 ± 1.5	0.853
Hypertension, n (%)	25 (68)	11 (52)	0.321
Best corrected visual acuity, logMAR			
Baseline	0.51 ± 0.25	0.54 ± 0.29	0.657
6 months	0.44 ± 0.24	0.64 ± 0.41	0.013
12 months	0.39 ± 0.21	0.71 ± 0.38	<0.001
Central retinal thickness, μm			
Baseline	380 ± 99	419 ± 93	0.132
6 months	282 ± 42	312 ± 93	0.169
12 months	290 ± 50	340 ± 116	0.061
Pseudophakia, n (%)	5 (14)	4 (19)	0.642
Severity of DR, NPDR:PDR, n (%)	22:15 (59:41)	12: 9 (57:43)	0.884
Perifoveal leakage on FA, area %	27.6 ± 11.8	36.7 ± 11.6	0.006
Optical coherence tomography angiography			
Timing of OCTA capture, months from baseline	7.8 ± 6.9 (median 7; range 0–24)	10.4 ± 8.4 (median 8; range 0–24)	0.286
Scan quality index	6.9 ± 0.9	6.9 ± 0.7	0.866
Parafoveal vessel density in SVP, %	40.1 ± 3.6	40.6 ± 3.7	0.614
Parafoveal vessel density in DVP, %	42.1 ± 5.2	41.4 ± 5.1	0.564
FD-300, %	43.5 ± 3.8	38.7 ± 4.1	<0.001
FAZ area, mm ²	0.33 ± 0.11	0.31 ± 0.08	0.353
FAZ perimeter, mm	2.44 ± 0.53	2.48 ± 0.56	0.820
FAZ a-circularity index	1.21 ± 0.11	1.27 ± 0.18	0.125
Duration of follow-up, months	32.0 ± 11.6 (range 13–54)	35.5 ± 11.9 (range 15–54)	0.322
Number of anti-VEGF injections			
Within the first year	4.9 ± 1.2	5.6 ± 1.3	0.037
Total	6.3 ± 2.1	12.8 ± 5.3	<0.001
Other treatments within study period			
Panretinal photocoagulation, n (%)	14 (38)	11 (52)	0.365
Focal retinal photocoagulation, n (%)	6 (16)	7 (33)	0.109
Intravitreal corticosteroid, n (%)	0 (0)	9 (43)	<0.001^c

^a Data are expressed as mean ± standard deviation (SD) unless otherwise indicated.

^b The difference between group 1 and group 2 was compared using generalized estimating equations (GEE) to account for the intraindividual correlation between the eyes from the same patient. The linking function was *identity* and distribution was *normal* for the continuous variable. The linking function was *logit* and distribution was *binomial* for the binary variable.

^c The difference between group 1 and group 2 was compared using Fisher's exact test.

DVP, deep vascular plexus; DR, diabetic retinopathy; FA, fluorescein angiography; FAZ, foveal avascular zone; FD-300, vessel density of the whole inner retinal layer within a width of 300 μm around the foveal avascular zone; HbA1c, glycated hemoglobin; logMAR, logarithm of the minimum angle of resolution; NPDR, nonproliferative diabetic retinopathy; OCTA, optical coherence tomography angiography; PDR, proliferative diabetic retinopathy; SVP, superficial vascular plexus; VEGF: vascular endothelial growth factor. Bold values indicate $P < 0.05$.

TABLE 2. Generalized Estimating Equations in Identifying Eyes with Suboptimal Clinical Course

Imaging Parameter (Unit)	Odds Ratio ^a	95% Confidence Interval	P Value ^b
Perifoveal leakage on FA (area %)	1.064	1.007–1.124	0.027
Parafoveal vessel density in SVP (%)	1.039	0.872–1.238	0.669
Parafoveal vessel density in DVP (%)	0.957	0.828–1.105	0.549
FD-300 (%)	0.733	0.620–0.867	<0.001
FAZ area (mm ²)	0.055	0–14.844	0.310
FAZ perimeter (mm)	1.028	0.348–3.034	0.960
FAZ a-circularity index	16.748	0.300–935.863	0.170

^a Odds ratio represents the change in the risk of suboptimal clinical course if the value of imaging parameter rises by one numerical unit in that variable.

^b After adjusting for age, sex, and severity of diabetic retinopathy.

