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Effect of Aflibercept on macular retinal layers and peripheral non perfusion in subjects with Proliferative Diabetic retinopathy

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A thesis submitted by

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KEY WORDS:

Diabetic Retinopathy;

Proliferative Diabetic Retinopathy;

Non-Perfusion Area;

Spectral Domain Optical Coherence Tomography;

Ultra-Widefield;

Effect of Aflibercept on macular retinal layers and peripheral non perfusion in subjects with Proliferative Diabetic retinopathy

Abstract:

Background: The relation between UWF fluorescein angiography (FA) and retinal non-perfusion were studied previously. The relationship between retinal thickness and retinal non-perfusion in subjects with diabetic retinopathy has not been studied using SD-OCT and UWF. Thus, in this current study we used SD-OCT to visualize the individual layers of retina and to evaluate whether the extent of non-perfusion is correlated with macular retinal layer thickness in subjects with PDR with no diabetic macular edema.

Methods: In this prospective longitudinal multicenter, randomized study a total of 36 eyes of thirty-six subjects were included in final analysis. Subjects with any history of anti-VEGF therapy, steroid therapy, pan-retinal photocoagulation or vitreoretinal surgery, macular edema, or SD-OCT determined central retinal thickness (CRT) of more than 320 μm in the study eye were excluded. All subjects underwent ETDRS BCVA testing, slit lamp and indirect ophthalmoscopy examination and diagnostic testing at all visits using Optos 200Tx (Optos plc, Dunfermline, United Kingdom), Heidelberg Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) with 9X image averaging. UWFA images were obtained and then transformed to stereographic projection images using proprietary manufacturer software. Regions of non-perfusion were identified and manually graded using Image J.

Results: The mean age of the subjects was 57 ± 13 years and 19 were female. The mean total retinal thickness was significantly lower at month 12 when compared to baseline 258.47 ± 23.52 μm vs. 285.19 ± 23.62 μm ($p < 0.001$). The thicknesses of NFL, GCL, IPL, ONL and EZ were significantly thinned at month 12 when compared to baseline ($p < 0.05$). Whereas OPL layer was significantly thickened ($p < 0.05$). No significant change was observed for INL and RPE at month 12 ($p > 0.05$). For both the treatment arms, the mean total NPA at month 12 (280 ± 143 mm^2) increased, but not significantly ($p=0.12$) differ, when compared with baseline (242 ± 169 mm^2). Ischemic index (ISI, %), however, was significantly ($p=0.009$) increased at month 12 (34 ± 17) when compared with baseline (27 ± 16).

Conclusion: The total NPA was significant and independently correlated with GCL and thinning of inner retinal layers continues even after treatment. The thickness of multiple retinal sublayers appears to decrease over time in eyes receiving intravitreal aflibercept for proliferative diabetic retinopathy despite an absence of a change in peripheral non-perfusion. The underlying pathophysiology for these reductions over time warrants further investigation.

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ABBREVIATIONS:

BCVA = Best-Corrected Visual Acuity;

CRT = Central Retinal Thickness;

DR = Diabetic Retinopathy;

DME = Diabetic Macular Edema;

ETDRS = Early Treatment Diabetic Retinopathy Study;

FA = Fluorescein Angiography;

ISI = Ischemic Index

PDR = Proliferative Diabetic Retinopathy;

NPDR = Non-Proliferative Diabetic Retinopathy;

NV = Neovascularization;

NVD = Neovascularization of the Disc,

NVE = Neovascularization Elsewhere;

PRP = Pan retinal Photocoagulation;

NPA = Non-Perfusion Area;

SD-OCT = Spectral Domain Optical Coherence Tomography;

UWF = Ultra-Widefield;

VEGF = Vascular Endothelial Growth Factor.

RISE = Ranibizumab injection in subjects with clinically significant in macular edema

RIDE = Ranibizumab injection in subjects with diabetic macular edema

Background:

