Visual Acuity Is Correlated with the Area of the Foveal Avascular Zone in Diabetic Retinopathy and Retinal Vein Occlusion

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Purpose: To determine if the area of the foveal avascular zone (FAZ) is correlated with visual acuity (VA) in diabetic retinopathy (DR) and retinal vein occlusion (RVO).

Design: Cross-sectional study.

Participants: Ninety-five eyes of 66 subjects with DR (65 eyes), branch retinal vein occlusion (19 eyes), and central retinal vein occlusion (11 eyes).

Methods: Structural optical coherence tomography (OCT; Spectralis, Heidelberg Engineering) and OCT angiography (OCTA; Avanti, Optovue RTVue XR) data from a single visit were analyzed. FAZ area, point thickness of central fovea, central 1-mm subfield thickness, the occurrence of intraretinal cysts, ellipsoid zone disruption, and disorganization of retinal inner layers (DRIL) length were measured. VA was also recorded. Correlations between FAZ area and VA were explored using regression models. Main outcome measure was VA.

Results: Mean age was 62.9 ± 13.2 years. There was no difference in demographic and OCT-derived anatomic measurements between branch retinal vein occlusion and central retinal vein occlusion groups (all P > 0.058); therefore, data from the 2 groups were pooled together to a single RVO group for further statistical comparisons. Univariate and multiple regression analysis showed that the area of the FAZ was significantly correlated with VA in DR and RVO (all P < 0.003). The relationship between FAZ area and VA varied with age (P = 0.026) such that for a constant FAZ area, an increase in patient age was associated with poorer vision (rise in logarithm of the minimum angle of resolution visual acuity). Disruption of the ellipsoid zone was significantly correlated with VA in univariate and multiple regression analysis (both P < 0.001). Occurrence of intraretinal cysts, DRIL length, and lens status were significantly correlated with VA in the univariate regression analysis (P ≤ 0.018) but not the multiple regression analysis (P ≥ 0.210). Remaining variables evaluated in this study were not predictive of VA (all P ≥ 0.225).

Conclusions: The area of the FAZ is significantly correlated with VA in DR and RVO and this relationship is modulated by patient age. Further study about FAZ area and VA correlations during the natural course of retinal vascular diseases and following treatment is warranted. Ophthalmology 2016;123:2352-2367 © 2016 by the American Academy of Ophthalmology.

Supplemental material is available at www.aaojournal.org.

Diabetic retinopathy (DR) is the leading cause of vision loss in the working-age population in developed countries.1 According to data from the National Eye Institute, there were approximately 7.7 million diagnosed cases of DR in the United States alone in 2010.2 Retinal venous occlusion (RVO), due to central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO), is the second most common retinal vascular disorder leading to significant vision loss, with an estimated number of global cases in 2010 exceeding 16.4 million.3 The prevalence of DR and RVO is projected to increase to pandemic portions over the next 30 years, as is the socioeconomic burden associated with these conditions.4 Thus, there is an urgent need to improve our understanding of the pathophysiologic mechanisms and anatomic correlates for significant visual loss due to these disorders.

The foveal avascular zone (FAZ) is a specialized region of the human retina that approximates the region of highest cone photoreceptor density and oxygen consumption.5,6 Histologic techniques7–9 and a range of in vivo imaging modalities10–19 have highlighted the variability in FAZ topology in normal human eyes. In healthy eyes, the size of
the FAZ does not seem to influence visual function, but the relationship between FAZ size and visual acuity (VA) in retinal vascular diseases remains a matter of conjecture. Much of our understanding concerning the relationship between FAZ topology and visual function has been attained from studies that utilized fluorescein angiography (FA) techniques to visualize the retinal circulation. Although these studies significantly aided our understanding of the pathogenic mechanisms leading to vision loss in retinal vascular diseases, they seldom accounted for the influence of other anatomic changes, such as ellipsoid zone (EZ) disruption, that may have also affected visual function.

Volumetric imaging using optical coherence tomography angiography (OCTA) permits rapid, noninvasive evaluation of FAZ dimensions and provides quantitative vascular information comparable to histologic examination. The technique of OCTA thus appears suited for delineating relationships between the morphometric properties of the FAZ and VA. In this report, a biomorphometric analysis incorporating OCTA and structural optical coherence tomography (OCT) data is used to determine the significant predictors of VA in DR and RVO. We show that FAZ area is an independent predictor of VA in DR and RVO. We also demonstrate that patient age modulates the relationship between FAZ area and VA in retinal vascular diseases. The results of this report, therefore, have important clinical applications.

Methods

This study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at North Shore Long Island Jewish Health System. Data were stored and managed in compliance with guidelines from the Health Insurance Portability and Accountability Act.

