

Accepted Manuscript

Retinal non-perfusion in the posterior pole is associated with increased risk of neovascularization in central retinal vein occlusion

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PII: S0002-9394(17)30310-0

DOI: [10.1016/j.ajo.2017.07.015](https://doi.org/10.1016/j.ajo.2017.07.015)

Reference: AJOPHT 10213

To appear in: *American Journal of Ophthalmology*

Received Date: 27 April 2017

Revised Date: 13 July 2017

Accepted Date: 14 July 2017

Please cite this article as: Nicholson L, Vazquez-Alfageme C, Patrao NV, Triantafyllopoulou I, Bainbridge JW, Hykin PG, Sivaprasad S, Retinal non-perfusion in the posterior pole is associated with increased risk of neovascularization in central retinal vein occlusion, *American Journal of Ophthalmology* (2017), doi: 10.1016/j.ajo.2017.07.015.

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Structured Abstract

Purpose:

To review the definition of ischaemic central retinal vein occlusion (CRVO) and stratify the risk of neovascular complication based on wider areas of visible retinal non-perfusion.

Design:

Retrospective consecutive case series and image analysis study

Methods:

Setting: Moorfields Eye Hospital, London, United Kingdom.

Study Population: 42 consecutive treatment naïve eyes with CRVO imaged with ultra-widfield angiography with a minimum of 12 months follow-up.

Observation Procedure: The spatial location and total area of retinal non-perfusion (measured in disc areas, DA) were determined using the validated concentric rings method. The area was corrected for projection distortion. The images were graded by two retinal physicians and average measurements used.

Main Outcome Measures: Development of neovascular complications.

Results:

The percentage of eyes developing new vessels increased from none in eyes with less than 10 DA of non-perfusion in total, to 14.3% in eyes with 10-30DA, 20.0% for 30-75DA and 80% risk with 75-150DA of non-perfusion. From 13 (31.0%) eyes with a perfused posterior pole (an area encompassing a five disc diameter radius centered at the fovea) and more than 10DA of non-perfusion isolated in the periphery (beyond the posterior pole), only one (7.7%) eye developed new vessels, OR 0.12 [95% CI:0.01, 1.03]. Comparatively, for 13 (31.0%) eyes with more than 10DA of non-perfusion in the posterior pole, 11 (84.6%) developed new vessels, OR 74.25 [95% CI: 9.26, 595.30], $p < 0.001$.

Conclusion:

With ultra-widfield angiography, we have ascertained that posterior pole non-perfusion of more than 10DA remains the key risk factor for new vessel development compared to areas of non-perfusion confined to the periphery.

(250 words)

Title Page**Title**

Retinal non-perfusion in the posterior pole is associated with increased risk of neovascularization in central retinal vein occlusion

Short title

Posterior non-perfusion associated with neovascularization

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Main Text

Introduction

Our understanding of the natural history of central retinal vein occlusion CRVO is based on the observations reported in the Central Vein Occlusion Study (CVOS). The study showed that 35% of non-perfused eyes and 10% of perfused eyes at baseline progressed to anterior segment neovascularization over a three year period with the majority within the first year.¹ In the control arm of the CVOS, among eyes with more than 10 disc areas of retinal capillary non-perfusion, the risk of developing anterior segment neovascularization was 35% with no prophylactic treatment and this risk reduced to 20% when prophylactic panretinal photocoagulation was administered.² Based on these findings, ischemic CRVO is defined as an eye with 10 disc areas or more of non-perfusion and the risk of anterior segment neovascularization increased significantly in eyes with more than 30 disc areas of non-perfusion.² The Standard Care vs. Corticosteroid for Retinal Vein Occlusion (SCORE) study report number 11, on the other hand, suggested the limits of 5.5 disc areas as an indicator of ischemia with an incidence of anterior segment neovascularization of 23.6% and only 5.9% with less than 5.5 disc areas of non-perfusion.³ The imaging methods used in these landmark studies were limited to the standard 30-degree seven field ETDRS protocol in SCORE and five field views using 60-degree images or eight standard views using 30-degree images in CVOS.^{2,4} Therefore, the areas of perfusion visualized were limited.

