PROGRESSIVE RETINAL NONPERFUSION IN ISCHEMIC CENTRAL RETINAL VEIN OCCLUSION

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Background: Serial wide-field fluorescein angiography was performed on eyes with preproliferative (ischemic) central retinal vein occlusion to evaluate retinal perfusion.

Methods: Serial wide-field fluorescein angiography was performed on 12 preproliferative central retinal vein occlusion eyes in the 3-year Rubeosis Anti-VEGF (RAVE) trial using the Staurenghi lens (Ocular Staurenghi 230SLO Retina Lens) with a scanning laser ophthalmoscope (Heidelberg HRA Spectralis). "Disk area" was defined anatomically for each eye.

Results: Mean total field of gradable retina was 290 disk areas (range, 178–452). All eyes demonstrated extensive areas of retinal nonperfusion; at baseline, mean area of retinal perfusion was 106 disk areas (range, 37–129), correlating with a mean of 46.5% perfused retinal area (range, 19.1–56.4%). The area of retinal nonperfusion increased in all eyes with a mean loss of approximately 8.1% of perfused retinal area per year (range, 4.3–12.4%), which corresponded to a mean 15-disk areas (range, 12–35) of retina evolving from perfused to nonperfused annually. The extent of baseline and final nonperfusion was not significantly different between eyes that developed neovascularization and eyes that did not.

Conclusion: In this population of severe central retinal vein occlusion eyes, profound retinal nonperfusion was observed with wide-field fluorescein angiography at baseline and the extent of nonperfusion progressed while undergoing anti-vascular endothelial growth factor therapy.

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Central retinal vein occlusion (CRVO) has classically been divided into two subtypes, ischemic and nonischemic, based on the presumed extent of damage to the retinal vasculature. Preproliferative (ischemic) CRVO often portends a poor prognosis,[1] and the Central Retinal Vein Occlusion Study defined such eyes as having >10 disk areas of capillary nonperfusion on a standardized photography protocol.[2] Classically regarded as a single insult, limited prospective studies have considered if progressive changes occur to the retinal circulation after an ischemic CRVO while undergoing anti-vascular endothelial growth factor (VEGF) therapy.[3,4]

In nonischemic CRVO and branch retinal vein occlusion, Early Treatment of Diabetic Retinopathy Study–defined standard posterior pole fundus fluorescein angiography revealed that anti-VEGF therapy may be protective against the development of retinal nonperfusion.[3] In comparison, more capillary nonperfusion was documented longitudinally in the posterior pole of CRVO eyes observed or treated with triamcinolone; at baseline, the majority of eyes were nonischemic in this trial (SCORE), with 0% to 3% of each cohort demonstrating >10 disk areas of capillary nonperfusion.[4]

In this prospective series, wide-field fluorescein angiography (WFFA) was performed serially in eyes with severe CRVO undergoing anti-VEGF therapy to assess peripheral retinal perfusion status.

Methods

Preproliferative CRVO eyes treated in the 3-year prospective Rubeosis Anti-VEGF (RAVE, NCT00406471) trial evaluating ranibizumab treatment

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were analyzed.[5] Serial WFFA was performed using the Staurenghi lens (Ocular Staurenghi 230SLO Retina Lens; Ocular Instruments Inc, Bellevue, WA) with a scanning laser ophthalmoscope (Heidelberg HRA Spectralis; Heidelberg Engineering, Heidelberg, Germany), allowing 150° imaging as measured from the center of the eye.[6]

A standard field for each eye's longitudinal series of WFFA images was defined so that the maximal consistent retinal area was analyzed for each eye at each time point. The area analyzed varied between patients but remained constant for any given eye at all time points. While considering all of the given eye's WFFA images, an unblinded author (D.E.C.) maximized the diameter of a circular field centered on the fovea and cropped it. Furthermore, if a peripheral region was questionable to grade, due to poor resolution, it was excluded from the standard field of each of that eye's longitudinal images. The images were then randomized for quantification.