Subjects

Consecutive cases of DR, BRVO, and CRVO seen between August 2014 and October 2015 by 2 retina specialists (L.A.Y. and K.B.F.) at Vitreous Retina Macula Consultants of New York were enrolled in this study. Treatment-naïve and treated cases were included. The diagnosis of diabetes mellitus was based on the results of fasting blood samples and glycosylated hemoglobin (HbA1c). Only patients with clinical signs of diabetic retinopathy, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) grading criteria, were included in this study (Fig 1). Patients with diabetes mellitus without evidence of diabetic retinopathy (level 10 scale ETDRS criteria) were not included in this study. Central retinal vein occlusion was determined by the presence of retinal vein dilation, retinal edema, or scattered superficial or deep hemorrhages with or without the presence of optic disc edema/hyperemia (Fig 1). Branch retinal vein occlusion was determined by the presence of the same clinical features as CRVO, but confined to a focal region in the retina corresponding to a specific arteriovenous crossing (Fig 1). Long-standing vein occlusion was determined by the presence of occluded or sheathed retinal veins and/or vascular anastomoses. With the exception of 4 cases (1 case of BRVO and 3 cases of DR), the clinical evaluation of all subjects included imaging with FA (Optos 200Tx [Optos, Dunfermline, Scotland, United Kingdom], Topcon TRC-50ix fundus camera [Topcon Imagenet, Tokyo, Japan], or Heidelberg Spectralis HRA+OCT [Heidelberg Engineering, Heidelberg, Germany]). Other inclusion criteria for this study included the following: (1) clear ocular media; (2) absence of significant refractive error; (3) absence of significant concurrent ocular diseases. Patients deemed to have unsatisfactory OCTA images such that FAZ area could not be reliably measured, as described below, were also excluded from this study. All subjects underwent slit-lamp biomicroscopy, dilated funduscopy examination, and measurement of pinhole VA on the day of OCTA imaging. Demographic and clinical information, including treatment history, lens status (phakic or pseudophakic), and duration of disease, was obtained from patient records.

Optical Coherence Tomography–Derived Measurements of Foveal Anatomy

All patients were imaged with Spectralis spectral-domain OCT (SD OCT) (Heidelberg Engineering, Heidelberg, Germany) on the day of OCTA imaging. A raster scan protocol centered at the fovea (range, 20° × 25°–30° × 20°) was used. The following assessments of foveal structure were attained using the macular volume scan (Figs 2 and 3):

1. Point thickness of the central fovea (point foveal thickness, PFT): Determined using the B-scan image of the central fovea and defined as the distance between the retinal pigment epithelium and inner limiting membrane (ILM). Calipers provided by the OCT software were used to determine this measurement. Point thickness of the central fovea was measured by 2 independent examiners (M.I. and J.M.) and the average measurement was used for statistical analysis.

2. Occurrence of intraretinal cystoid changes: Categorically graded as being present or absent by 2 independent observers (M.I. and J.M.). Intraretinal cysts were identified using previously determined OCT criteria and were defined as the occurrence of round or oval hyporeflective spaces arranged in linear aggregates at the level of the inner nuclear or outer nuclear/Henle fiber layers. When the evaluation was inconsistent between the 2 graders, a third masked reader (C.B.) made the final arbitration.

3. Integrity of the ellipsoid zone in the central fovea: The central 3 mm of the fovea, as circumscribed by the ETDRS grid, was evaluated. Disruption of the EZ was categorically graded as being present or absent by 2 independent observers (M.I. and J.M.). An absent grading was denoted if there was any disruption of the EZ on OCT. When the evaluation was inconsistent between the 2 graders, a third masked reader (C.B.) made the final arbitration.

4. Central 1-mm subfield thickness (CST): Recorded from the retinal thickness ETDRS grid generated by Spectralis software (Heidelberg Engineering, Heidelberg, Germany). The occurrence of diabetic macular edema, defined as a central foveal thickness of greater than 275 μm (ETDRS central subfield), was also evaluated in the diabetic cohort.

5. Disorganization of the retinal inner layers (DRIL) length: Previously reported definitions of DRIL were used. Disorganization of the retinal inner layers length was defined as the horizontal extent (in microns) for which any boundaries between the ganglion cell–inner plexiform layer complex, inner nuclear layer, and outer nuclear layer...
could not be identified. The horizontal extent of DRIL within each B-scan image within the central 3 mm of the fovea, circumscribed using the ETDRS grid, was assessed. Average of measurements was used to derive a global DRIL measurement for the eye. Global DRIL measurement was determined by 2 independent examiners (M.I. and C.B.) and the average measurement was used for quantitative analysis.

6. Foveal pit volume: This measurement was determined by applying the Cavalieri principle of stereologic analysis as illustrated in Figure 3. The following steps were used to calculate foveal pit volume:

i. The rim of the foveal pit on each B-scan image was determined using ImageJ (a publicly available image processing program developed by Wayne Rasband,
specifically, the first point away from the foveal center (in nasal and temporal directions) where the slope of the ILM was measured to be zero was defined as the rim. The angle tool on ImageJ was used to determine this measurement.

ii. The area confined by the ILM and a straight line connecting the 2 points denoting the foveal pit rim on the B-scan image, as determined in step (i), was then calculated using manual planimetry.

iii. The volume of the foveal pit between 2 adjacent OCT slices (herein called a segment) was then determined using this equation:

\[
Vol = d \left( \frac{A_x + A_{x+1}}{2} \right)
\]

where: \(Vol\) = volume of foveal pit within segment bounded by OCT slice number \(x\) and \(x+1\),

\(d\) = distance between adjacent slices in \(\mu m\)

iv. Foveal pit volume was calculated by summing the volumes of individual segments. The number of segments in the OCT volume was \((n+1)\), where \(n\) = total number of slices that spanned the foveal pit.

Measurement of the Area of the Foveal Avascular Zone

The area of the FAZ was measured using OCTA. The instrument used was based on the Optovue RTVue XR Avanti (Optovue, Inc, Fremont, CA) to obtain split-spectrum amplitude-decorrelation angiography images. This instrument has an A-scan rate of 70,000 scans per second, using a light source centered on 840 nm and a bandwidth of 45 nm. Each OCTA volume contains 304×304 A-scans with 2 consecutive B-scans captured at each fixed position before proceeding to the next sampling location. The scan area was...
Each OCTA volume is acquired in approximately 3 seconds and 2 orthogonal OCTA volumes were acquired to perform motion correction to minimize motion artifacts arising from microsaccades and fixation changes.