Ultra-widefield imaging has allowed visualization of up to three times the area captured with a standard 7-field imaging protocol in a single capture.⁵ So the area of retinal capillary non-perfusion observed using ultra-widefield angiography is also significantly greater.⁶ Therefore, a question has risen as to whether the definition of ischemic CRVO needs to be revised in light of the greater area of non-perfusion noted on ultra-widefield angiography. Using a standard 10 disc areas of non-perfusion interchangeably with different imaging modalities poses a problem. Tsui et al proposed an ischemic index (non-perfused retina/gradable retina) on ultra-widefield angiography, and reported that eyes that develop neovascularization have a mean ischemic index of 75% in contrast to eyes that did not with a mean ischemic index of only 6%.⁷ The authors also concluded that eyes with an ischemic index of 45% have a greater likelihood of developing neovascularization.⁷ However, the images used were not corrected for three dimensions to two dimension projection errors leading to a magnification of the periphery compared to the center as well as a horizontal stretch.^{8,9} Efforts have been made to correct for these artifacts and Optos, Dunfermline, United Kingdom has incorporated a correction into the V2 Vantage Pro software which now produces a stereographically corrected image.¹⁰ Despite this improvement, we have found that images are still enlarged in the periphery by using 3-D printed model eyes.¹¹

Furthermore, there is evidence that anti-VEGF therapy does modify the natural history of CRVO. Therefore, the guidance obtained from the CVOS study whereby it was acceptable to wait for the development of neovascularization before performing

panretinal photocoagulation cannot be translated into practice because intravitreal anti-VEGF treatment may mask or delay the development of neovascularization. The high-risk period of the first three months is now shifted with anti-VEGF therapy. The RAVE study reported the risk of neovascularization is not ameliorated but only delayed.¹² It has also been reported that following regular intravitreal anti-VEGF treatment, the treatment free interval prior to the development of neovascular events was 6.2 ± 7.3 months.¹³ This further highlights the necessity to risk stratify patients for either prophylactic panretinal photocoagulation or more intensive follow-up upon cessation or extension of the interval of anti-VEGF therapy. Using a 10DA of non-perfusion as a guide with an incidence of new vessels of 35% will result in 65% of patients having unnecessary intensive follow-up clinic visits or prophylactic treatment.

Therefore, to enhance our understanding of the development of neovascularization in eyes with CRVO and to risk-stratify our patients better when ultra-widefield angiography is used, we performed a retrospective analysis of corrected ultra-widefield angiography images of eyes with CRVO to evaluate the area and distribution of retinal non-perfusion that is most predictive of neovascularization.

Methods

This retrospective consecutive case series and image analysis study was performed in the National Institute for Health Research Moorfields Biomedical Research Centre and the Institute of Ophthalmology, University College London, United Kingdom. Local institutional review board approval was obtained for analysis of anonymised data and the study was conducted in accordance with the tenets of the Declaration of Helsinki (Registration number 15006).

Inclusion criteria and data collection

A total of 1659 patients with a diagnosis of CRVO were identified from the medical retina service database. From this group, eyes that had been imaged with Optos ultra-widefield angiography using standard local protocols were identified. Images were excluded if they were not of sufficient clarity, defined as the ability to clearly discern perfused from nonperfused capillaries. The observation period was a minimum of 12 months from diagnosis or until the development of neovascularization. Eyes treated with intravitreal agents or prophylactic panretinal photocoagulation either previously or during the observation period were also excluded from the analysis. The effect of anti-vascular endothelial growth factor (VEGF) on the area of retinal non-perfusion is debatable with contradictory reports published and therefore patients with prior treatment were excluded.^{14,15} The definition of neovascular complications in our study were iris new vessels, new vessels at the angles, disc new vessels, retinal new vessels and vitreous hemorrhage. Treatment was permitted after the development of neovascular changes. Eyes that develop neovascularization must have had ultra-widefield angiography within two months of the date of documented neovascular complication to reduce the likelihood of underestimating the area of non-perfusion should an eye convert to an ischemic form following diagnosis.

Image acquisition

The ultra-widefield angiography was performed with the Optos 200TX (Optos Plc, Dumfermline, Scotland) ultra-widefield system using a standard protocol after intravenous bolus infusion of 5 mL of 20% fluorescein sodium. The protocol consisted of acquiring images in transit phase (up to 45s), arteriovenous phases (1-2min), and late frames at 3-4 minutes and 7-8 minutes. A single investigator (L.N.) identified the best macula centered fluorescein angiography (FA) image in the arteriovenous phase from the FA series of each eligible eye.

