

REVIEW ARTICLE



Retinal non-perfusion in diabetic retinopathy

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Diabetic retinopathy is a major cause of vision loss worldwide and areas of retinal non-perfusion (RNP) are a key pathologic feature of the vascular component of diabetic retinopathy. While there is a need for a more complete understanding of the natural history of RNP development and progression, overall, increasing RNP has been closely linked with worsening DR severity. Both traditional and novel approaches to quantitative image assessment are being explored to advance our understanding of the vascular, physiologic and functional changes associated with progressive RNP. Retinal ischemia secondary to RNP leads to tissue hypoxia and changes in the expression of a host of signalling molecules. Current anti-vascular endothelial growth factor and steroid pharmaceutical agents appear to be unable to reperuse areas of RNP, but may be able to slow the progressive longitudinal accumulation of RNP with regular retreatments. There remains a tremendous unmet need for pharmacotherapies that can slow RNP progression and ultimately reperuse areas of the non-perfused retina. Towards this end, novel targets including the semaphorin family are being investigated.

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INTRODUCTION

Diabetic retinopathy (DR) remains one of the most common causes of blindness among working-age people in developed countries. DR leads to visual loss primarily through diabetic macular oedema (DMO) and proliferative DR (PDR), both relatively late manifestations of DR.

A multitude of cellular and clinical characteristics associated with DR have been described, including loss of pericytes, thickening of endothelial cell basement membranes, and development of microaneurysms. Many of these alterations contribute to the breakdown of the blood-retinal barrier and to loss of normal retinal vasculature. This vascular loss appears to initially affect retinal capillary beds and can progress to involve both larger arterioles and veins. These areas of retinal non-perfusion (RNP) are typically not apparent on funduscopic examination or colour fundus photography, but are readily visualized with angiography (Fig. 1), and can lead to inadequate blood flow to the metabolically active retina.

RNP NATURAL HISTORY

Natural history studies are foundational for understanding any disease process. These critical studies help to define the underlying disease process and may result in new therapeutic opportunities. One recent example is age-related macular degeneration (AMD)-associated geographic atrophy (GA). Numerous natural history studies, coupled with analyses of the genetic underpinnings of GA and histopathologic analyses of eyes with various stages of AMD, were critical for setting the stage for the

multitude of ongoing pharmaceutical trials investigating treatments to slow GA progression by targeting the complement cascade. Similarly, specific stages of DR were defined by the early treatment diabetic retinopathy study (ETDRS) DR severity scale (DRSS) [1], and this scale helped to refine our understanding of the natural history of DR progression from NPDR to PDR.

In comparison, while multiple studies have considered the presence of RNP in eyes with DR and correlated RNP with various biomarkers, there remains a notable lack of knowledge regarding the natural history of RNP in DR, especially in the peripheral retina. The following two analyses have reported outcomes of RNP changes longitudinally in the absence of ongoing treatment.

In 2018, a post hoc analysis of the phase 3 RIDE and RISE (a Study of Ranibizumab Injection in Subjects with Clinically Significant Macular Edema with Centre Involvement Secondary to Diabetes Mellitus (DM)) trials by Reddy et al. quantified macular nonperfusion (MNP) through 2 years [2]. All eyes had vision loss due to DMO with no history of laser, intraocular corticosteroids, or anti-vascular endothelial growth factor (VEGF) drugs administered within 3 months prior to enrolment. At baseline, macular nonperfusion (MNP) was detected in 26.3% of sham eyes. MNP was measured at baseline, month 12, and month 24 by overlaying the ETDRS grid over fluorescein angiography (FA) images of the macula (field 2 of ETDRS 7-standard field images). The percentage of capillary loss was estimated and converted to disc areas (DA) by a central reading centre. Through month 24, a steady non-significant increase in MNP area was observed among sham eyes: 0.17 ± 0.43 , 0.22 ± 0.63 , and 0.27 ± 0.59 DAs at baseline, month 12, and month 24, respectively.