Angiography information displayed is the average of the decorrelation values when viewed perpendicularly through the thickness being evaluated. If the image processed with motion-correction software demonstrated artifact involving the FAZ in the form of doubling of vascular structures or sideways shearing, then the case was excluded from analysis. Only OCTA images with signal strength above 50 were included in this study.

Image analysis to calculate FAZ area was performed using Adobe Photoshop CC (Adobe Systems, Inc., San Jose, CA) and our previously reported technique. Images from screen capture program integrated in the AngioView software, representing the full thickness of the retina (inner and outer capillary networks...
combined), were used for quantitative analysis. Gain and contrast were adjusted if necessary, using the brightness/contrast function, to allow clear delineation of the FAZ. The magic wand tool was then used to manually demarcate the boundaries of the FAZ after the innermost capillaries in the fovea were identified. We used a tolerance of 15 and repeatedly used the magic wand tool to select the FAZ. If the selection spilled outside of the FAZ, the excess selection was trimmed using the lasso tool. If there were regions in the FAZ where pixel value exceeded the selected pixel value plus tolerance, then they were not selected by the magic wand tool. In these instances, the lasso tool was also used to manually select pixel outliers. The area measurement function was then used to determine the area of the FAZ in pixels$^2$ and this value was converted to micrometers squared (µm$^2$) using scale conversion. The FAZ area was determined by 2 independent examiners (M.I. and J.M.) and the average measurement was used for quantitative analysis.

Statistical Analysis

Data were summarized with descriptive statistics. Univariate regression models estimated using generalized estimating equations (GEE) were fit using FAZ area, foveal pit volume, age, lens status (phakic or pseudophakic), PFT, CST, disease (DR or RVO), sex (male or female), occurrence of intraretinal cysts, occurrence of EZ disruption, DRIL length, or previous treatment (no treatment, laser photocoagulation, intravitreal anti-VEGF injection, vitrectomy, or combination therapy) as a single predictor and VA as the outcome. Treatment was a single categorical variable. The reference group for treatment was anti-VEGF therapy, vitrectomy, or combination therapy, while the other 3 were dummy variables (laser photocoagulation, intravitreal anti-VEGF injection, combination therapy, and vitrectomy) to be directly included in the regression model, and also contain the same information as the single categorical variable. The reference group for treatment was the cohort of subjects that had not received any previous treatment.

Generalized estimating equations were used to account for correlations of observations from the same subject, as some patients had bilateral imaging. Interactions between FAZ area and age/CST/disease (DR or RVO)/integrity of the EZ/occurrence of intraretinal cysts/DRIL length/foveal pit volume were then explored using regression analysis (Appendix, available at www.aaojournal.org).

Results from univariate regression models and also models assessing interactions were then used to create a final multivariate model in which VA was the outcome. A summary of all the models explored in this study is provided in the Appendix. Visual acuity measurements were converted to logarithm of the minimum angle of resolution (logMAR) units before analysis. A $P$ value less than or equal to 0.05 was considered significant. Statistical analysis was performed using R 3.1.2 and Stata 12. Descriptive results in this manuscript are provided as mean ± standard deviation.

Results

General

A total of 95 eyes from 66 subjects (36 male and 30 female) were analyzed. The mean age of the cohort was 62.9±13.2 years. Mean VA was 0.25±0.24 logMAR (mean Snellen acuity of 20/35). A total of 53 right eyes and 42 left eyes were evaluated. Twenty-six eyes were pseudophakic. The cohort comprised 65 eyes with DR and 30 eyes with RVO (19 with BRVO and 11 with CRVO). The diabetic cohort comprised 13 eyes with mild DR, 22 eyes with moderate DR, 6 eyes with severe DR, and 24 eyes with proliferative DR according to the ETDRS scale. Twenty-five eyes in the entire cohort had not received any previous treatment, 25 eyes had previously received intravitreal anti-VEGF therapy, 17 eyes had received laser photocoagulation, 3 eyes had undergone vitrectomy, and 25 eyes had received a combination of the above nonsurgical treatments. The condition of diabetic macular edema, defined as a central subfield thickness greater than 275 µm, was present in 38 of 65 diabetic eyes (58.4%). Mean signal strength of OCTA images for the cohort was 65.2±5.1.

Summary statistics for FAZ area, lens status, VA, duration of disease, PFT, CST, frequency of intraretinal cysts, foveal pit volume, DRIL length, and frequency of EZ disruption for the entire cohort are provided in Table 1. There was no difference in demographic and OCT-derived anatomic measurements (all $P ≥ 0.058$) between BRVO and CRVO groups (Table S1, available at www.aaojournal.org); therefore, data from the 2 groups were pooled together to a single RVO group for further statistical comparisons. Interobserver disagreement on the status of the ellipsoid zone or intraretinal cysts required a tie-breaker examination in 3 and 4 instances, respectively.

Mean age ($P = 0.822$), VA ($P = 0.974$), PFT ($P = 0.050$), CST ($P = 0.056$), DRIL length ($P = 0.252$), foveal pit volume ($P = 0.060$), frequency of pseudophakia ($P = 0.274$), and frequency of EZ disruption ($P = 0.473$) was not significantly different between the DR and RVO groups. Duration of disease was significantly greater in the DR group compared with the RVO group (91.8 vs. 34.9 months; $P < 0.001$). The frequency of treatment variables was significantly greater in the DR group than in the RVO group ($P = 0.042$). Mean FAZ area was significantly greater in the DR group ($P = 0.019$). A greater proportion of eyes in the RVO demonstrated intraretinal cysts on OCT (46.7% vs. 24.6%; $P = 0.033$).