Diabetic retinopathy (DR) is a microvascular complication that can lead to vision loss due to increased vessel permeability, retinal ischemia and neovascularization in the working age group.[1,2] The most advanced stage of DR is proliferative diabetic retinopathy (PDR), which develops as retinal ischemia, causing the existing vasculature to develop abnormal new vessels (hypoxia).[3] Left untreated, these hypoxic changes can cause vascular bleeding, vitreous hemorrhage, traction, and retinal detachment[4] secondary to neovascularization on the optic disc (NVD) or neovascularization elsewhere (NVE), leading to vision loss.[5] Pharmaceutical agents that specifically inhibit Vascular Endothelial Growth factor (VEGF) including the FDA-approved agents aflibercept (Eylea, Regeneron) and ranibizumab (Lucentis, Genentech), as well as the off-label us of repackaged bevacizumab (Avastin, Genentech) have revolutionized the management of all forms of DR. Multiple prospective, randomized trials focusing on the management of DME have demonstrated that anti-VEGF therapy can significantly blunt the progression of non-proliferative DR (NPDR) to PDR. For example, PDR events were reduced in the Ranibizumab injection in subjects with clinically significant in macular edema (RISE)/ Ranibizumab injection in subjects with diabetic macular edema (RIDE) phase 3 trials at 2 years from approximately 34% with sham treatment to 11% with monthly ranibizumab treatment.[6,7] The role of anti-VEGF pharmaceuticals in the management of DR may be much more important than simply blunting progression to PDR and improving DR severity as assessed by color fundus photography. VEGF blockade may be fundamentally impacting the underlying disease pathophysiology of progressive retinal non-perfusion. Consistent with this, in Ranibizumab injection in subjects with clinically significant in macular edema (RISE)/ Ranibizumab injection

in subjects with diabetic macular edema RIDE, the development of angiographically-identified NPA was significantly reduced with monthly VEGF blockade.[8]

Examination of the peripheral retina and retinal ischemic status may be a key factor in the evaluation of PDR. Several ultra-wide field (UWF) imaging studies have demonstrated that extensive and clinically important pathology may be present in the peripheral retina.[9–12] Wessel et al.[11] reported finding 3.9 times more non-perfusion and 1.9 times more neovascularization using Ultra Wide Field (UWF) than in traditional Early Treatment of Diabetic Retinopathy Study (ETDRS) seven-field images. Unoki et al[13] reported that areas of capillary nonperfusion resulting from severe nonproliferative or proliferative diabetic retinopathy showed morphologic changes in retinal structure.

A number of studies have been published on UWF fluorescein angiography (FA) and retinal non-perfusion; but, to our knowledge, only a few of these studies showed any relationship between retinal thickness and retinal non-perfusion in subjects with diabetic retinopathy.[15] The relationship between retinal layer thickness and peripheral non-perfusion in diabetic retinopathy has not been studied using spectral-domain optical coherence tomography (SD-OCT) and UWF FA. Thus, in this current study we used SD-OCT to visualize the individual layers of retina and to evaluate whether the extent of non-perfusion is correlated with macular retinal layer thickness in subjects with PDR with no diabetic macular edema and to evaluate the longitudinal changes in individual retinal layers after anti VEGF treatment in PDR with no DME.

Methods:

In this prospective longitudinal multicenter, randomized study (ClinicalTrials.gov Identifier: NCT 02863354) 40 eyes of forty subjects were included. An Institutional Review Board approval was obtained and is complied with the Declaration of Helsinki and the requirements of the Health Insurance Portability and Accountability Act (HIPAA). All subjects signed the HIPAA authorization, written informed consent for treatment and participation in the study was obtained before enrollment. To be eligible for the study, patients had to be ≥ 18 years old, with early treatment-naïve PDR secondary to DM (Type 1 or 2) in the study eye. Participants were required to have ETDRS best corrected visual acuity (BCVA) of 19 (20/400 Snellen equivalent) or more letters determined by protocol trial lens refraction at 4 meters, and with substantial non-perfusion (defined as greater than 20 disc areas) in the study eye were enrolled into the study.

Subjects with a previous history of anti-VEGF therapy, steroid therapy, pan-retinal photocoagulation or vitreoretinal surgery and those with any history of macular edema, or SD-OCT determined central retinal thickness (CRT) of more than 320 μm in the study eye were excluded. All subjects underwent ETDRS BCVA testing, slit lamp and indirect ophthalmoscopy examination and diagnostic testing at all visits using Optos 200Tx (Optos plc, Dunfermline, United Kingdom), Heidelberg Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) with a volume/cube acquisition protocol (20 x 20, 49 lines, 768 A-scans per line) with nine-times image averaging.

Retinal image acquisition:

Study eyes were dilated using tropic amide 1% and phenylephrine 2.5%, and UWF pseudo color images were captured after dilation using the Optos 200Tx centered on the fovea. After

intravenous administration of fluorescein dye, UWF fluorescein angiography (FA) images were obtained during the early (45 seconds), middle (2 minutes and 30 seconds), and late (5 minutes) phases of the angiogram, and steered peripherally (nasally, temporally, superiorly, and inferiorly) in all three phases.