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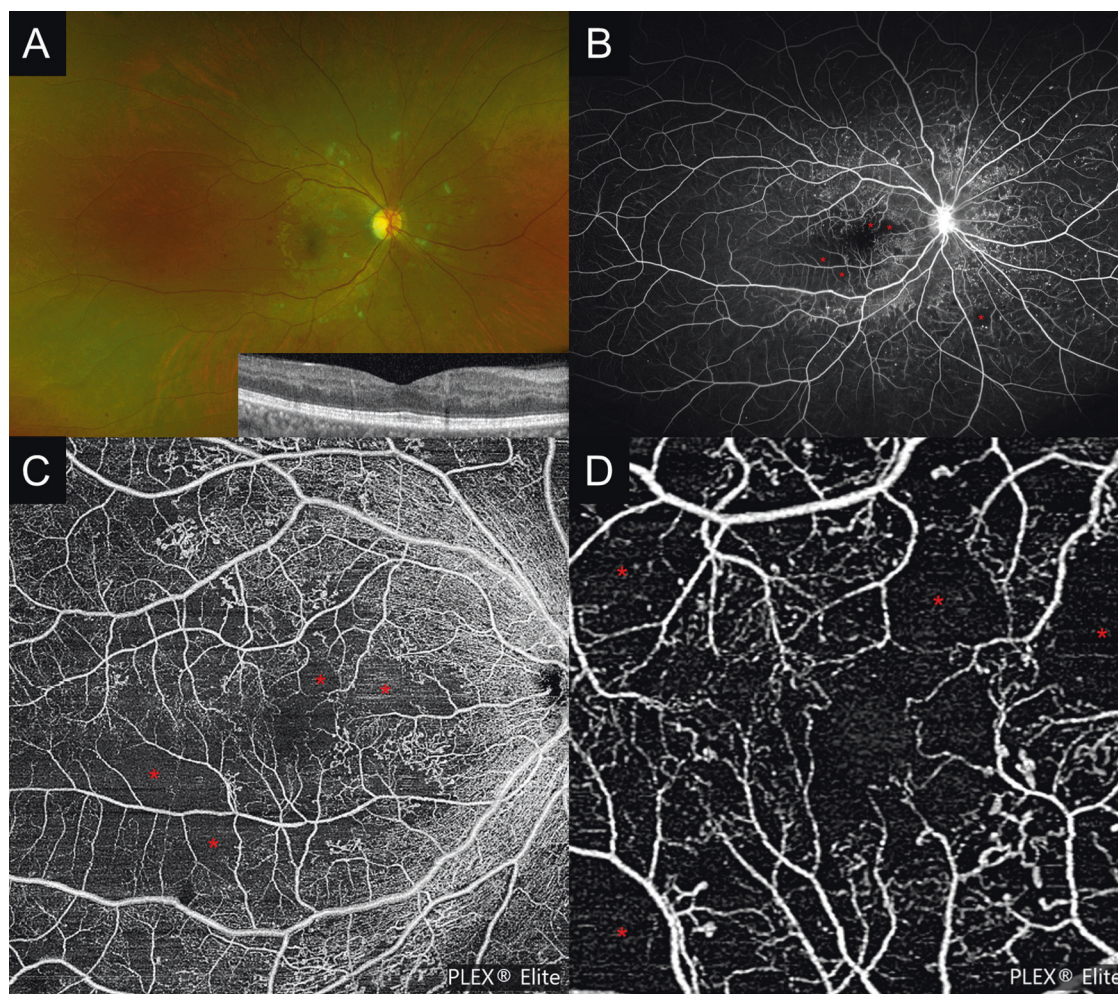


Fig. 1 A 25-year-old female with type 1 diabetes mellitus and proliferative diabetic retinopathy was enrolled in the prospective PRIME trial (ClinicalTrials.gov; NCT03531294). Baseline imaging included (A) ultra-widefield (UWF) colour fundus photography with optical coherence tomography (OCT) line scan through the fovea (bottom right inset); B UWF fluorescein angiography; (C) 9 mm × 9 mm swept-source OCT angiography; and (D) 3 mm × 3 mm swept-source OCT angiography. Extensive areas of retinal nonperfusion are visualized on both fluorescein and OCT angiography (red asterisks).

Another study, AFFINITY (Efficacy of Intravitreal Aflibercept Injection for Improvement of Retinal Nonperfusion in Diabetic Retinopathy) [3], analysed RNP changes longitudinally among 20 eyes with regions of RNP but without centre-involved DMO who were observed without treatment over a 1-year period. Nonperfusion was assessed using ultra-widefield FA (UWFA) and reported as an index (total area of RNP divided by total retinal area [TRA]). Through one year of follow up, no significant increase in the RNP index was reported for this small cohort.

Both the AFFINITY study and the RISE/RIDE analysis have substantial limitations, and larger prospective longitudinal studies of RNP are needed.