Image processing

A correction factor was applied for the flattening of the 3-dimensional image to a 2-dimensional image using a non-commercial research tool under development by Optos at the time of the study but now available as part of the Optos V2 Vantage Pro software. The concentric rings template was applied to each image according to previously described methods.¹⁶ In brief, this validated method incorporates a macular ring with a radius of 2.5 disc diameters (DD) and five additional concentric rings (rings 1-5), each with a 2.5DD increment in radius.¹⁶ Each of the six rings (Ring M and 1-5) were divided into 12 segments (Figure 1). Each segment is graded as upgradeable, not-perfused or perfused if 50% or more of the segment is involved.¹⁶ In addition to quantifying non-perfusion, the concentric rings method allows documentation of location of non-perfusion. The area of each cell in each concentric ring was modified based on the enlargement factor identified using 3-D printed model eyes.¹¹ The enlargement factor for rings M, 1, 2, 3, 4, and 5 were 1.08, 1.20, 1.34, 1.54, 1.81 and 1.97 respectively.¹¹ Therefore, the modified area of each segment in each ring is now represented in Table 1. We did not use the eye-steering images in our cohort. We feel the montaged image using the three images (superior, central, inferior) introduces projection distortions that as yet has not been studied or validated as the location of the same area of the retina is distorted differently in different images given the different angular location.¹¹ Therefore, we elected to only use the central image as the projection distortion in this has previously been studied and we have made the appropriate corrections.¹¹

Data collection and analysis

Measurement of retinal nonperfusion from ultra-widefield angiography was performed using the concentric rings method. The location and area of retinal non-perfusion (measured in disc areas, DA) were determined using the concentric rings method.¹⁶ The macular ring and ring 1 corresponds to the posterior pole while rings 2 to 5 corresponds to the periphery. The images were graded by two retinal physicians and average measurements were used to increase reliability. Alternative methods to quantify retinal non-perfusion include the ischemic index but this method only quantifies retinal non-perfusion as a ratio and does not give us the pattern of ischemia.⁷ We elected to quantify non-perfusion as disc areas rather than in mm² as historically we have been accustomed to disc areas as a measure of retinal non-perfusion on fluorescein angiograms and therefore comparisons can be made simple.

Statistical analysis

Descriptive statistics were used to present the data. The agreement between the two graders was expressed as intraclass correlation coefficients. The association between patterns of retinal non-perfusion and the development of neovascularization

was studied using a 2X2 contingency table and applying the Fisher's Exact test. A Kaplan-Meier curve was used to report the time to neovascular events in our cohort. Differences between means were assessed with a Mann Whitney U Test. Risks were expressed as odds ratio and applying a two tailed Fisher's Exact test to assess statistical significance. Statistical significance was set at 0.05.

Results

A total of 114 consecutive patients with central retinal vein occlusion and imaged with ultra-widefield angiography that attended Moorfields Eye Hospital, London, United Kingdom, between January 2014 and December 2014, were assessed. There were 42 treatment naïve non-diabetic eyes with central retinal vein occlusion from 41 patients with gradable UWF-A and a minimum of 12 months follow-up was identified and the development of new vessels in that period was recorded.

The mean (+SD) age at diagnosis of patients in this study was 61.2 ± 16.5 years. There were 24 (58.5%) males and 20 (47.6%) were right eyes. The median duration between diagnosis and UWF-A was 17 days. The Intraclass correlation coefficient (ICC) for grading areas non-perfusion among the two graders was 0.951.

Area of non-perfusion

The mean area of non-perfusion in the whole cohort was $78.4\text{DA} \pm 104.4\text{ DA}$ (range, 0-403.9 DA). Thirteen out of 42 eyes (30.95%) developed neovascular complications. The mean area of non-perfusion in eyes complicated by neovascularization was 177.4 DA [95% CI: 102.2, 252.6]. As for eyes with no neovascularization, (29 eyes), the mean area of non-perfusion was 34.1DA [95% CI: 14.2, 53.9]. This difference was statistically significant, $p < 0.001$

There were 9 (21.4%) eyes that had no areas of non-perfusion while 12 (28.6%) eyes had less than 10 disc areas of non-perfusion. The incidence of new vessels was 0% with less than 10 DA of non-perfusion. This increases with increasing area of retinal non-perfusion as described in Table 2. In eyes with less than 30 disc areas of retinal non-perfusion, 5.3% developed new vessels as opposed to 52.2% of eyes with more than 30 disc areas of retinal non-perfusion developing neovascularization. This is further detailed in Table 3.

Location

When each ring was assessed specifically, the incidence of developing new vessels is more than 80% if three or more cells in a single ring for rings M, 1 or 2 in the concentric rings were graded as not perfused. This is also reflected in eyes with more than 10 disc areas of non-perfusion in rings M and 1 whereby the incidence is also more than 80%. The risk of neovascularization is further detailed in Table 4 where posterior pole involvement relates to a high risk of developing new vessels.