UWF FA Image projection:

Uncorrected native images from the baseline visit were exported and sent to the Doheny Image Reading Center (Doheny Eye Institute, Los Angeles, California) for grading. Images were then transformed to stereographic projection images using proprietary software available from the manufacturer. This projection technique was accomplished by ray tracing every pixel through a combined optical model of the Optos 200Tx and a Navarro UWF model eye with an axial length of 24 mm.[8] This optical model represented the projection used by the Optos 200Tx scanning laser ophthalmoscopy platform to create the 2-dimensional Optomap.

• **Non-Perfusion Area Analysis:**

Regions of non-perfusion were identified by the absence of retinal arterioles and capillaries with hypo-fluorescence relative to the background. Two masked, trained, reading center-certified UWF FA graders (S.B.V and M.G.N) independently analyzed the image according to standardized reading center grading protocols at Doheny Image Reading Center.[16] Graders were allowed to adjust the contrast and brightness to optimize visualization of the areas of non-perfusion. Areas with an absence of 4^{th} order and higher-order vessels was considered as evidence of NPA (Figure 1 A). 1^{st}, 2^{nd}, and 3^{rd} order vessels adjacent to non-perfusion areas were included in the area measurements using a middle-phase FA frame. Using Image J version 1.49b (US National Institutes of Health, Bethesda, MD), the graders manually delineated the peripheral

extent of the visible retina and the borders of the non-perfusion area (NPA) (Figure 2); the software then automatically calculated the number of pixels within the regions. The number of pixels of capillary non-perfusion was calculated as a percentage of the total number of pixels of NPA within the visible retina. The manually annotated regions were then exported as a binary mask; these masks were later transformed into stereoscopic projection and their area was automatically calculated in mm^2 by summing the size of all pixels using software provided by the manufacturer (Optos plc). Total non-perfusion area and macular non perfusion area (3mm radius ring centered to the fovea) were generated at baseline and month 12 visits (Figure 1B). Ischemic index was calculated as the ratio of NPA to the total visible retina.[17] If there was a difference in NPA > 20% between graders, the graders met in open adjudication to agree on a single consensus result for each case. When differences smaller than this threshold were present, the results of the two graders were averaged to yield a final result for subsequent correlative analyses.

• SD- OCT Retinal Layer Thickness Analysis:

Uncorrected native images from all visits were exported and sent to the Doheny Image Reading Center (Doheny Eye Institute, Los Angeles, California) for grading. Raw data from Heidelberg Spectralis OCT was imported into the previously described and validated reading center custom grading software (OCTOR).[18] Then various retinal layers, i.e., vitreous top, nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), ellipsoid zone (EZ), retinal pigment epithelium (RPE), choroid and total retina were segmented in each B-scan of the macular volume and their thicknesses were measured (Figure 3).

The space extending between the ILM-inner and NFL-outer is defined as *nerve fiber layer*; NFL-outer to GCL- outer is defined as *ganglion cell layer*; the GCL-outer to IPL-outer is defined as *inner plexiform layer*; IPL-outer to INL-outer is defined as *inner nuclear layer*; INL-outer to OPL-outer is defined as *outer plexiform layer*; OPL-outer to ONL-outer is defined as *outer nuclear layer*; EZ to RPE inner is defined as *ellipsoid zone thickness*; RPE-inner to RPE-outer is *retinal pigment epithelium*, choroid-inner to choroid-outer is *choroid* and ILM- inner to RPE-inner is defined as *total retina*. The automated segmentation was followed up with manual correction for any segmentation errors in detail. Retinal thickness maps were generated for all 49 B-scans for all subjects.

Statistical analysis:

In final analysis, 36 eyes were included, as 4 eyes of four subjects were excluded from the study due to missed follow-up (2 subjects) and diseased (2 subjects). Descriptive statistics were analyzed for total study sample. The mean differences at baseline and month 12 visits were calculated using paired sample t test. Similarly, the change analysis at month 12 visit between cohorts were analyzed using paired sample t test. For baseline data, correlations between the non-perfusion area (total and macular non perfusion area) and individual retinal layers thickness was analyzed using bivariate Pearson correlations. And multivariate analysis was performed using regression analysis. Statistical results were expressed as *P* values. A *P* value of less than 0.05 was considered statistically significant. All statistical analysis were performed using SPSS 18.0 statistical software (SPSS, Chicago, IL).