THE EXTENT OF RETINAL NONPERFUSION IN DIABETIC RETINOPATHY

Even among patients with DM without clinical manifestations of DR, it appears that a meaningful proportion manifest signs of retinal vascular impairment and RNP as detected by optical coherence tomography-angiography (OCTA). In 2015, a prospective observational study analysed OCTA images from 61 eyes of 39 patients with DM without clinical evidence of DR and compared them to 28 control eyes from 22 age-matched healthy subjects [4]. This study reported a significant increase in the size of the foveal

avascular zone in diabetic eyes versus control eyes ($p = 0.04$) and noted that 21% of diabetic eyes contained capillary nonperfusion adjacent to the fovea compared to 4% of control eyes ($p = 0.03$). Another prospective OCTA analysis observed decreases in both the superficial and deep retinal vessel densities adjacent to the fovea in diabetic eyes compared to healthy controls [5].

Once clinical manifestations of DR are present, overall, more severe DR severity appears to be associated with more extensive RNP. Several analyses have specifically considered the extent of RNP in eyes with different DR severity levels.

In a retrospective analysis by Silva et al., 68 eyes ranging from no DR to high-risk PDR were evaluated (no DR, 8.8%; mild NPDR, 17.6%; moderate NPDR, 32.4%; severe NPDR, 17.6%; PDR, 19.1%; high-risk PDR, 4.4%) [6]. An increase in the area and proportion of RNP was observed with worsening DR severity, though this association appeared to plateau between PDR and high-risk PDR. In a second study by Sędziak-Marcinek et al., 4%, 51% and 49% of eyes with RNP were classified as having mild, moderate, and severe NPDR, respectively, versus 49%, 51%, and 0% of eyes without RNP [7], indicating an increased likelihood of RNP in eyes with more severe NPDR. An additional study by Ehlers et al. demonstrated a similar progressive increase in RNP with increased DRSS; UWFA was utilized to determine the ischaemic index among 339 eyes with various DR severity levels, and an ischaemic index of

0.95%, 1.37%, 2.80%, and 9.53% in eyes with mild, moderate, severe NPDR, and PDR were observed respectively [8]. Another study retrospectively analysed the prognostic value of the central RNP index (RNP in the central retina divided by the total central retina area) and the peripheral RNP index (RNP in the peripheral retina divided by the total peripheral retina area) among 78 treatment naïve eyes with DR severities ranging from mild NPDR to PDR [9]. Overall, the peripheral RNP index was significantly increased among eyes with severe NPDR (median, 4%) and PDR (median, 8%) compared to mild or moderate NPDR (median, 1%; mild/moderate NPDR vs. severe NPDR, $p = 0.002$; mild/moderate NPDR vs PDR, $p = 0.008$), and the central RNP index was significantly increased among eyes with PDR (median, 6%) compared to eyes with mild or moderate NPDR (median, 2%; $p = 0.007$). There was also a statistically significant linear correlation between the central and peripheral indices among eyes with severe NPDR ($R^2 = 0.141$, $p = 0.041$) and PDR ($R^2 = 0.311$, $p = 0.025$).

Consistent with more advanced DR stages being associated with more extensive RNP, in the phase 1/2 DAVE (Efficacy and Safety of Intravitreal Injections Combined with PRP for CSME Secondary to Diabetes Mellitus) trial involving 40 eyes with severe NPDR ($n = 34$) or PDR ($n = 6$), the global mean area of RNP was $196.1 \pm 123.4 \text{ mm}^2$, or $31 \pm 19\%$ of the TRA observed by UWFA [10]. Additionally, in the RECOVERY trial involving 40 eyes with PDR (DRSS level 61 to 75), at baseline, RNP comprised $25.8 \pm 15.3\%$ of the TRA observed by UWFA [11].

Cumulatively, these studies highlight a consistent association between RNP and DR, indicate that RNP is common even within eyes without evidence of clinical DR, and suggest that on average as DR severity worsens, so does the extent of both central and peripheral RNP.

LOCATION OF RETINAL NONPERFUSION IN DIABETIC RETINOPATHY

The location of RNP may also be an important factor to consider in DR. Multiple analyses have defined retinal zones on UWFA as the posterior retina (within a 10 mm radius from the fovea), the mid-peripheral retina (10–15 mm from the fovea), and the far peripheral retina (>15 mm from the fovea). Overall, when compared according to ischaemic index (ISI; total RNP divided by the TRA), the more peripheral retina appears to contain a greater proportion of RNP compared to more posterior retina.