Mean DRIL length for the cohort was 235.2±506.4 µm. There was a moderate positive correlation between FAZ area and DRIL length (Pearson correlation coefficient = 0.3961) that was statistically significant ($P < 0.001$; Fig 4).

It was possible to calculate foveal pit volume in only 73 of 95 eyes. In the remaining eyes, retinal thickening and intraretinal cystic changes distorted the shape of the foveal pit such that it was not possible to reliably identify its margins (Fig 5). Mean foveal pit volume for the eyes that were measured was 0.14±0.11 mm$^3$. A moderate positive correlation between FAZ area and foveal pit volume ($P = 0.001$) was identified, with a Pearson correlation coefficient of 0.3744 (Fig 6).

Association of Foveal Avascular Zone Area and Other Variables with Visual Acuity

Results from univariate GEE analyses are summarized in Table 2. Foveal avascular zone area ($P < 0.001$), the occurrence of EZ disruption ($P < 0.001$), the occurrence of intraretinal cysts ($P = 0.018$), lens status ($P = 0.002$), and DRIL length ($P < 0.001$) were significantly associated with VA. Age ($P = 0.600$), PFT ($P = 0.283$), CST ($P = 0.226$), foveal pit volume ($P = 0.237$), disease (DR or RVO; $P = 0.489$), female sex ($P = 0.225$), and history of previous treatment were not associated with VA (all $P ≥ 0.072$).

Point foveal thickness and CST were highly correlated ($r = 0.893$) and similarly associated with VA. Therefore, CST was used as a predictor in subsequent multiple regression models and PFT measurements were excluded from further analyses.
Interactions Between Foveal Avascular Zone Area and Other Variables

The results of multivariate GEE analyses where interactions between FAZ area and another predictor were explored using VA as the outcome are summarized in Table 3. The only interaction that was observed to be significant was the interaction between FAZ area and age (P = 0.026). Interactions between FAZ area and CST (P = 0.615), FAZ area and disease (DR or RVO; P = 0.598), FAZ area and the occurrence of intraretinal cysts (P = 0.453), FAZ area and the integrity of the EZ (P = 0.655), FAZ area and DRIL length (P = 0.245), and FAZ area and foveal pit volume (P = 0.810) were not significant.

Predictive Model of Visual Acuity Using Foveal Avascular Zone Area and Other Variables

Because FAZ area, the occurrence of intraretinal cysts, the occurrence of EZ disruption, DRIL length, and lens status were found to be significant predictors of VA in univariate GEE analyses, they were used in the final predictive model. Age was also used in the final predictive model because the interaction between age and FAZ area was found to be significant in multivariate GEE analyses. We also wanted to determine if the type of retinal vascular disease (DR or RVO) would be a significant predictor of VA, so this variable was also included in the final model. The final model was therefore defined as follows:

Outcome: VA. Predictors: FAZ area, age, disease (DR or RVO), occurrence of intraretinal cysts, occurrence of EZ disruption, lens status (phakic or pseudophakic), DRIL length, and an interaction between FAZ area and age.

The results of this model are summarized in Table 4. FAZ area (P = 0.003), age (P < 0.001), EZ disruption (P < 0.001), and the interaction between FAZ area and age (P < 0.001) were significant predictors of VA. Disease (DR or RVO), lens status (phakic or pseudophakic), DRIL length, and the occurrence of intraretinal cysts were not found to be significant predictors of VA using this model (all P ≥ 0.210).

Figure 7B is a graphical illustration of the final regression model demonstrating how the relationship between FAZ area and mean VA varies with age. Data from subjects above the mean age of the cohort (62.9 years) are denoted in red and those below the mean age are denoted in blue. Statistical techniques previously described by Cohen et al. were used to generate simple slopes for different age groups using the predictive model. The predicted slope of the relationship between FAZ area and VA for a population 1 standard deviation above the mean age of the cohort (dashed line), the mean age of the cohort (continuous line), and 1 standard deviation below the mean age of the cohort (dotted line) are provided. The slopes of the 3 lines are different, thereby providing evidence that the relationship between FAZ area and VA varies with age.

Case Illustrations

A comparison of VA from 2 patients with DR of similar age and OCT-derived anatomic measurements (aside from FAZ area) are presented in Figure 8.

Case A is the right eye of a 70-year-old female patient that was diagnosed with type 2 diabetes mellitus 26 years ago. Intraretinal cysts were seen on SD OCT, PFT was 239 μm, the integrity of the EZ was judged to be intact, and DRIL was present. The area of the FAZ was measured as 0.44 mm² and the VA was recorded as 20/40.

Case B is the left eye of a 71-year-old male patient that was diagnosed with type 2 diabetes mellitus 29 years previously. Intraretinal cysts were seen on SD OCT, PFT was 254 μm, the EZ was intact, and DRIL was present. The area of the FAZ was measured as 0.76 mm² and the VA was recorded as 20/100.