There were 13 (31.0%) eyes that had more than 10DA of non-perfusion isolated in the periphery with a completely perfused posterior pole. Of these eyes, 1 (7.7%) developed new vessels. 13 (31.0%) eyes had more than 10DA of non-perfusion in

the posterior pole and of these, 11 (84.6%) developed new vessels. This association was found to be statistically significant, $p=0.0002$ (Table 5). Among these 13 eyes with more than 10DA of non-perfusion in the posterior pole, 6 (46.2%) developed anterior segment neovascularization.

In a sub-analysis of macular non-perfusion, among the 12 eyes with non-perfusion in Ring M, 9 (75%) developed new vessels. In contrast, among the 17 eyes with retinal non-perfusion not involving Ring M, no eyes developed new vessels. A Kaplan-Meier curve for time to development of neovascular complications for our cohort is presented in Figure 2.

We present two cases in our cohort, figure 3, which demonstrates the differences in isolated peripheral non-perfusion and posterior pole non-perfusion and its association with the development of neovascularization. The eye in example 1 has a lesser area of retinal non-perfusion compared with example 2, however, the posterior pole is involved in example 1 with three segments in Ring 1 being non-perfused. This eye did develop retinal new vessels. The eye in example 2 on the other hand, despite having a larger area of non-perfusion albeit no segments in Rings M, 1 or 2 that are not perfused, did not develop new vessels.

Discussion

This is the first study reporting the incidence of neovascularization in central retinal vein occlusion based on a corrected area of retinal non-perfusion in ultra-widefield angiography as well as the distribution of retinal ischemia. As we are now imaging a significantly wider area of the retina, the traditional concept of 10 disc areas of retinal non-perfusion as a definition of an ischemic central retinal vein occlusion in ultra-widefield angiography can be improved. In our cohort, eyes with areas of retinal non-perfusion between 10-30 disc areas, 85.7% did not experience neovascularization. Increasing the threshold to 30 disc areas of non-perfusion appears a better indicator with 5.3% developing new vessels if less than 30 disc areas were involved and 52.2% if this was exceeded.

Despite the quantity of non-perfusion, the distribution of retinal non-perfusion is a sensitive determinant of neovascularization. Involvement of the posterior pole appears to harbor a significant risk of developing new vessels with 84.6% of eyes with more than 10 disc areas in the posterior pole (rings M and 1) experiencing this complication with an odds ratio of 74.3. We have also found that involvement of the posterior pole is related to a larger total area of retinal non-perfusion as well. Interestingly, should the posterior pole remain perfused, despite large areas of non-perfusion in the periphery (more than 10 disc areas), the risk of neovascular complications remains very low, neovascularization incidence of 7.7% and odds ratio of 0.12. This further reduces the significance of a global 10 disc areas of non-perfusion as a definition for an ischemic retinal vein occlusion as these patients are unlikely to develop neovascular complications. This could be explained by the fact that the peripheral retina is thinner and inner retinal ischemia which could be compensated by the choroid has less of an impact as opposed to ischemia in the

posterior pole. In regards to the implication on panretinal photocoagulation, as posterior non-perfusion is frequently associated with a larger total area of non-perfusion which is inclusive of the periphery, there is still place for peripheral panretinal photocoagulation. However, this also helps explain the finding that despite peripheral panretinal laser, persistent new vessels in diabetic retinopathy exist and 20% of prophylactically treated eyes in the CVOS study still progressed to significant neovascular events.² This implies that in these very ischemic eyes with resistant cases of neovascularization, panretinal laser involving the posterior pole may be required.

The quantity of retinal non-perfusion in each ring from the concentric rings method is an alternative method to risk stratify the incidence of neovascularization. The area of each segment progressively increases with each subsequent ring towards the periphery as described in Table 1. The significance of three segments or more of retinal non-perfusion in a single ring appears to confer to an increased incidence of neovascularization which is can translate to a practical method to utilize in clinical practice.

The importance of making adjustments to the peripheral distortion in ultra-widefield imaging is also essential in our understanding of the area of non-perfusion. Tsui et al identified a mean ischemic index of 75% among eyes that develop neovascularization.⁷ While, in our cohort, the corresponding ischemic index taking into account the correction factors described, the mean ischemic index was 52% for eyes that develop new vessels.