For example, in the DAVE trial, while total RNP was greatest in the mid-peripheral retina (47.7% of total RNP versus 32.6% in the posterior zone and 19.7% in the far peripheral retina, $p < 0.001$), ISI increased with increasing distance from the fovea, with an ISI of 0.22, 0.37, and 0.43 in the posterior, mid-peripheral and far peripheral retina, respectively ($p = 0.005$) [10]. When considered by quadrants instead of concentric circular zones, a non-uniform RNP distribution has also been identified [12]; ISI was found to be greater in the inferonasal quadrant ($p = 0.011$), with 30.3% of the total RNP being identified in that quadrant [12]. Overall, these results appeared to be generally consistent with other studies describing the distribution of RNP in eyes with DR [6, 11, 13, 14].

In another analysis, the DAVE study group stratified areas of RNP as “with leakage” or “without leakage” and found that the absolute area of RNP without leakage was increased in the midperiphery compared to the posterior zone and the far periphery [15], but RNP with leakage was increased in the posterior zone compared to the mid and far peripheries; when indexed, RNP with leakage had an ISI of 0.13, 0.12, and 0.05 in the posterior, mid-peripheral, and far peripheral retina, respectively ($p < 0.001$). This has been hypothesized to potentially be related to higher metabolic activity within the posterior zone, leading to an increased susceptibility to vascular leakage.

Considering RNP spatial location at an even more granular level, Ishibazawa et al. [16], retrospectively described the location of RNP in relation to individual retinal arteries and veins using OCTA images of 63 eyes with NPDR or PDR. Overall, the arterial-adjacent RNP area appeared to be significantly larger than the venous-adjacent RNP area for all DR severity stages, although this difference became smaller as total RNP increased and DR severity worsened. The authors hypothesized that this decreasing ratio may be attributable to the progression of vascular damage associated with worsening DR severity. From a pathogenesis perspective, initial vascular damage from DR is thought to occur predominantly on the arterial side; as vascular damage accumulates, the venous side subsequently becomes involved. A local hypoxic microenvironment triggers the upregulation of an array of pro-angiogenic and pro-inflammatory cytokines that result in the recruitment of leukocytes to the endothelium, a process that appears to occur more commonly in post-capillary venules compared to arterial vessels [17]. The recruitment of leukocytes may result in blockages of the microvasculature, known as leukostasis, potentially further exacerbating the damage to and hypoperfusion of downstream vascular beds. Thus, as DR severity progresses, venous capillary nonperfusion may increase, resulting in a decreasing ratio of arterial-adjacent RNP vs venous-adjacent RNP.

CORRELATION OF NONPERFUSION WITH DIABETIC MACULAR OEDEMA AND OTHER DIABETIC RETINOPATHY BIOMARKERS

Several studies have correlated the extent of RNP with various diabetic eye disease states and biomarkers.

While there does not appear to be a simple linear correlation between the extent of RNP and the severity of DMO, when more nuanced phenotypes are considered, there does appear to be a complex relationship between RNP, DMO, and leakage. For example, in the DAVE trial, both extent of global RNP and ISI were not associated with the central macular thickness (CMT) or macular volume [10, 12]. Interestingly, however, RNP in the midperiphery and in the temporal retina both was found to be negatively associated with CMT. As it has been hypothesized that the development of RNP may lead to increased VEGF production and subsequent DMO, this relationship would be predicted to result in a positive correlation between RNP and CMT. One explanation to reconcile the observation that increasing RNP in specific regions appears to be negatively correlated with the extent of oedema is that frank RNP may occur downstream of initial pathologic VEGF upregulation or even possibly as a consequence of VEGF upregulation.

Other analyses of eyes with RNP have demonstrated additional associations between RNP, CMT, and retinal leakage. In a prospective study by Sędziak-Marcinek et al. involving 49 eyes with RNP and 49 eyes without RNP, the extent of RNP was correlated with vascular leakage in all three retinal zones (posterior, midperiphery, and far periphery) [7]. Among eyes with any RNP, the probability of leakage increased by 26-, 60-, and 5-fold in the far periphery, midperiphery, and posterior zones, respectively. Within the DAVE data set, when RNP was subcategorized as “with leakage” or “without leakage”, it was found that RNP with leakage was positively correlated with CMT, and RNP without leakage was negatively correlated with CMT [15]. Intuitively, this aligns with our knowledge of the pathophysiology of DR since DMO is thought to occur due to retinal leakage caused by pathologically increased permeability of the inner blood-retinal barrier. Thus, though these areas with a leakage are relatively nonperfused, they may retain some functional tissue that can produce cytokines that drive vascular leakage and oedema in adjacent regions. Alternatively, when areas of RNP have no remaining functional tissue to produce these cytokines, including VEGF, associated leakage and oedema may not be observed.