Discussion

We utilized OCT-derived anatomic measurements and regression models to determine if FAZ area is a significant predictor of VA in DR and RVO. Although robust statistical evaluations demonstrated consistent findings across multiple analyses, we emphasize that the strength of our conclusions is modulated by the limited sample size of our cohort. The major findings of this study are as follows: (1) FAZ area is significantly correlated with VA in diabetic retinopathy and retinal vein occlusion; (2) age modulates the relationship between FAZ area and VA such that for a constant FAZ area a relatively older group of patients will have poorer vision (i.e., a higher mean logMAR visual acuity).
The FAZ is a specialized region of the human macula that contains the highest density of cone photoreceptors. With respect to metabolic activity, oxygen consumption within the macula, per gram of tissue, is also greater than most other organs in the human body. Cells within the FAZ are principally nourished by the choroid and during physiologic conditions the choroidal circulation is able to meet the metabolic demands of the FAZ. Therefore, it is

Figure 4. Relationship between foveal avascular zone (FAZ) area and disorganization of retinal inner layers (DRIL) length. FAZ area was significantly and positively correlated to DRIL length, as illustrated by the clinical images of 2 different patients (A, B). The area imaged by optical coherence tomography angiography is denoted by the green inset on each fluorescein angiogram image and the area of the FAZ in each eye is highlighted in red shade. DRIL length was defined as the horizontal extent (in microns) for which any boundaries between the ganglion cell—inner plexiform layer complex, inner nuclear layer, and outer nuclear layer could not be identified.
not surprising that a correlation between FAZ size and VA in the normal human eye has not been reported. Samara et al. quantified the area of the superficial FAZ in 70 normal eyes using OCTA, and although there was significant variation in FAZ area (range, 0.071–0.827 mm²), the VA of every eye in their cohort was recorded as 20/20. Similarly, Laatikainen and Larinkari measured the diameter of the FAZ in 167 healthy eyes using FA. Despite great variation in FAZ diameter (range, 0.25–0.85 mm; approximated from graphical data presented in their manuscript), the VA of all subjects in their study was recorded as 20/25 or better.

Yu et al. showed that the inner retina was rendered relatively anoxic following experimental retinal arterial occlusion, thereby providing evidence that vasculogenic insults to the fovea perturb the delicate balance between oxygen supply and consumption within the inner retina. Because the choroid is unable to sufficiently oxygenate the inner

Figure 5. Distortion of foveal pit morphology due to retinal structural changes. Color (A), fluorescein angiography (B), and optical coherence tomography (OCT) angiography (C) images of the macula of a patient with diabetic retinopathy are presented. Marked thickening of the central fovea is evident on the thickness map generated by Spectralis software (Heidelberg Engineering, Heidelberg, Germany; D) and the foveal pit is not discernible upon 3-dimensional visualization of the structural OCT (E) data. B-scan images (I–III) of the central fovea demonstrate disruption of the foveal pit due to intraretinal cystic changes. Sites from which B-scan images were acquired are denoted on the color image (white lines) and the area studied using OCT angiography is denoted on the fluorescein angiogram (yellow inset). It was not possible to calculate foveal pit volume in these eyes, but the area of the foveal avascular zone (red shade) was readily measurable.
retina following retinal vascular injury, it is plausible that FAZ size, and therefore the area of the macula devoid of a retinal blood supply, would correlate to the degree of visual dysfunction in DR and RVO. However, previous reports concerning the relationship between FAZ size and VA in retinal vascular diseases have reached discordant conclusions. Arend et al\textsuperscript{21} compared FAZ size between diabetic patients with decreased VA (20/50 or worse) and unaffected VA (median VA of 20/25) and showed that the FAZ was enlarged by 73% in eyes with decreased VA. In their study, scanning laser video-fluorescein angiograms were used to quantify FAZ topology and fundus photography was used to grade the occurrence of macular edema. Remky et al\textsuperscript{22} evaluated FAZ size, using digitized FA images acquired with a scanning laser ophthalmoscope, in 24 eyes with acute CRVO and compared it to measurements from 98 healthy volunteers. Although FAZ size was increased in the CRVO group (0.32±0.17 mm\textsuperscript{2}) compared with the control group (0.22±0.07 mm\textsuperscript{2}), it was not found to be correlated with VA. Parodi et al\textsuperscript{41} also used FA techniques to compare FAZ area between 20 patients with BRVO and 41 control subjects. Mean FAZ area was shown to be greater in eyes with BRVO (0.56±0.34 mm\textsuperscript{2}) compared with controls (0.26±0.07 mm\textsuperscript{2}). Their study also showed that VA impairment due to BRVO was correlated with FAZ enlargement. Most of these studies only examined the association between FAZ size and VA using FA and omitted the influence of other disease-induced structural changes, such as EZ disruption,\textsuperscript{23} DRIL length,\textsuperscript{35} and intraretinal cystic changes,\textsuperscript{42} in their statistical models. This may be one explanation for the discrepancies in findings between previous FA-based studies.

Optical coherence tomography angiography is a relatively new imaging modality that utilizes flow properties within a defined volume of tissue to visualize vascular structures and therefore obviates dye administration.\textsuperscript{43} Over the past year, there have been a considerable number of studies that have reported the spectrum of retinal vascular changes due to DR, BRVO, and CRVO using OCTA.\textsuperscript{24,44–47} With regard to quantitative evaluation, recent evidence also suggests that OCTA is a reliable technique for measuring FAZ area that compares favorably

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**Figure 6.** Relationship between foveal avascular zone (FAZ) area and foveal pit volume in diabetic retinopathy and retinal vein occlusion. A scatterplot demonstrates a significant relationship, with a Pearson correlation coefficient of 0.3744.