Results:

A total of thirty-six eyes of 36 subjects with PDR were included in this study. The mean age was 57 ± 13 years and 19 were female. The mean total retinal thickness was significantly lower at month 12 when compared to baseline 258.47 ± 23.52 μm vs. 285.19 ± 23.62 μm ($p < 0.001$). The thicknesses of NFL, GCL, IPL, ONL and EZ were significantly thinned at month 12 when compared to baseline ($p < 0.05$). Whereas OPL layer was significantly thickened at month 12 compared to baseline ($p < 0.05$). No significant change was observed for INL and RPE at month 12 ($p >0.05$). The individual retinal layers thickness measurements at baseline, month 12 and change at month 12 were shown in table 1.

For the entire study sample, the mean total NPA at month 12 (280 ± 143 mm^2) increased, but not significantly ($p = 0.12$) differ, when compared with baseline (242 ± 169 mm^2). Ischemic index (ISI, %), however, was significantly ($p=0.009$) increased at month 12 (34 ± 17) when compared with baseline (27 ± 16). Meanwhile, total area of neovascularization (NV), which was calculated by combining neovascularization of the disc (NVD) and neovascularization elsewhere (NVE), decreased dramatically from 20 mm^2 to 1.02 mm^2 ($P = 0.004$, As shown in Table 2).

Correlation Analysis between retinal layers and total NPA:

The relation between total NPA and the thickness of various retinal layers was studied using univariate correlation analysis which showed a significant correlation between total NPA and thickness of the NFL ($r = -0.34$, $p = 0.03$), GCL ($r = -0.48$, $p = 0.002$), IPL ($r = -0.46$, $p = 0.003$), and INL ($r = -0.31$, $p = 0.04$). We also observed significant relation between total NPA and total retinal thickness ($r = -0.38$; $p= 0.01$). Similar results were observed for macular non perfusion also as shown in Table 3. The total NPA showed no significant correlation with the thickness of the OPL, ONL, EZ, and RPE ($p > 0.05$). All parameters (NFL, GCL, IPL, and INL thicknesses)

which were significant on univariate analysis ($p \leq 0.05$) were included in the multivariate analysis. Only GCL thickness remained independently correlated with total NPA [$R^2 = 0.23$, $p = 0.002$] and ischemic index [$R^2 = 0.12$, $p = 0.03$].

Discussion:

In the current study, we evaluated the effect of intravitreal aflibercept on individual retinal layers thickness at macula and the total non-perfusion across the entire retina as determined by UWF FA. The thickness of multiple retinal sublayers appears to decrease over time in eyes receiving intravitreal aflibercept for PDR.

We have excluded subjects with any evidence of macular edema from study enrollment, and thus confounding effect of exudation of the measurement of retinal sublayer thickness was eliminated. The correlation of VEGF production with resultant macular edema and the extent of retinal non-perfusion is well known.[19] It has been studied in diseases like branch retinal vein occlusion by Singer et al.[20]

Previous studies have described positive relation between capillary loss and macular edema.[21–23] In current study, we showed a significant reduction of inner retinal layers (NFL till the OPL) thickness after anti VEGF treatment and also showed thinning of inner retina layers with respect to the total non-perfusion area. Total (macular and peripheral) non perfusion area was significantly correlated with inner retinal layers, more interestingly multivariate analysis showed only GCL is independently correlated with total NPA. Rabiolo et al[24] reported a significant correlation between the ischemic index and area of FAZ measured at full thickness and superficial layers and also showed primary involvement of inner retinal layers with respect to disease severity. An OCT based study by Sim et al[25] showing an inverse correlation between the FAZ area and the RNFL thickness also confirmed the involvement of inner retinal layers, as shown by various other studies.[26–28] We observed significant thinning of retinal layers like NFL, GCL, IPL, ONL and EZ at 1 year follow up and significant increase in thickness of OPL and

RPE and also observed increase in total NPA, which indicates with respect to severity of NPA inner retinal layers are effected at most.

This study highlights the association and early loss of inner retinal layers in eyes with peripheral retinal capillary loss in eyes with PDR. We also observed a significant progressive accumulation of non-perfusion through 1 year in the entire population. This indicates that the NPA underlying DR is a progressive process that appears to be largely not reversible with aflibercept treatment.