Interestingly, this is also supported by research that has found a stronger association of intraocular VEGF levels to quantitative leakage index parameters compared to ischaemic index areas alone [18].

Additional studies are needed to more precisely inform our understanding of the relationship between RNP and DMO. OCTA offers many potential advantages. First, it is able to image retinal vasculature within different retinal layers at resolutions not possible with FA. Second, OCTA does not demonstrate leakage, allowing areas of RNP to be more readily discerned [19, 20]. Such increased accuracy may afford more reliable correlations between DMO, and biomarkers of interest including RNP. In 2016, Mané et al. retrospectively analysed OCTA images to measure areas of capillary nonperfusion in the superficial and deep capillary plexi around regions of oedema [21]. This study demonstrated that in both vascular beds, the majority of intraretinal cystoid spaces were associated with regions of surrounding capillary dropout with decreased capillary density; furthermore, when DMO resolved spontaneously or following treatment with anti-VEGF, surrounding areas of capillary nonperfusion were not similarly resolved.

A multitude of additional DR biomarkers including VEGF levels, neovascularization, predominantly peripheral lesions (PPLs), and retinal fractal dimension has also been correlated with RNP. Most intuitively, VEGF levels, area of neovascularization, and a number of neovascular lesions have all been reported to be positively correlated with RNP area among eyes with PDR [18, 22]; specific neovascularization of the disc appears to be associated with RNP of the posterior zone as well. PPLs [6] have also been reported to be associated with an increased risk of DR progression [23]. When a specific type of DR lesions, such as haemorrhages or microaneurysms, is determined to be more extensive in an extended field (peripheral field not included in the standard ETDRS fields) compared to its corresponding/adjacent standard ETDRS field, the DR is considered to be predominantly peripheral. In a prospective study by Silva et al., the presence of PPLs appeared to be associated with a 30–60% increase in retinal non-perfusion [23]. Finally, fractal dimension, a measure of the complexity of vascular branching patterns, has been reported to be negatively associated with mean RNP, an association identified in all three retinal zones [24]. This finding may be due to vascular pruning of retinal vessels and loss of their branching complexity with progressive RNP. Furthermore, enhancements in image feature extraction technology including deep learning and radiomics are enabling the generation of highly detailed vascular segmentation masks [25, 26]. Using these vascular segmentation masks from UWFA, quantitative metrics, for example, local measures of “zero vessel density,” may be able to be utilized to differentiate eyes according to disease severity. These biomarkers may help facilitate a more in-depth quantitative assessment of RNP.

PHARMACOTHERAPY IMPACT ON RETINAL NON-PERFUSION

Retinal ischaemia secondary to RNP leads to tissue hypoxia and increased local levels of multiple signalling molecules such as HIF-1 α , VEGF-A, and Angiopoietin-2 (Ang-2) [18]. Aqueous fluid analysis and phenotype correlation with quantitative UWFA analysis have also demonstrated a correlation between increased TIMP-1 and AGPTL4 and ischaemic index [18]. Randomized controlled trials have demonstrated that pharmacologic blockage of VEGF-A with aflibercept (Eylea, Regeneron), ranibizumab (Lucentis, Genentech) and bevacizumab (Avastin, Genentech), can be effective for the treatment of DMO [27], PDR [28], and, more recently, non-proliferative DR (NPDR) [29, 30]. Through these trials, it has repeatedly been demonstrated that VEGF blockade is remarkably effective at improving retinal oedema and regressing pathologic neovascularization. Critically, however, we have also

learned that inhibition of VEGF can impact far more than just retinal oedema and neovascularization.

Anti-VEGF treatment impact on diabetic retinopathy severity

First, anti-VEGF pharmacotherapy can meaningfully blunt the progression of NPDR to PDR. For example, within the RISE/RIDE phase 3 program, PDR events were reduced at 2-years from approximately 34% with sham to ~11% with monthly ranibizumab dosing [31, 32]. Furthermore, when the control arm of RISE/RIDE was crossed over to monthly ranibizumab dosing after 2 years of sham injections, the slope of progression to PDR was blunted and subsequently continued in parallel with the arms initially randomized to ranibizumab [31, 32]. Analogously, and possibly mediated by their anti-VEGF effect, intravitreal corticosteroids also slow progression to PDR. For example, at the 3-year endpoint of the paired phase 3 FAME trials among DMO patients, ~29% of sham-treated patients compared to ~17% of fluocinolone acetonide (FAC)-treated patients progressed to PDR [33].