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**Table 2.** Results of Simple Regression Models (Generalized Estimating Equations) Using Visual Acuity as the Outcome

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>P Value</th>
<th>95% CI</th>
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<tr>
<td>FAZ area (mm\textsuperscript{2})</td>
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<td>0.07</td>
<td>&lt;0.001</td>
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<td>Age (yrs)</td>
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<td>0.002</td>
<td>0.600</td>
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<td>Lens status</td>
<td>0.1735</td>
<td>0.0566</td>
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<td>Point foveal thickness ((\mu)m)</td>
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<td>0.0003</td>
<td>0.283</td>
<td>(−0.0003, 0.001)</td>
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<td>Central 1-mm subfield thickness ((\mu)m)</td>
<td>0.0006</td>
<td>0.0005</td>
<td>0.226</td>
<td>(−0.0004, 0.002)</td>
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<tr>
<td>Disease (DR or RVO)</td>
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<td>0.06</td>
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<td>Female</td>
<td>0.07</td>
<td>0.05</td>
<td>0.225</td>
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<td>Intraretinal cysts</td>
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<td>0.06</td>
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<td>0.06</td>
<td>&lt;0.001</td>
<td>(0.22, 0.45)</td>
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<td>DRIL length ((\mu)m)</td>
<td>0.00018</td>
<td>0.000046</td>
<td>&lt;0.001</td>
<td>(0.00009, 0.0027)</td>
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<td>Foveal pit volume (mm\textsuperscript{3})</td>
<td>0.2641</td>
<td>0.232</td>
<td>0.237</td>
<td>(−0.1732, 0.7015)</td>
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<td>Treatment</td>
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<tr>
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<td>0.051</td>
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<td>0.06</td>
<td>0.091</td>
<td>(−0.02, 0.22)</td>
</tr>
<tr>
<td>Combination therapy*</td>
<td>0.127</td>
<td>0.071</td>
<td>0.072</td>
<td>(−0.01, 0.27)</td>
</tr>
<tr>
<td>Vitrectomy</td>
<td>0.003</td>
<td>0.032</td>
<td>0.918</td>
<td>(−0.059, 0.066)</td>
</tr>
</tbody>
</table>

CI = confidence interval; DR = diabetic retinopathy; DRIL = disorganization of the retinal inner layers; FAZ = foveal avascular zone; RVO = retinal vein occlusion; VEGF = vascular endothelial growth factor.

*Two or more of the listed treatments.
to histology. Carpineto et al quantified FAZ area in 60 eyes using OCTA and demonstrated a high intraobserver intersession concordance correlation coefficient (mean 0.99) and high interobserver intrasession concordance correlation coefficient (mean 0.99). The advantages of OCTA for reliably evaluating FAZ size were exploited in a number of recent studies that quantified the topologic features of the FAZ in DR and RVO. Salz et al used a prototype swept-source OCT to delineate the innermost vessels surrounding the avascular zone in DR, which they denoted as the foveal nonflow zone, and showed that the size of the foveal nonflow zone was significantly greater in eyes with proliferative DR compared with normal eyes and those with nonproliferative DR. Takase et al used OCTA to show that FAZ area was significantly greater in the DR group compared with a control group but, similar to the report by Salz et al, correlations between FAZ size and VA were not performed. Samara et al evaluated 17 eyes with BRVO and compared FAZ area measurements between the eye with BRVO and the fellow normal eye from the same subject. They found that FAZ area in BRVO was significantly enlarged at the level of the deep capillary network only. They also reported that the area of the deep FAZ correlated positively with logMAR VA. The important work by Samara et al however, did not consider the influence of other disease-induced anatomic changes, such as EZ disruption and intraretinal cysts, which may have also influenced VA. Therefore, it was not possible to determine if VA change was correlated to FAZ size or was due to other retinal anatomic changes that may have confounded the analysis. Similarly, a number of other studies that have reported the correlation between FAZ size and VA also did not account for the effects of confounding variables.

In this study, FAZ area together with other OCT-derived anatomic measurements such as PFT, CST, intraretinal cysts, DRIL length, and the integrity of the EZ were used to determine the significant predictors of VA in DR and RVO. Important clinical variables such as age, sex, and disease type (DR or RVO) were also used in the analysis. We attained FAZ measurements using the entire volume scan and did not stratify capillary networks into superficial and deep networks to avoid errors associated with image segmentation—Shahlaee et al previously showed that interobserver agreement did not meet the lowest acceptable grader agreement for isolated measurements of the deep vascular network using OCTA. We demonstrate that FAZ area is a significant predictor of VA in DR and RVO. Univariate and multiple regression analysis using GEEs revealed that irrespective of disease type (DR or RVO), FAZ area was significantly correlated with VA, thereby implicating a degree of overlap in the pathways

Table 3. Multiple Regression Models (Generalized Estimating Equations) Exploring Interactions Between Foveal Avascular Zone Area and Other Variables Using Visual Acuity as the Outcome