DVP, deep vascular plexus; FA, fluorescein angiography; FAZ, foveal avascular zone; FD-300, vessel density of the whole inner retinal layer within a width of 300 μ m around the foveal avascular zone; SVP, superficial vascular plexus. Bold values indicate $P < 0.05$.

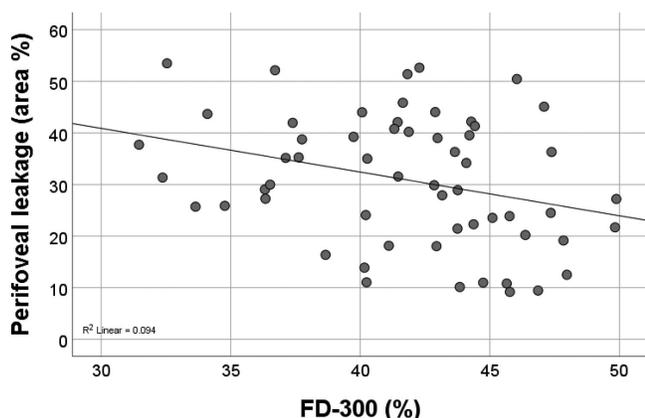


FIGURE 5. Scatter plot showing the relationship between FD-300 and perifoveal leakage.

DISCUSSION

Our results suggested that foveal microvascular integrity, represented by FD-300 on OCTA and perifoveal leakage on FA, was associated with the clinical course in patients with DME receiving anti-VEGF treatment. Furthermore, we demonstrated that FD-300 was negatively correlated with perifoveal leakage.

Most DME patients required repeat anti-VEGF injections.⁴ PRN regimen is commonly adopted.^{15,17,18} However, frequent clinical visits for monitoring recurrence is needed. Recent treat-and-extend studies have showed that approximately 41% to 67% of patients were able to extend their injection interval to ≥ 16 weeks at two years, while other patients still required frequent injections in four to 12-week intervals.^{19,20} Early identification of particular treatment responses could help optimize treatment strategy and minimize treatment burden (e.g., follow-up interval, switching to corticosteroid). Therefore, the treatment response in this study was classified by *achieving a treatment-free interval \geq four months within two years*.

In practice, a physician may switch to intravitreal corticosteroid if DME persists after the anti-VEGF loading phase.^{7,8} Early switching in nonresponders could benefit visual outcome.^{5,6} Therefore, these patients, together with those who require frequent anti-VEGF injections, were all classified into group 2 in our study. Our data showed that group 2 had a substantially higher number of anti-VEGF injections than group 1 within the study period. In addition, comparing to group 1, eyes in group 2 had worse BCVA at

six and 12 months. These results support that our classification scheme could be useful for indicating the medium- to long-term treatment burden and visual outcome in patients with DME.

FD-300 is a new OCTA-derived biomarker which measures the vessel density within 300 μ m around the FAZ. This study suggested that decreased vessel density over this juxtafoveal region was associated with persistent or frequent recurrent DME. Microvascular alterations could be either causative or secondary to DME. Previous studies suggested that the integrity of microvasculature around the FAZ may play a critical role in the homeostasis of fluid.²¹ Lower FD-300 could represent more severe damage in juxtafoveal capillaries, resulting in fluid accumulation. On the other hand, chronic fluid accumulation itself could aggravate microvascular pathological changes.²¹ Low FD-300 could also imply chronic macular edema, thus requiring a higher number of anti-VEGF injections.

Prior research reported that SVP vessel density could be a predictor for visual improvement after the loading phase.²² Microvascular impairment in the DVP could also be associated with less CRT reduction after the loading phase.²³ However, SVP and DVP vessel densities showed no difference between the two groups in this study. This may be attributed to different study designs. Instead of evaluating the response after the anti-VEGF loading phase, we focused on the medium- to long-term clinical course. The protocols for SVP/DVP segmentation were also different. In fact, one of the advantages of using FD-300 as an OCTA biomarker is that segmentation of SVP/DVP is not required, thus minimizing possible bias from segmentation error commonly found in DME.²⁴

The FAZ area, FAZ perimeter, and FAZ a-circularity index on OCTA also did not show prognostic values in this study. A possible explanation is that the enrolled eyes were with diabetic retinopathy of different severity, which may lead to remarkable variations in the FAZ area, FAZ perimeter, and a-circularity index.²⁵

Theoretically, it is better to use baseline OCTA for analysis. However, a main difficulty is that patients with DME usually have poor visual acuity and fixation before treatment; this leads to inadequate OCTA image quality. In contrast, using OCTA images at the macular edema remission (or minimized) stage is acceptable for the following reasons.