Notably, aflibercept treatment within RECOVERY did appear to have a biological impact on NPA. The documentation of reduced NPA development with anti-VEGF dosing is consistent with analyses of the RIDE/RISE dataset in which ranibizumab dosing slowed both development and progression of NPA, compared to sham treatment.[8] However, despite the presumably valuable clinical impact of slowing NPA accumulation in RIDE/RISE data, NPA accumulation was not halted and area of NPA did not appear to decrease in treated eyes across the population[8], consistent with the findings of RECOVERY.

It is noteworthy that treatment naïve PDR eyes with such dramatic NPA were adequately controlled, without development of DME or progressive PDR or need for rescue PRP, through aflibercept treatment. The Diabetic Retinopathy Clinical Research Network (DRCR.net) protocol S employed 4-6 monthly ranibizumab loading doses for PDR management before anti-VEGF re-treatments were individualized.[29] Clinically, intravitreal anti-VEGF dosing can dramatically decrease the extent of visible vascular abnormalities such as intra-retinal hemorrhages, microaneurysms, intra-retinal microvascular abnormalities and venous beading, all considered when quantifying a particular photography-based grade.

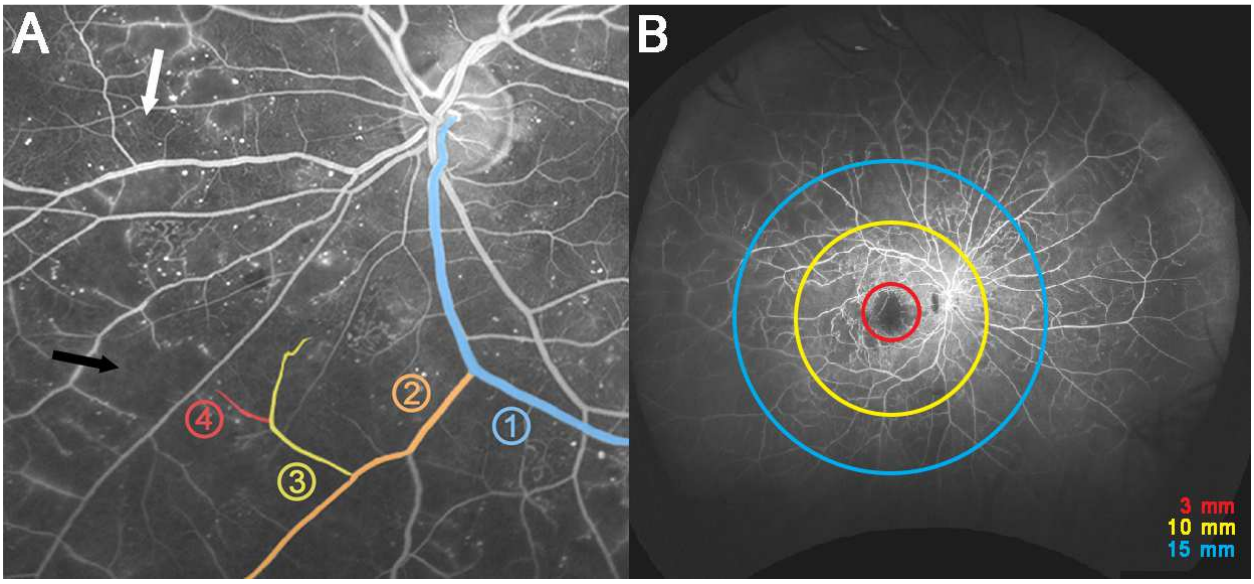
At a deeper level NPA has proven to be a clinical marker with important prognostic value. Specifically, in the face of consistent and intensive anti-VEGF treatment, some eyes will nonetheless experience DR severity worsening. Through 3 years in RIDE/RISE study, approximately 18%, or nearly 1 in 5 subjects, developed a PDR event despite monthly ranibizumab dosing; in this context, the only statistically significant prognostic factor identified for progression to a PDR event was the presence of baseline NPA.[7] NPA has also been identified as a significant prognostic indicator of progression to PDR among eyes with DME treated with fluocinolone acetonide.[30] The current work indicates that location of NPA may also be clinically relevant. A statistically significant progression of NPA within the largest zone, 15 mm indicate that retinal vasculature in the mid-periphery may be particularly sensitive to progressive vascular damage.

Strengths of the current study are, this is the first study showing the effects of peripheral retinal capillary loss on central retinal layer thickness till date with laborious manual segmentation of all individual retinal layers. And another strength of the study was the use of stereographically projected images for ischemic index calculation, to nullify the effect of peripheral image distortion and magnification. This gives the precise measurements of the ischemic index, as was shown by Tan et al[19]. Restrictions of this randomized trial include the limited number of subjects and inherent challenges in measuring NPA from fluorescein angiographic images.