As a caveat highlighting the inherent limits of current pharmacotherapies, despite apparently adequate treatment, some patients will progress from NPDR to PDR. For example, in RIDE/RISE despite intensive, monthly anti-VEGF dosing, ~18%, or nearly 1 in 5 patients, developed a PDR event through 3 years of follow-up [31, 32]. Critically, among all of the baseline factors analysed, the presence of RNP within the posterior pole was the only significant prognostic indicator identified that correlated with progression to PDR when using ranibizumab [32] or FAC [33]. Enrolling eyes with NPDR without DMO, the PANORAMA and DRCR-W trials have observed similar protective effects with regular anti-VEGF dosing. Specifically, at 2-years within the phase 3 PANORAMA trial, the likelihood of developing PDR was reduced by more than 75%, from 31% with sham treatment to 7–9% with aflibercept dosing [29]. Within the DRCR-W dataset, at 2-years the cumulative probability of developing PDR was 14% with aflibercept compared to 33% with sham [30].

Second, anti-VEGF pharmacotherapy not only slows the progression of DR, but it can also improve DR severity score levels based on colour fundus photography in a substantial proportion of eyes both with [31, 32, 34] and without [29, 30] baseline DMO. For example, among NPDR eyes without DMO, PANORAMA found that at 2-years 50–62% of aflibercept treated eyes experienced ≥ 2 step DRSS improvements compared to 13% of sham-treated eyes; in DRCR-W, at 2-years 45% of aflibercept treated eyes experienced ≥ 2 step DRSS improvements compared to 14% of sham-treated eyes

Anti-VEGF treatment impact on retinal non-perfusion

At a more basic level, the role of anti-VEGF pharmaceuticals appears to be more important than simply blunting progression to PDR and improving DR severity on fundus photography. VEGF blockade may be able to fundamentally impact the underlying disease pathophysiology of progressive RNP. Multiple datasets have considered this endpoint.

The first large dataset to illustrate that VEGF-A blockade may have an impact on RNP development was RISE/RIDE, in which the development of angiographically-identified RNP was significantly reduced with monthly VEGF blockade [35]. In RISE/RIDE, FAs were evaluated by a masked reading centre for the presence and extent of RNP within the macula. Among eyes with no baseline RNP, the development of RNP was significantly reduced at 2 years from approximately 30% with sham treatment to <10% with monthly ranibizumab dosing. Similarly, sham-treated eyes demonstrated a faster rate of increasing RNP compared to ranibizumab treated eyes.

Likewise, outcomes from VISTA also demonstrate beneficial anti-VEGF mediated changes to the retinal vasculature, primarily again showing evidence of slowing of the worsening of FA-identified RNP [36]. Retinal perfusion status was evaluated at the

quadrant level by FA based on the presence of any non-perfusion in each quadrant by a masked reading centre. Through 2 years, aflibercept-treated patients demonstrated greater improvement and less worsening of retinal perfusion compared to control patients. For example, from baseline to 2-years, improvement in perfusion status was observed in 15% of control patients compared to 40–45% of aflibercept treated patients and worsening in perfusion status was observed in 25% of control patients compared to 9% of aflibercept treated patients.

The RECOVERY randomized trial involving 40 patients was designed to prospectively investigate the change in RNP, as well as the prospect of retinal reperfusion, with regular aflibercept treatments and evaluate for the possibility of a dose-dependent response of aflibercept on RNP evolution among eyes with PDR [11]. A key finding at the 1-year primary endpoint was that aflibercept treatment did appear to have a biological impact on RNP in a dose-dependent fashion; mean RNP did not increase among eyes dosed with monthly aflibercept, while in contrast, mean RNP increased significantly among eyes dosed with quarterly aflibercept. This suggests that continuous VEGF inhibition is superior to intermittent VEGF inhibition with regard to reducing the progression of RNP in DR. The PERMEATE clinical trial similarly evaluated the impact of aflibercept therapy on quantitative UWFA parameters, including ischaemic index. In the eyes of DMO within PERMEATE, the ischaemic index remained stable over 1 year without significant change [37].