The need for ultra-widefield imaging is then brought into question as a standard ETDRS seven field will be sufficient to image the posterior pole thus potentially identifying these patients that are high risk for developing neovascular complications. In figure 4, we have superimposed the concentric rings with a standard seven field coverage. Rings M and 1 are shaded and is included in the standard seven field imaging. Therefore, this implies that standard seven-field imaging is sufficient to predict neovascularization and 10DA within the posterior pole remains a very important threshold. Knowledge of the area and pattern of non-perfusion in ultra-widefield imaging is required if this imaging modality is used. The ultra-widefield system still provides a whole image that is in-phase as opposed to seven different phases montaged into a single image. It has also been reported to identify significantly more new vessels and retinal pathology compared to standard seven field imaging.¹⁷ We need to add that the standard seven field also incorporates almost 8 segments in ring 2 and almost three segments in ring 3. The inclusion of parts of rings 2 and 3 in the standard seven field may result in a larger number of eyes classified as ischemic using standard seven fields but less than 10DA if just studying the posterior pole. This may explain why a lower percentage progress to anterior segment neovascular events, 35%, in the CVOS study compared to our cohort, 45% anterior segment neovascularization and 84.6% any neovascular events.

The limitations of our study include the retrospective nature of the study and the exclusion of treated eyes that may introduce a recruitment bias towards a lower

tendency for neovascularization. However, our cohort had an incidence of neovascularization at 31%, and the standard deviation for the area on non-perfusion was high at 104DA indicating a good spread of patients with CRVO were included. Gonioscopy was only performed if deemed necessary by the examining clinician. Therefore, early angle new vessels may have been missed but we believe this is very unlikely. We also acknowledge the numbers included in the study are small. However, ultra-widefield angiography has only been used in routine practice in the last few years and so we believe that these findings are timely.

In conclusion, posterior pole non-perfusion in CRVO is more prone to new vessel development compared to isolated peripheral non-perfusion. Posterior pole retinal non-perfusion is also associated with a larger area of retinal non-perfusion in total. Moreover, our understanding of anti-VEGF induced disease modification and the risk of neovascularization when some eyes are withdrawn from anti-VEGF therapy is only becoming more apparent. Therefore, we believe that the observations of this study will help identify patients at risk of developing delayed anterior neovascularisation on ultrawide imaging before commencing anti-VEGF therapy so that they can either be treated with panretinal photocoagulation or followed up closely for early identification of neovascularization.

Acknowledgements / Disclosures

a (Funding Support): No funding was received for the work submitted

b (Financial Disclosures): All authors have no financial or commercial interest relevant to the subject matter.

Dr Nicholson, Dr Vazquez-Alfageme, Dr Patrao and Dr Triantafyllopoulou have nothing to disclose.

Prof Bainbridge has received research grants from Novartis (Surrey, United Kingdom) and Bayer (Leverkusen, Germany), and received consultancy fees from Astellas and MeiraGTx Ltd.

Dr Hykin has received Grants from Novartis (Surrey, United Kingdom), Allergan (Irvine, California) and Bayer (Leverkusen, Germany); is on the advisory board and receives speaker fees from Novartis and Bayer.

Prof Sivaprasad has received Grants from Novartis (Surrey, United Kingdom), Allergan (Irvine, California) and Bayer (Leverkusen, Germany); is on the advisory board and receives speaker fees from Novartis, Allergan, Roche and Bayer.

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Figure Captions

Figure 1: The concentric rings template with 6 concentric rings, Ring M and Ring 1 representing the posterior pole, Ring 2-5 representing the periphery.

Figure 2: Kaplan-Meier time to neovascular event curve for eyes with central retinal vein occlusion that developed neovascular complications.

Figure 3: Ultra-widefield fluorescein angiogram image of two eyes with central retinal vein occlusion with the concentric rings superimposed, example 1(left) and example 2 (right). The segments of the rings are colored to demonstrate segments that are perfused (green), not perfused (Red) and upgradeable (purple). The development of new vessels and the total area of non-perfusion is also included.

Figure 4: The concentric rings and standard seven field area superimposed on a corrected ultra-widefield image with the overlap between the standard seven field with rings M and 1 (pink) and Rings 2 and 3 (orange) marked.

Tables of Contents Statement

This study reviewed the area and pattern of retinal non-perfusion as imaged using ultra-widefield angiography in eyes with central retinal vein occlusion and its association with neovascularization. The authors identified that posterior pole non-perfusion is associated with a larger total area of retinal non-perfusion and a higher incidence and risk of neovascularization.

Table 1: The original and modified area per segment using the correct conversion factor for each ring in the concentric rings method.