In conclusion, the thickness of multiple retinal sublayers appears to decrease over time in eyes receiving intravitreal aflibercept for proliferative diabetic retinopathy despite an absence of a change in peripheral non-perfusion. The underlying pathophysiology for these reductions over time warrants further investigation

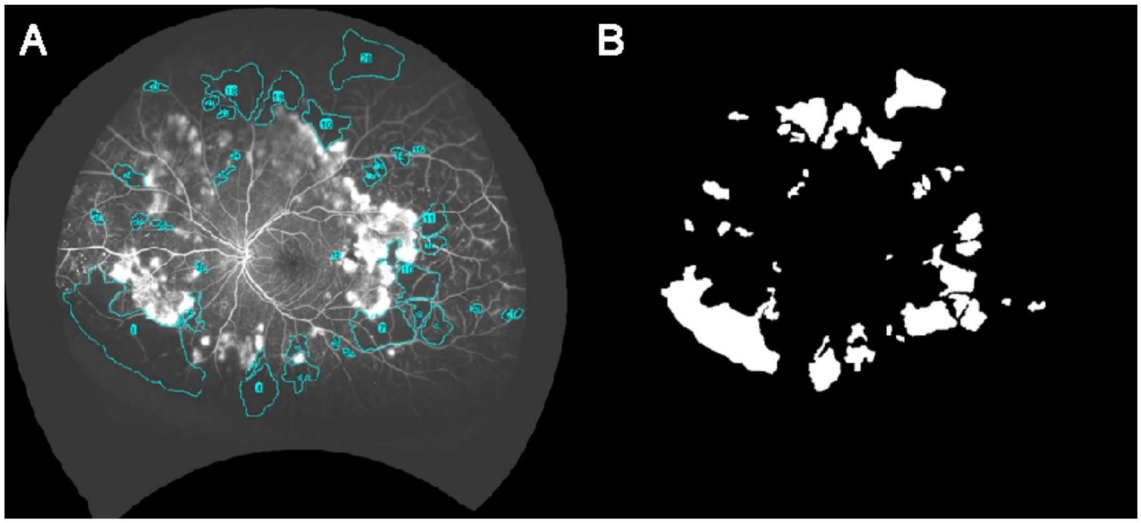
Figures and Legends:

Figure 1: Definition of Retinal Non-Perfusion



A. Non-perfusion Area (NPA) was defined as an absence of 4th-order (red) and higher-order vessels. 1st-, 2nd-, and 3rd-order vessels were illustrated in blue, orange and yellow respectively. The area with an absence of 4th-and-higher-order vessels (black solid arrow) was considered non-perfused whereas the area identified with the white solid arrow was considered perfused due to visible 4th-and-higher-order vessels. **B.** Concentric rings (centered on the fovea) were used to measure areas of NPA: 3-mm area (red; 3-mm radius, excluding the fovea), 10-mm area (yellow; 3- to 10-mm radius), 15-mm area (blue; 10- to 15-mm radius).