<table>
<thead>
<tr>
<th>Interaction Terms</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>P Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ area × Age</td>
<td>0.014</td>
<td>0.006</td>
<td>0.026</td>
<td>(0.002, 0.026)</td>
</tr>
<tr>
<td>FAZ area × CST</td>
<td>−0.001</td>
<td>0.002</td>
<td>0.615</td>
<td>(−0.004, 0.002)</td>
</tr>
<tr>
<td>FAZ area × Disease</td>
<td>−0.081</td>
<td>0.154</td>
<td>0.958</td>
<td>(−0.382, 0.220)</td>
</tr>
<tr>
<td>FAZ area × Integrity of EZ</td>
<td>0.063</td>
<td>0.14</td>
<td>0.655</td>
<td>(−0.213, 0.338)</td>
</tr>
<tr>
<td>FAZ area × Intraretinal cysts</td>
<td>0.098</td>
<td>0.131</td>
<td>0.453</td>
<td>(−0.159, 0.355)</td>
</tr>
<tr>
<td>FAZ area × DRIL length</td>
<td>−0.002</td>
<td>0.0001</td>
<td>0.245</td>
<td>(−0.0004, 0.0001)</td>
</tr>
<tr>
<td>FAZ area × Foveal pit volume</td>
<td>0.135</td>
<td>0.561</td>
<td>0.810</td>
<td>(−0.965, 1.234)</td>
</tr>
</tbody>
</table>

CI = confidence interval; CST = central 1-mm subfield thickness; DRIL = disorganization of the retinal inner layers; EZ = ellipsoid zone; FAZ = foveal avascular zone.

Table 4. Results of the Final Multiple Regression Model where the Outcome Was Visual Acuity and the Predictors Included Foveal Avascular Zone Area, Age, Disease, Occurrence of Ellipsoid Zone Disruption, Occurrence of Intraretinal Cysts, Lens Status, Disorganization of the Retinal Inner Layers, and the Interaction Between Foveal Avascular Zone Area and Age

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>P Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ area (mm²)</td>
<td>−0.703</td>
<td>0.239</td>
<td>0.003</td>
<td>(−1.171, −0.235)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>−0.007</td>
<td>0.002</td>
<td>0.001</td>
<td>(−0.011, −0.003)</td>
</tr>
<tr>
<td>Disease (DR or RVO)</td>
<td>0.041</td>
<td>0.043</td>
<td>0.344</td>
<td>(−0.044, 0.126)</td>
</tr>
<tr>
<td>Ellipsoid zone disruption</td>
<td>0.245</td>
<td>0.057</td>
<td>&lt;0.001</td>
<td>(0.133, 0.357)</td>
</tr>
<tr>
<td>Intraretinal cysts</td>
<td>−0.002</td>
<td>0.043</td>
<td>0.962</td>
<td>(−0.087, 0.083)</td>
</tr>
<tr>
<td>Lens status (phakic or pseudophakic)</td>
<td>0.077</td>
<td>0.062</td>
<td>0.210</td>
<td>(−0.044, 0.126)</td>
</tr>
<tr>
<td>DRIL length</td>
<td>0.000</td>
<td>0.000</td>
<td>0.240</td>
<td>(−0.000, 0.001)</td>
</tr>
<tr>
<td>FAZ area × Age</td>
<td>0.012</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>(0.006, 0.018)</td>
</tr>
<tr>
<td>Constant</td>
<td>0.531</td>
<td>0.147</td>
<td>&lt;0.001</td>
<td>(0.244, 0.818)</td>
</tr>
</tbody>
</table>

CI = confidence interval; DR = diabetic retinopathy; DRIL = disorganization of the retinal inner layers; FAZ = foveal avascular zone; RVO = retinal vein occlusion.
by which retinal vascular diseases lead to visual dysfunction.

Foveal homeostasis is critically regulated by retinal glia. Astrocytes and Muller cells are the predominant glia subtypes in the primate macula. From an embryological standpoint, the pattern of astrocyte development closely approximates retinal vascularization and a number of studies have shown that glial cells codistribute with blood vessels in the retina. Therefore, it is expected that eyes with a relatively larger FAZ also have a lower number of glia in the macula. During physiologic states, the concentration of glial and vascular elements in the fovea in those with larger FAZs may be sufficient to maintain visual function. However, following insults that perturb the structural and biochemical systems that ordinarily support macular homeostasis, these mechanisms may rapidly decompensate; more so when there is an associated increase in energy demands by regional neurons. We postulate that eyes with larger FAZs have an increased susceptibility to visual dysfunction due to the lower baseline concentrations of glial and vascular elements. We also postulate that such eyes are more vulnerable to imbalances in energy supply/demand relationships during disease states and may suffer a greater level of injury than an eye with a smaller FAZ, following an insult of comparable magnitude. We emphasize, however, that our hypothesis is entirely speculative and focused work with histologic correlation is required for validation of these concepts.

The other major finding in this study is that age modulates the relationship between FAZ area and VA in DR and RVO. For a constant FAZ area, mean logMAR VA was found to be higher in a relatively older age group. Age-related decline in VA is known to occur in patients absent of ocular disease; however, there is little evidence to suggest that it is due to age-related changes in FAZ topology. In the study by Laatikainen and Larinkari, a gradual enlargement in FAZ size was noted until the fifth decade of life in healthy eyes after which significant increases were not detected. Their study concluded that the size of the FAZ did not affect VA in healthy eyes. Further work is required to understand the cellular basis for the interaction between FAZ area and age in retinal vascular diseases. Again, disease-induced alterations to Muller cells and astrocytes may underlie this interaction.

Disorganization of inner retinal layers is an OCT-derived biomarker that is predictive of baseline VA and VA after resolution of diabetic macular edema. Histologic correlations for DRIL have not been performed, but DRIL is thought to correlate to regions where synaptic connections of bipolar, horizontal, and amacrine cells within the inner retina have been disrupted. The inner retina is predominantly nourished by the retinal circulation; therefore, it is plausible that vasculogenic insults at the level of the inner retina will result in observable retinal structural changes that manifest as DRIL on OCT. The data in our study support this speculation, as we found a significant positive correlation between FAZ size and DRIL length. Although our results implicate an important relationship between FAZ size and DRIL, it will be essential to validate these findings by defining the temporal relationships between these 2 variables.