In 2019, a small case series analysed reperfusion in eyes with treatment-naïve DMO following three monthly anti-VEGF injections using widefield OCTA and FA [20]. Nonperfusion was evaluated at baseline and one month following the third anti-VEGF injection. Overall, no significant areas of reperfusion were observed following the treatment regimen with either imaging modality; additionally, OCTA was found to be more accurate for identifying areas of RNP compared to FA, especially in regard to capillary nonperfusion. Another case series reported similar findings among patients with DR treated with three monthly anti-VEGF injections [38]; while improvements in DRSS level were observed following treatment, no reperfusion of arterioles or venules was observed.

Overall, while RNP development may be able to be slowed with monthly anti-VEGF dosing, the RECOVERY, RIDE/RISE, and VISTA datasets all indicate that progressive RNP may continue to develop in a meaningful proportion of patients despite regular anti-VEGF dosing, and angiographically-obvious reperfusion was not common. In addition, it is worthwhile to recognize the challenges of identifying ischaemic alterations in the eye that are undergoing anti-VEGF therapy on FA and UWFA. The dramatic reduction in leakage associated with anti-VEGF therapy creates significant changes in contrast and imaging features that make subtle ischaemia more difficult to readily detect [37]. New and emerging technologies, including deep-learning segmentation of retinal vasculature and image interrogation, may provide new opportunities for enhanced reliability and assessment [26].

REPERFUSION OF AREAS OF RETINAL NON-PERFUSION: POSSIBLE MECHANISM OF ACTION AND NOVEL APPROACHES

Changes in vascular perfusion in DR appear to be a dynamic process. In fact, reperfusion of previously nonperfused retina has been reported in the context of both the natural history of DR [39, 40] as well as following ocular-specific interventions including PRP [41, 42] and intravitreal anti-VEGF therapy [36, 43]. Most published cases have demonstrated small areas of reperfusion, at best.

Although one potential irreversible cause of retinal nonperfusion may be apoptosis of endothelial cells in retinal capillaries [44], another potentially reversible mechanism that could lead to

apparent retinal reperfusion is the alleviation of a leukostatic plug [45]. Pathologic levels of VEGF can contribute to leukostasis, which could theoretically obstruct vascular flow in the absence of permanent vascular collapse or closure. Pharmacologic anti-VEGF therapy may allow for the dissolution of such plugs and restoration of vascular flow through the previously, temporarily, occluded vessel. Of interest, studies in oncology have reported improvements in perfusion following systemic anti-VEGF treatment that have been observed to decrease hyperpermeability and transiently remodel aberrant tumour vasculature toward a more phenotypically normal state [46, 47]. Regrowth of physiologically normal retinal vasculature through areas of previously dead vasculature secondary to DR following anti-VEGF pharmacotherapy appears to be notably uncommon.

Consistent with this, while intravitreal anti-VEGF dosing can dramatically decrease the extent of visible vascular abnormalities associated with DR such as intraretinal haemorrhages, reperfusion of areas of RNP is typically not observed in these eyes. Towards, this end, new pharmacotherapies are needed that could achieve physiologic reperfusion of non-perfused retina and multiple companies are pursuing this endpoint including Sema Therapeutics [48], Boehringer Ingelheim [49], and Perfuse Therapeutics [49].

Semaphorins are a family of well-conserved, neuronal guidance proteins that are involved in a wide variety of signalling pathways, including axonal growth cone guidance, immune function, embryonic development, and adult circulatory vascular maintenance [50]. Generally, systemic levels of Semaphorins are low in healthy adults, though increased levels have been reported among patients with DM [51].

The class 3 Semaphorin, *Sema3a*, has specifically been linked to retinal and kidney dysfunction in patients with DM [52, 53]. *Sema3a* is a diffusible, disulphide-linked homodimer that is secreted by retinal ganglion cells during periods of prolonged hypoxia and can bind to neuropilin-1 (Nrp-1), a known receptor for VEGF-A [50]. Importantly, *Sema3a* and VEGF-A do not appear to compete for binding to Nrp-1, but rather can bind simultaneously at two distinct extracellular sites, propagating cytoskeletal collapse and angiogenesis, respectively. While *Sema3a* and VEGF-A exhibit apparently conflicting angiogenic properties, both have also been shown to increase vascular permeability upon binding to Nrp-1 [52].

Specifically related to areas of RNP, *Sema3a* has been hypothesized to be a factor that may be preventing revascularization of nonperfused tissues through its anti-angiogenic effects [53]. As *Sema3a* accumulates in and around areas of RNP, new vessels may be inhibited from forming within the hypoxic tissues, a mechanism that has also been hypothesized to be relevant to patients with stroke or spinal cord injury [54, 55]. This may be a protective response in which metabolic resources are shunted away from unsalvageable tissues towards remaining viable tissues.