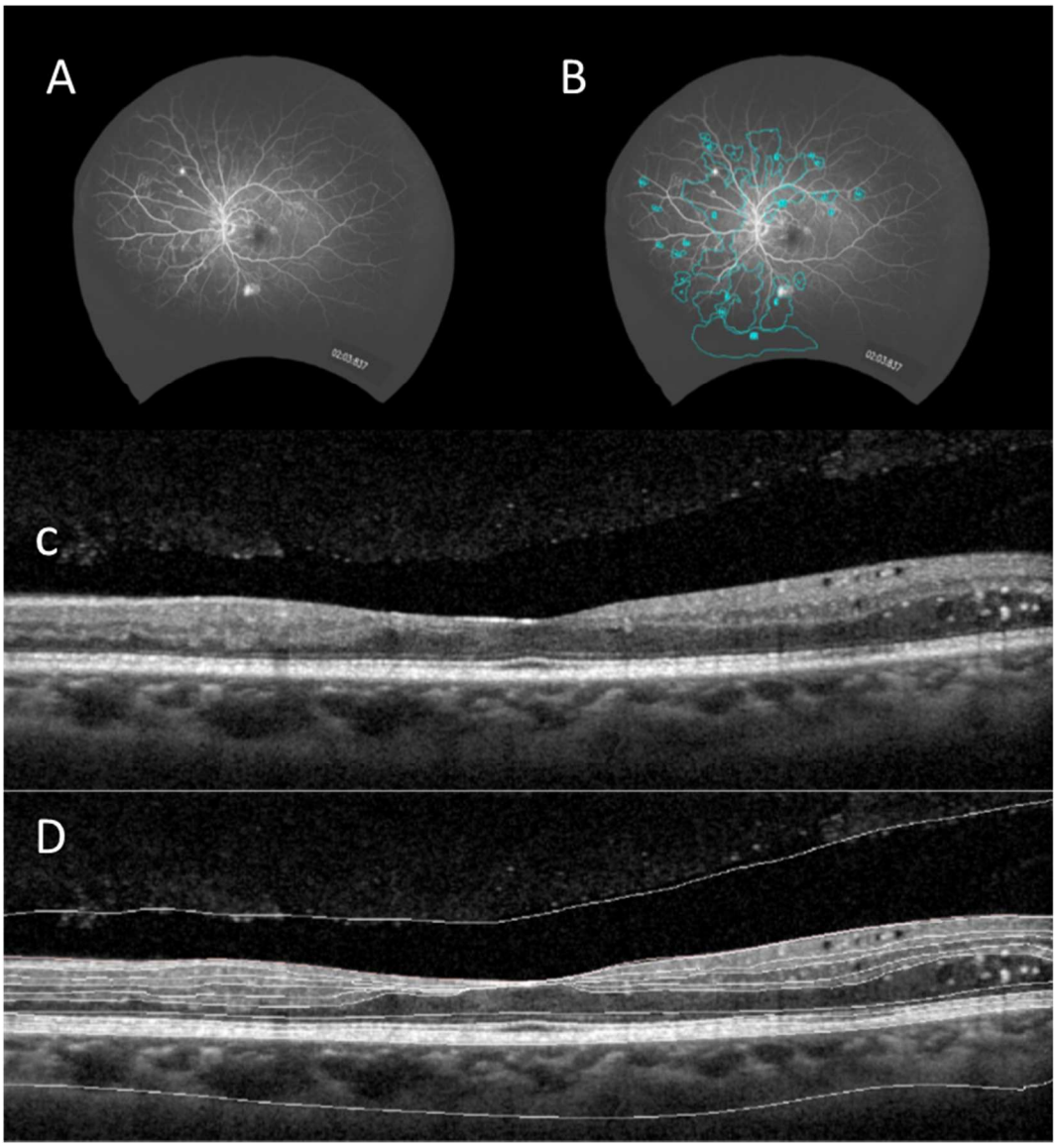
Figure 2: Summation of Areas of retinal non-perfusion



A. Non-perfusion Area (NPA) were delineated using Image J on ultra-widefield fluorescein angiography image. B. Binarized images were used to calculate the NPA.

Figure 3: UWF Images segmented and unsegmented UWF Images and SD-OCT B-scans.

Note total retinal thickening in the temporal region due to ischemia in the non-perfused area.



(A) Ultra-Wide Field (UWF) image, (B) Segmented UWF image, (C) Optical Coherence tomography (OCT) image, (D) Segmented OCT image.