The FAZ is required for foveal development and complete foveal excavation. Astrocytes and Muller cells are the predominant glia subtypes

Figure 7. Correlation between area of the foveal avascular zone (FAZ) and visual acuity (VA). A, A scatterplot of the entire cohort is provided. B, A graphical illustration of the final regression model demonstrating how the relationship between FAZ area and mean VA varies with age is presented. Blue and red circles represent subjects below and above the mean age of the cohort (62.9 years), respectively. The predicted slope of the relationship between FAZ area and VA for a population 1 standard deviation above the mean age of the cohort (dashed line), the mean age of the cohort (continuous line), and 1 standard deviation below the mean age of the cohort (dotted line) are provided. The slopes of the 3 lines are different, thereby providing evidence that the relationship between FAZ area and VA varies with age. BCVA = best-corrected visual acuity.
formation of the FAZ before the foveal pit during embryologic development suggests that the size of the FAZ regulates the degree of foveal excavation.\textsuperscript{53} Dubis et al\textsuperscript{18} calculated foveal pit volume in 42 subjects using OCT techniques and demonstrated that it was significantly correlated with FAZ area. Because foveal pit volume is intrinsically related to FAZ area, foveal pit volume might be considered a useful biomarker of visual function in retinal vascular disease. However, in this study we found that it was not possible to reliably measure foveal pit volume in 22 of 95 eyes (23.2\%) due to retinal thickening and intraretinal cystic changes that subsequently distorted the contour of the foveal pit. Furthermore, foveal pit volume was not shown to be significantly associated with

\textbf{Figure 8.} Case illustrations. Patient A and patient B are of similar age and demonstrate similar foveal anatomic changes due to diabetic retinopathy, with the exception of the area of the foveal avascular zone. Patient B has a significantly larger foveal avascular zone area and also a lower visual acuity (VA).
VA using regression analysis. Collectively, these findings suggest that FAZ area has greater utility as a biomarker of visual function than foveal pit volume in DR and RVO. Arguably, FAZ area is also easier to quantify than foveal pit volume in the clinical setting using current image analysis software.

We acknowledge several limitations of this study, including its retrospective design and the limited number of subjects. Prospective studies of larger sample size will be important to reaffirm the findings of this report. Another limitation of this study is the nonstandardized manner in which VA measurements were attained. Future studies that aim to evaluate the relationship between VA and FAZ area should utilize standardized VA measurement protocols such as those used in clinical trials. Also, because image artifacts due to poor fixation or media opacities may preclude clear visualization of the macula, it may not be possible to measure FAZ area using OCTA in all patients with retinal vascular disease. Additionally, when capillary flow rates fall below the threshold detected by OCTA algorithms, the terminal capillary ring may not be completely visualized in certain instances and FAZ area measurements may therefore be influenced. We also emphasize that the etiopathology of retinal vascular diseases, particularly DR, is complex. Several important predictors that have been shown to correlate with disease progression and visual loss, such as age of disease onset, duration of disease, HbA1c measurements, renal disease, and hypertension, were not used in the regression analyses. Some of these parameters can be difficult to reliably discern from patient medical records. Furthermore, The Diabetes Control and Complication Trial showed that approximately 11% of the variation in retinopathy risk was associated with HbA1c readings and duration of diabetes, suggesting that 89% of the variation in risk was due to factors independent of HbA1c. Because OCT is widely used in the clinical management of retinal vascular diseases, the purpose of this report was to demonstrate that FAZ area is a useful metric for grading the functional severity of maculopathy due to DR and RVO. Automated algorithms and advances in OCTA software may facilitate rapid acquisition of FAZ area measurements, thereby enabling clinicians to routinely integrate morphologic information about the FAZ into clinical practice. Because FAZ area is also correlated to foveal capillary density and foveal intercapillary distances in some conditions, by extension, it is possible that these latter quantitative metrics may also be correlated with VA. We emphasize that biomorphometric modeling performed in this study utilized data from a single visit; therefore, the results of this study cannot be used to determine the risk of disease progression or predict response to treatment. The previous study by Sim et al showed that the rate of FAZ enlargement ranged between 5% and 10% of baseline FAZ area per year in eyes with diabetic macular ischemia; therefore, further study about FAZ area and VA correlations during the natural course of retinal vascular diseases and following treatment is warranted.

References

Footnotes and Financial Disclosures

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Data acquisition and/or research execution: Balaratnasingam, Inoue, Ahn, McCann, Dhrami-Gavazi, Yannuzzi
Obtained funding: Not applicable
Overall responsibility: Balaratnasingam, Inoue, Ahn, Yannuzzi, Freund

Abbreviations and Acronyms:
BRVO = branch retinal vein occlusion; CRVO = central retinal vein occlusion; CST = central subfield thickness; DR = diabetic retinopathy; DRIL = disorganization of the retinal inner layers; ETDRS = Early Treatment Diabetic Retinopathy Study; EZ = ellipsoid zone; FA = fluorescein angiography; FAZ = foveal avascular zone; GEE = generalized estimating equations; ILM = inner limiting membrane; LogMAR = logarithm of the minimum angle of resolution; OCT = optical coherence tomography; OCTA = optical coherence tomography angiography; PFT = point foveal thickness; RVO = retinal venous occlusion; SD OCT = spectral-domain optical coherence tomography; VA = visual acuity; VEGF = vascular endothelial growth factor.

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