Two retina specialists (C.C.W. and D.M.B.) independently graded the WFFA images using the Photoshop CS6 (Adobe Inc, San Jose, CA) "Magic Wand" tool to select regions of nonperfusion; an edge-detection algorithm, the tool allows manual definition of a tolerance range of pixel intensities and subsequent selection of a pixel in a region of nonperfusion. This selected pixel serves as the baseline pixel intensity for the selection tool. The algorithm then identifies surrounding pixels in the range ± the defined tolerance. The selection propagates outward contiguously, with a sensitivity defined by the tolerance, until the region of nonperfusion is selected. The larger the tolerance, the further the selection will propagate with each selection. Vasculature and regions of retinal perfusion are brighter (greater pixel-intensity), thus serving as barriers to the selection. Tolerance values were defined manually by the grader and changed based on the sensitivity required to delineate different areas. Another customizable option for the Magic Wand tool that was used was "3 × 3 Averaging": on clicking in a region of nonperfusion, the surrounding pixel's intensities are averaged to create the baseline intensity used by the selection algorithm. Once all areas of nonperfusion were selected, they were extracted and the number of nonperfused pixels were quantified with ImageJ (Version 1.42q, http://rsb.info.nih.gov/ij/, NIH) and divided by the total number of pixels in the eye's standard field. After the data were unmasked, the percentage of perfused and nonperfused retina was calculated. Disk areas of nonperfusion were also quantified. One disk area was defined for each eye as the number of pixels composing the optic disk. Statistical comparisons were performed with Pearson product–moment correlation coefficient (r) and the Mann–Whitney *U* test (U) where appropriate.

By protocol when assessable, all eyes in the RAVE trial had a relative afferent pupillary defect quantified in log units by a retina specialist (D.M.B.) using neutral density filters.[7] Linear regression (R^2) was used to analyze the correlation between the quantified relative afferent pupillary defect and extent of retinal nonperfusion.

Results

Sequential WFFA was performed in 12 eyes during 36 months of treatment of ischemic CRVO in the RAVE trial (Table 1). Within the standardized fields generated for each eye, the mean total field of gradable retina was 290 disk areas (range, 178–452). Pearson's interrater agreement was calculated (r = 0.63, P = 0.002). All eyes demonstrated extensive areas of retinal nonperfusion, predominately affecting the peripheral circulation; at baseline, mean area of retinal perfusion was 106 disk areas (range, 37–129), correlating with a mean of 46.5% of retinal area being graded as perfused retina (range, 19.1–56.4%).

The area of retinal nonperfusion increased in all eyes with a mean loss of approximately 8.1% of perfused retinal area per year (range, 4.3–12.4%), which corresponded to a mean 15-disk areas (range, 12–35) of retina evolving from perfused to nonperfused annually (Figure 1). Two clinical examples are illustrated in Figure 2.

When consecutive 1-year intervals were analyzed, the mean rate of retinal perfusion loss was 16.3% (range, 4.2–22.0%; mean 29.2-disk areas, range 7–40 disk areas) in Year 1, 4.2% (range, −1 to 10%; mean 8-disk areas, range −2 to 18 disk areas) in Year 2, and 3.6% (range, −3% to 4%, mean 6-disk areas, range −5 to 7 disk areas) in Year 3.

Five of 12 eyes (42%) developed either anterior or posterior segment neovascularization during follow-up of the RAVE trial. Although numerically greater, the extent of baseline and final nonperfusion was not statistically significantly different between eyes that did develop neovascularization and eyes that did not. Specifically, mean baseline and final extent of nonperfusion were 74.0% (SD, 7.4%) and 82.6% (SD, 9.1%) for eyes that developed neovascularization compared with 70.4% (SD, 6.8%) and 79.6% (SD, 7.2%) for eyes that did not (U = 23, P = 0.43 and U = 22, P = 0.53, respectively). Similarly, there were no statistically significant differences in the extent of retinal nonperfusion in eyes that developed anterior segment compared with posterior segment neovascularization.

Baseline quantified relative afferent pupillary defect measurements varied from 0.6 to 1.5 log units. There

Table 1. Patient and Study Eye Characteristics

Patient characteristics	
Age (mean/range), years	64/41–78
Eye (OD/OS)	6/6
Gender (M/F)	7/5
Ranibizumab injections (mean/range)	19/9–32
ETDRS best-corrected visual acuity (mean/range)	
Baseline letters	15/0–37
Final letters	26/0–53
Gradable field size (mean/range)	
Pixels	294,971/75,439–567,163
Disk areas	290/178–452
Area of nonperfusion (mean/range)	
Baseline%	53.5/43.6–80.9
Final%	78.2/59.0–89.3
Baseline pixels	166,697/35,848–374,468
Final pixels	202,805/45,589–378,203
Baseline disk areas	184/111–284
Final disk areas	213/125–308

ETDRS, Early Treatment Diabetic Retinopathy Study; OD, right eye; OS, left eye.

was no correlation between relative afferent pupillary defect and the extent of baseline or final retinal nonperfusion ($R^2 = 0.03$ and <0.01).