Tables

Table 1: Thickness of retinal layers and choroid at baseline and month 12 visits

Layer **thickness** (µm) N= 36 eyes	**Baseline Mean ± SD (Range)**	**Month 12 Mean ± SD (Range)**	**Change at Month 12 Mean ± SD (Range)**	***p*-value**
Nerve Fiber Layer	38.72 ± 7.39 (27.2 - 51.1)	33 ± 5.08 (25.1 - 41.7)	-2.74 ± 14.72 (-20.7 - 41.7)	**0.005**
Ganglion Cell Layer	41.6 ± 4.44 (33.6 - 48.8)	34.75 ± 5.02 (22.6 - 41.5)	-3.66 ± 11.97 (-12.5 - 34.1)	**<0.001**
Inner Plexiform Layer	33.41 ± 3.11 (27.4 - 38.6)	29.95 ± 3.43 (22.7 - 33.9)	-0.9 ± 10.57 (-7.5 - 33.4)	**<0.001**
Inner Nuclear Layer	28.12 ± 3.06 (22.3 - 34.6)	27.01 ± 1.68 (23.7 - 29.4)	1.06 ± 8.83 (-7.1 - 29.3)	0.1
Outer Plexiform Layer	32.15 ± 3.17 (25.1 - 35.7)	31.14 ± 3.66 (23.9 - 36.3)	1.47 ± 10.54 (-5.6 - 35.9)	**<0.001**
Outer Nuclear Layer	57.59 ± 5.24 (48.8 - 65.3)	51 ± 9.35 (33.2 - 62.7)	-2.16 ± 18.66 (-19.8 - 54.1)	**0.02**
Ellipsoid Zone	46.66 ± 49.73 (20.6 - 158)	23.77 ± 3.8 (19.1 - 29.1)	-24.27 ± 66.18 (-240.7 - 19.1)	**<0.001**
RPE	30.8 ± 1.28 (28.6 - 33)	30.26 ± 5.58 (24.1 - 44.3)	1.84 ± 13.26 (-7.2 - 44.3)	0.14
Choroid	259.22 ± 3.24 (205.2-318.2)	230.86 ± 20.33 (181 - 253.8)	-8.43 ± 87.57 (-94.9 - 245.1)	0.04
Total Retina Thickness	285.19 ± 23.62(249.6 -324.6)	258.47 ± 23.52(209.1 - 277.1)	-4.78 ± 86.3 (-52.9 - 277.1)	**<0.001**

SD-Standard Deviation; p-significance levels; statistically significant levels were in bold

Table 2: Baseline and Month 12 Non Perfusion Area and Neovascularization data for total study eyes (N= 36 Eyes)

	Baseline Mean ± SD (Range)	**Week 52 Mean ± SD (Range)**	***p-value***
Total retinal area (mm2)	909 ± 185 (650 – 1213)	834 ± 131 (629 — 1212)	0.16
Non perfusion in 3 mm (mm2)	2 ± 2 (1 – 4)	1.11 ± 4.73 (0 — 28.29)	0.25
Non perfusion in 10 mm (mm2)	32 ± 24 (1 – 95)	48.92 ± 52.46 (0.3 — 296.42)	0.06
Non perfusion in 15 mm (mm2)	128 ± 78 (6 – 323)	173.12 ± 109.61 (38.18 — 611.54)	0.006
Total Non perfusion area (mm2)	242 ± 169 (60 – 774)	280.1 ± 142.99 (68.39 — 817.43)	0.12
Ischemic Index (%)	27 ± 16 (6 – 67)	34 ± 17 (8 — 100)	0.009
Total NVE/NVD area (mm2)	20 ± 35 (1 – 188)	1.02 ± 2.88 (0 — 12.66)	0.004

NVE- Neovascularization Elsewhere; NVD- Neovascularization of the Disc

Table 3: Retinal Layers Thickness and Univariate Correlation Analysis

Layers	Thickness (µm)	Total Capillary Non Perfusion r[*p*]	Macular Capillary Non Perfusion r[*p*]	Ischemic Index r[*p*]
Nerve Fiber Layer	37 ± 6 (27 - 54)	-0.34 **[0.03]**	-0.26 [0.10]	-0.33 [0.03]
Ganglion Cell Layer	41 ± 5 (24 - 50)	-0.48 **[0.002]**	-0.55 **[<0.001]**	-0.30 [0.05]
Inner Plexiform Layer	34 ± 4 (23 - 42)	-0.46 **[0.003]**	-0.51 **[0.001]**	-0.30 [0.06]
Inner Nuclear Layer	29 ± 3 (20 - 36)	-0.31 **[0.04]**	-0.51 **[0.001]**	-0.16 [0.36]
Outer Plexiform Layer	32 ± 3 (23 - 36)	-0.29 [0.71]	-0.54 **[<0.001]**	-0.18 [0.27]
Outer Nuclear Layer	60 ± 8 (46 - 81)	0.01 [0.93]	0.12 [0.46]	0.05 [0.72]
Ellipsoid Zone	25 ± 3 (21 - 30)	-0.11 [0.48]	-0.11 [0.49]	-0.18 [0.28]
RPE	31 ± 2 (27 - 34)	-0.11 [0.49]	-0.04 [0.79]	-0.11 [0.51]
Choroid	258 ± 50 (158 - 369)	0.05 [0.73]	-0.17 [0.27]	0.14 [0.40]
Total Retina Thickness	286 ± 23 (233 - 344)	-0.38 **[0.01]**	-0.42 **[0.007]**	-0.27 [0.08]

RPE-Retinal Pigment epithelium**; µm-**micro-meter**; r**-correlation coefficient; ***p*****-**Statistical Significance

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