Supporting its role in suppressing revascularization of areas of RNP, preclinical mouse models have observed that suppression of *Sema3a* results in increased rates of revascularization of avascular zones [53]. Furthermore, upon injection of recombinant *Sema3a* into the vitreous, pre-retinal neovascularization within mouse models of ischaemia has been reported to be reduced, suggesting that intraretinal *Sema3a* may not only suppress revascularization of areas of RNP but also may simultaneously drive neovascularization towards the vitreoretinal interface at the edges RNP regions [53, 56, 57].

Thus, *Sema3a* is a potential target to consider for inhibition in an attempt to promote revascularization of areas of RNP. Sema Therapeutics is exploring antibodies against *Sema3a* as a therapeutic for multiple exudative retinal diseases [48]; their lead compound, ST-102, is a bispecific recombinant trap protein that binds both VEGF-A and *Sema3a* and is currently in pre-clinical testing for the treatment of DMO [48, 58].

SUMMARY

What was known before

- Retinal non perfusion (RNP) is a key pathologic feature of diabetic retinopathy (DR).
- RNP is common, even within eyes without evidence of clinical DR.
- On average as DR severity worsens, so does the extent of RNP.
- Anti-vascular endothelial growth factor (VEGF) pharmacotherapy meaningfully blunts the progression of non-proliferative DR to proliferative DR, and improves DR severity score (DRSS) levels based on color fundus photography in a substantial proportion of eyes both with and without DME.
- Overall, while RNP development may be able to be slowed with monthly anti-VEGF pharmaceutical bolus dosing, multiple prospective datasets have reported that RNP may continue to accumulate in a meaningful proportion of eyes despite regular anti-VEGF dosing.
- While intravitreal anti-VEGF dosing can dramatically decrease the extent of visible vascular abnormalities associated with DR such as intraretinal hemorrhages leading to improvement in DRSS levels, reperfusion of areas of RNP is typically not observed in these eyes.

What this study adds

- Highlights the need for prospective, longitudinal studies to better define the natural history of RNP development and progression in DR.
- Summarizes our current understanding of the correlation between RNP and imaging biomarkers including neovascularization area and location, predominantly peripheral lesions and retinal fractal dimension.
- Describes DR phenotype correlations including extent of RNP with panretinal quantitative assessments and aqueous cytokine levels.
- Highlights the need for new pharmacotherapies with new mechanisms of action to achieve consistent, clinically meaningful reperfusion of non-perfused retina in DR.

CONCLUSION

RNP is a key finding that is closely linked to DR severity. Beyond DR, changes in perfusion status have been implicated in many other ocular diseases including retinal vascular pathologies such as retinal venous occlusive disease and sickle cell retinopathy, as well as diseases that may be driven in part by a change in inner choroidal perfusion such as AMD. There remains a tremendous unmet need for a better understanding of the natural history of RNP development and progression in DR, optimized approaches to panretinal quantitative assessment, and pharmacotherapies that can slow RNP progression and ultimately reperfuse areas of the non-perfused retina.

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AUTHOR CONTRIBUTIONS

All authors had significant contributions to the drafting, critical revision, and supervision of the writing of the current manuscript. C.C.W. confirms final responsibility for the decision to submit for publication.

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The authors report the following conflicts of interest: C.C.W.: consultant: Adverum, Aerie Pharmaceuticals, Allergan, Apellis, Arctic Vision, Arrowhead Pharmaceuticals, Bausch + Lomb, Bayer, Bionic Vision Technologies, Chengdu Kanghong Biotechnology, Clearside Biomedical, EyePoint Pharmaceuticals, Genentech, Gyroscope, IVERIC Bio, Kato Pharmaceuticals, Kodiak Sciences, Long Bridge Medical, NGM Biopharmaceuticals, Novartis, OccuRx, Ocular Therapeutix, ONL Therapeutics, Opthea Limited, Oxurion, Palatin, PolyPhotonix, RecensMedical, Regeneron, RegenXBio, Roche, SAI MedPartners, Takeda, Verana Health; research support: Adverum, Aerie Pharmaceuticals, Aldeyra, Alimera Sciences, Allergan, Amgen, Apellis, Asclepix, Bayer, Boehringer Ingelheim, Chengdu Kanghong Biotechnology, Clearside Biomedical, Gemini, Genentech, Graybug Vision, Gyroscope, IONIS Pharmaceutical, iRENIX, IVERIC bio,

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ADDITIONAL INFORMATION

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