Ring	Original area per segment, DA	Enlargement Factor	Modified area per segment, DA
M	2.00	1.08	1.85
1	6.25	1.20	5.21
2	10.42	1.34	7.78
3	14.58	1.54	9.47
4	18.75	1.81	10.36
5	22.92	1.97	11.63

Table 2: The incidence and risk of neovascularization in central retinal vein occlusion based on total area of non-perfusion in ultra-widefield angiography.

Area of non-perfusion, in disc areas	Number of eyes, n	No neovascularization, n (%)	Neovascularization, n (%)	Odds Ratio, (95% CI ^a)	p-value
< 10	12	12 (100.0)	0	0	0.008
10-30	7	6 (85.7)	1 (14.3)	0.32 (0.03, 2.97)	0.405
30-75	10	8 (80.0)	2 (20.0)	0.48 (0.09, 2.65)	0.466
75-150	5	1 (20.0)	4 (80.0)	12.44 (1.23, 126.18)	0.026
>150	8	2 (25.0)	6 (75.0)	11.57 (1.91, 70.24)	0.006

^aConfidence Interval

Table 3: Incidence and risk of neovascularization in eyes with central retinal vein occlusion based on 10 and 30 disc areas of retinal non-perfusion in ultra-widefield angiography.

Area of non-perfusion, in disc areas	Number of eyes, n	Neovascularization, n (%)	Odds Ratio, (95% CI ^a)	p-value
< 10	12	0	0	0.008
>10	30	13(43.3)	∞	0.008
<30	19	1 (5.3)	0.05 (0.01,0.45]	0.002
>30	23	12 (52.2)	19.64 (2.23, 172.60]	0.002

^aConfidence Interval

Table 4: The incidence and risk of neovascular complications based on number of eyes with more than 10 disc areas of non-perfusion and three or more cells of non-perfusion in each concentric ring.

Ring	Eyes with >10 DA in a single ring, n	Neovascular complications, n (%)	Odds ratio, (95% CI ^b)	p-value	Eyes with ≥ 3 cells graded as not perfused, n	Neovascular complications, n (%)	Odds ratio, (95% CI ^b)	p-value
Ring M	6	5 (83.3)	17.5 (1.8, 172.2)	0.007	7	6 (85.7)	24.0 (2.5, 233.1)	0.002
Ring 1	13	11 (84.6)	74.3 (9.3, 595.3)	<0.001	9	8 (88.9)	44.8 (4.6, 440.8)	<0.001
Ring 2	16	12 (75)	75 (7.5, 745.8)	<0.001	13	11 (84.6)	74.3 (9.3, 595.3)	<0.001
Ring 3	17	12 (75)	12.6 (2.8, 56.1)	<0.001	12	9 (75)	19.5 (3.6, 104.4)	<0.001
Ring 4	19	8 (42.1)	2.6 (0.7, 10.1)	0.192	11	6 (54.5)	4.1 (0.96, 17.6)	0.066
Ring 5 ^a	12	7 (58.3)	5.6 (1.3, 24.0)	0.026	2	2 (100.0)	∞	0.091

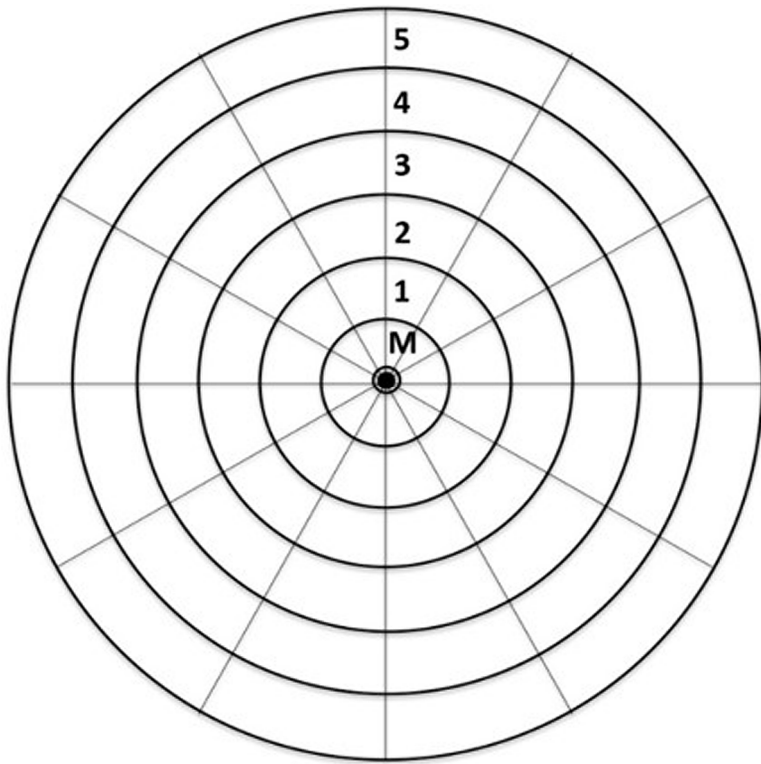
^aThe results in Ring 5 are variable due to the larger number of ungradable cells in that area.