Discussion

In this population of severe CRVO eyes, profound retinal nonperfusion was observed with WFFA at baseline and this nonperfusion progressed despite undergoing anti-VEGF therapy. The rate of perfusion loss decreased from the first year through the 3 years of follow-up. As sequential WFFA imaging was not available in the previous natural history studies by Hayreh et al[8] and the Central Retinal Vein Occlusion Study,[2] it is unknown whether anti-VEGF treatment alters the extent of retinal nonperfusion or whether the observations in the current report represent a natural progression over time in eyes with preproliferative CRVO. As anti-VEGF medications do not alter the actual occlusion but merely block the permeability and neovascular effects of cytokines released by ischemic retina, capillary dropout may be unaffected by anti-VEGF treatments and progress in retinas where the arterial circulation is severely compromised secondary to the venous blockage. Alternatively, the anti-VEGF therapy while required to decrease macular edema and optimize visual acuity gains may contribute in some way to the progressive capillary dropout observed. Finally, despite the progressive retinal nonperfusion observed in this study, VEGF blockade may in fact reduce the progression of retinal nonperfusion, as this study did not evaluate untreated eyes longitudinally.

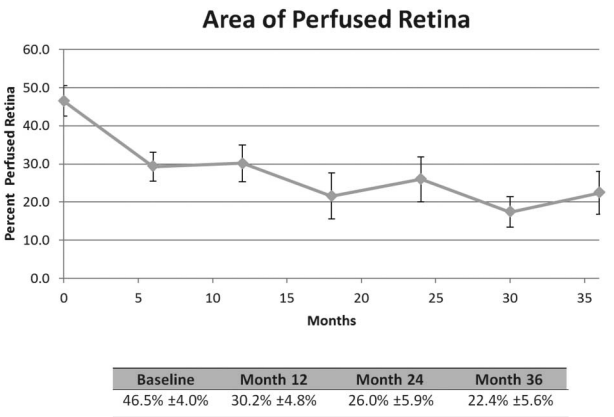

Fig. 1. Longitudinal mean percent area of retinal perfusion with error bars (standard error).

Of interest, a subanalysis of the CRVO and branch retinal vein occlusion eyes enrolled in the CRUISE and BRAVO trials by Campochiaro et al[3] described a protective effect of anti-VEGF treatment on retinal vascular perfusion. In comparison, this work demonstrates progressive loss of retinal perfusion in eyes with severe CRVO while receiving anti-VEGF therapy; the same anti-VEGF medication, ranibizumab, was used in both the Campochiaro analysis and in this study. Factors that may contribute to the differences observed between these studies include the fact that eyes from this study had severe ischemic retinal vein occlusion compared with eyes in the BRAVO and CRUISE trials, which were restricted to nonischemic retinal vein occlusion; additionally, this study evaluates wide-field retinal perfusion, whereas the Campochiaro analysis of BRAVO and CRUISE eyes was isolated to the Early Treatment of Diabetic Retinopathy Study–defined posterior pole grid.

Preproliferative CRVO eyes carry an especially poor prognosis, with >50% ultimately developing neovascular complications,[8] presumably related to progressive loss of retinal perfusion. Despite the significant perfusion loss observed in many eyes in this study, mean vision improved in these eyes.[5] Importantly, while VEGF-blockade can transiently reverse some degree of neovascularization, it seems that such treatment does not affect the natural history of this severe disease and such eyes require long-term clinical monitoring.[5]

The Early Treatment of Diabetic Retinopathy Study protocol established the first standard grading of capillary nonperfusion using fluorescein angiography.[9] A grid dividing the macula into 7 quantifiable regions