First, the DME could have been in existence for a long time before diagnosis. In this instance, even if the baseline OCTA image were used, it could not avoid the effect of heterogeneous disease duration. Second, better OCTA image quality could be obtained at the macular edema remission

(or minimized) stage and the higher quality image would enable a more reliable quantitative analysis. Third, recent studies have shown no significant changes on macular vessel density and FAZ metrics between pre- and posttreatment OCTA in DME, at least in the short term.^{13,26}

Persistent DME was more prevalent in the suboptimal clinical course group. While one may be concerned that the intraretinal fluid could cause artificially lower FD-300 values, prior studies suggest that macular vessel density and FAZ metrics could still be reproducible independent of the presence of cystoid edema.^{13,26,27} Our own experience is that quantitative analysis is unreliable in severe macular edema, but the measurement of vessels around FAZ could still be reliable if the macular edema is mild. A representative case was shown in Figure 1(C, D).

FA is a useful tool for evaluating the integrity and permeability of retinal microvasculature.⁹ However, the dynamic change and diffuse intensity gradient of fluorescein leakage on FA images posed difficulties in quantification and thus limited its clinical usefulness. This study developed a simple and automated method for quantifying the severity of perifoveal fluorescein leakage using widely available standard FA images and a publicly available software (ImageJ). The ICC was 0.764 in validation, which indicated an acceptable reliability. Perifoveal area was selected for analysis because quantitative vascular changes in the posterior pole have been shown to be more critical than those in the peripheral part of the retina.²⁸

Severe perifoveal leakage on FA was associated with persistent or frequent recurrent DME in our study. FA is a sensitive tool for identifying changes in the blood-retinal barrier. Localized fluorescein leakage could be found in the preretinopathy stage from patients with type 2 diabetes.²⁹ The fluorescein leakage could result from increased intravitreal VEGF level.³⁰ Vascular or cellular injuries around FAZ may also contribute to the severity of fluorescein leakage.^{21,31} This could be supported by our observation that more severe fluorescein leakage is negatively correlated with lower FD-300. More severe perifoveal fluorescein leakage could represent more severe microvascular damage, which could lead to recalcitrant DME and a higher number of required anti-VEGF injections.

Among 13 patients with both eyes included, 11 patients (85%) had two eyes that behaved similarly and two patients (15%) had two eyes that behaved differently in terms of response to anti-VEGF. We conducted GEE analysis among these 26 eyes and found that there was no significant difference among the two eyes within same patient in term of response to treatment ($P = 0.130$). In other words, the two eyes from the same patient could respond similarly to anti-VEGF treatments.

This study has several limitations. First, an extensive list of variables may skew the findings toward false positive results. However, while many variables were collected in our demographic data to help better represent our subject characteristics, only seven variables (listed in Table 2) were used as the main factors under investigation. Because of the small sample size, Bonferroni correction was not performed. The correction would reduce type I error at the expense of increasing type II error.³² Second, being a retrospective study, the compliance of treatment protocol is not as strict as that of a clinical trial. However, the real-world setting of this study could generate useful clinical applications. Third, OCTA images with poor quality were not eligible for analysis. This could restrict the generalizability of results.

In conclusion, the present findings demonstrated that foveal microvascular integrity was associated with anti-VEGF treatment response and clinical course in patients with DME. Quantitative parameters, including lower FD-300 on OCTA and more severe perifoveal leakage on FA were associated with persistent or frequent recurrent DME in anti-VEGF treatment. A negative correlation could be found between FD-300 and perifoveal leakage. This indicated that anatomical and functional changes of foveal microvasculature may occur in a parallel manner. Both parameters could be potential biomarkers for determining the prognosis and treatment response in DME.

Acknowledgments

Supported by grants from Chang Gung Memorial Hospital (CMRPG2J0211 and CMRPG2J0212).

The data is not publicly available due to the involvement of human participants. Nevertheless, upon reasonable request, the data is available from the corresponding author after obtaining Chang Gung Memorial Hospital Institutional Review Board approval.

The imaging processing codes are available from the corresponding author upon reasonable request.

Disclosure: **W.-H. Huang**, None; **C.-C. Lai**, None; **L.-H. Chuang**, None; **J.C.-C. Huang**, None; **C.-H. Wu**, None; **Y.-T. Lin**, None; **L. Yeung**, None

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