^bConfidence Interval

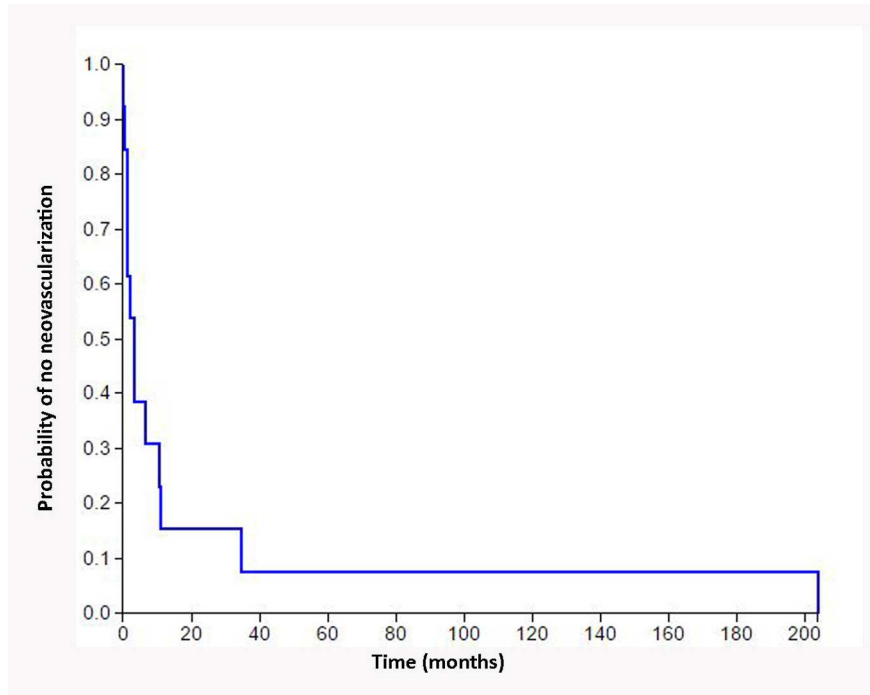
Table 5: The incidence and risk of neovascularization based on the pattern of retinal non-perfusion, a completely perfused posterior pole (Ring M and 1) with more than 10 disc areas of non-perfusion peripherally and more significant posterior pole retinal non-perfusion.

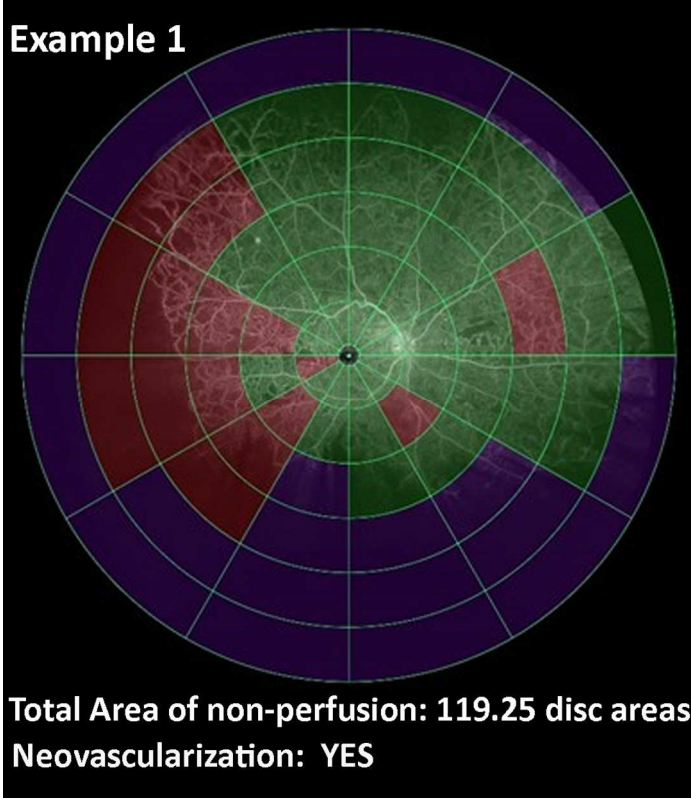
Area of non-perfusion	Number of eyes	Mean total area of non-perfusion	Number (Percentage) of neovascular complications	Odds Ratio (95% CI ^a)	p-value
Completely perfused Ring M and 1 and >10DA of non-perfusion in Rings 2-5	13	43.2 95% CI (18.7, 67.7)	1 (7.7%)	0.12 (0.01, 1.03)	0.036
>10DA of non-perfusion in Ring M and 1	13	193.2 95% CI (122.4, 264.1)	11 (84.6%)	74.25 (9.26, 595.30)	<0.001