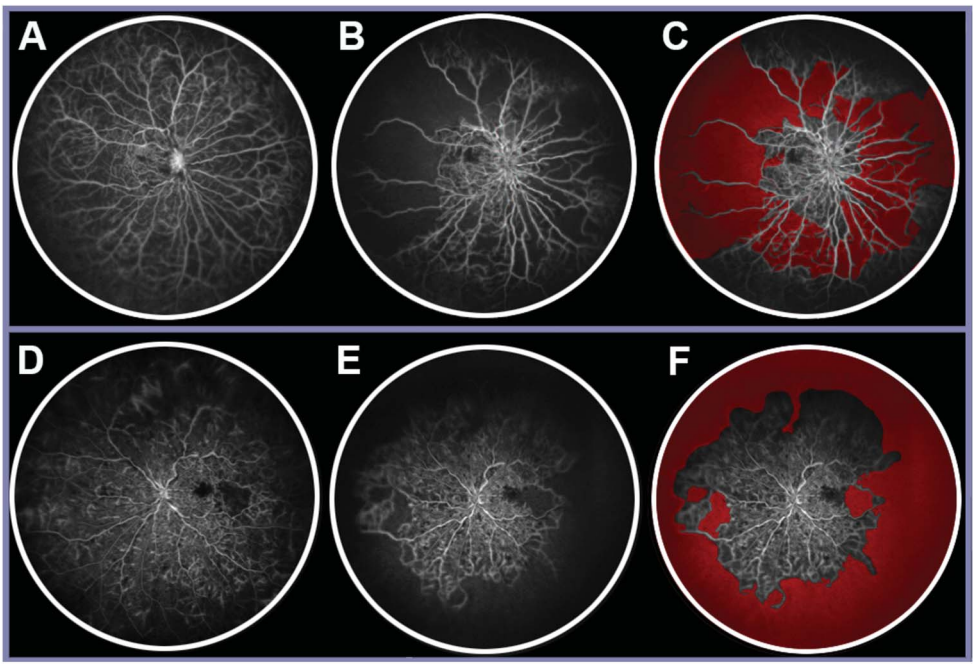

Fig. 2. Case examples of increased areas of retinal nonperfusion while undergoing anti-VEGF therapy during the RAVE trial. Patient 5 (**A**) baseline wide-field fluorescein angiography (WFFA) reveals 50% (185/370 disk areas) perfused retina and (**B**) WFFA performed 1 year later, after 9 intravitreal treatments with ranibizumab demonstrates substantial reduction in the area of retinal perfusion (25%, 93/370 disk areas), (**C**) Highlighted in red, the area of retinal nonperfusion quantified in Panel B. Patient 17 (**D**) baseline WFFA reveals 48% (220/458 disk areas) perfused retina and (**E**) WFFA performed 1 year later, after 9 intravitreal treatments with ranibizumab demonstrates substantial reduction in area of retinal perfusion (34%, 141/458 disk areas), (**F**) Highlighted in red, the area of retinal non-perfusion quantified in Panel E.

and an arbitrary scale from 0 ("absent") to 4 ("severe") was defined by examples. For more than 2 decades, reading centers have used this approach. In this report, we describe a digital selection algorithm that allows efficient identification of nonperfused regions. However, our digital processing was limited by the resolution, field of view, and consistency of images generated with the Staurenghi lens.

This single-arm uncontrolled analysis also suffers from the limitations inherent in quantifying retinal pathology. First, the retina is a 3-dimensional structure similar to a hemisphere, and the images analyzed in this study were flat projections; thus, distortions of surface area progress along a radial gradient.[10] This can be compounded by differential distortions due to individual refractive error. Spaide[11] has attempted to correct for the distortions due to retinal concavity using a model eye of average dimensions before quantifying retinal surface area. Second, delineating the exact border between areas of perfused and nonperfused retina down to precisely which pixel is or is not perfused is challenging. Similarly, in any analysis of retinal perfusion, findings that can simulate the appearance of retinal nonperfusion must be considered, including any lesion that would cause blockage on fluorescein angiography such as intraretinal hemorrhages, cotton-wool spots, pigment clumping, and any media opacity. The development of new techniques for quantifying retinal perfusion and pathology are needed to facilitate a more complete understanding of retinal vascular changes over time.

Key words: central retinal vein occlusion, RAVE, quantification, nonperfusion, anti-VEGF, perfusion, ischemia, capillary dropout, wide-field, Ischemic Index, preproliferative, ranibizumab, fluorescein angiography.

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