^aConfidence Interval



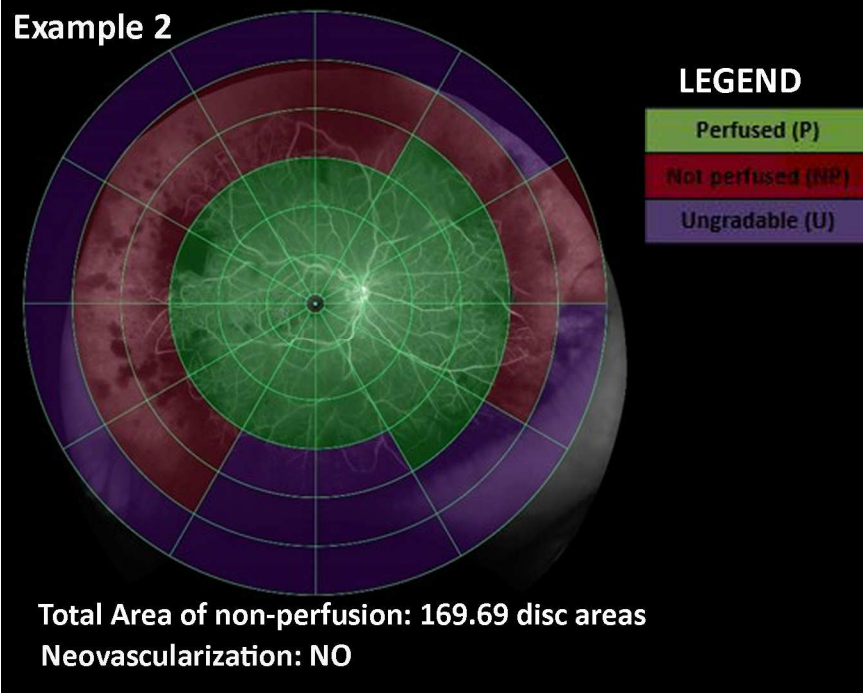
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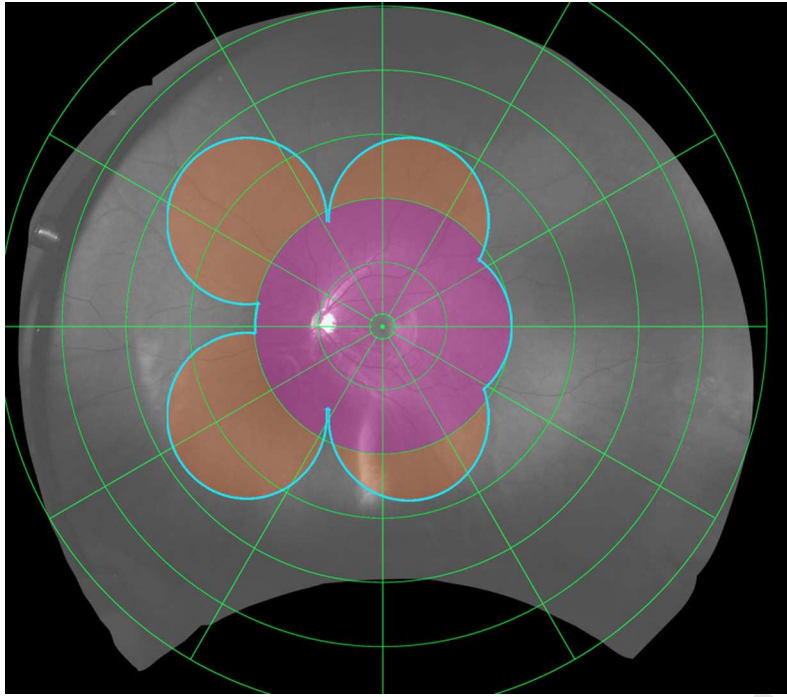


Example 1

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Example 2





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