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REVIEW ARTICLE

Perspectives of diabetic retinopathy—challenges and opportunities

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Diabetic retinopathy (DR) may lead to vision-threatening complications in people living with diabetes mellitus. Decades of research have contributed to our understanding of the pathogenesis of diabetic retinopathy from non-proliferative to proliferative (PDR) stages, the occurrence of diabetic macular oedema (DMO) and response to various treatment options. Multimodal imaging has paved the way to predict the impact of peripheral lesions and optical coherence tomography-angiography is starting to provide new knowledge on diabetic macular ischaemia. Moreover, the availability of intravitreal anti-vascular endothelial growth factors has changed the treatment paradigm of DMO and PDR. Areas of research have explored mechanisms of breakdown of the blood-retinal barrier, damage to pericytes, the extent of capillary non-perfusion, leakage and progression to neovascularisation. However, knowledge gaps remain. From this perspective, we highlight the challenges and future directions of research in this field.

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BACKGROUND

The number of people with diabetes worldwide is set to rise beyond 592 million in <25 years. However, ~175 million people remain undiagnosed and many of them present with complications [1].

Diabetic retinopathy (DR) is a frequent complication of diabetes that may lead to visual impairment, making it one of the most feared complications of diabetes. On colour fundus photographs, DR progresses from non-proliferative DR (NPDR) to proliferative DR (PDR) while diabetic macular oedema (DMO) can occur at any stage of DR. About one-third of people with diabetes have DR and 10% have vision-threatening complications (VTDR), comprising of DMO and PDR [1].

Over decades of research, the focus has been to reduce the rate of blindness due to VTDR. Therefore, in clinical research, DR outcomes are broadly classified into 'any DR' and VTDR based on 7-field colour fundus photographs [2]. However, advances in this field have improved our understanding of diabetic retinal disease. For example, there are earlier changes in the retina than those visible in colour photographs and multimodal imaging has highlighted several features that have not been considered in the severity level of DR. Investigational agents are now being evaluated to prevent the development and progression of earlier stages of DR. In this perspective, we highlight the challenges and opportunities in this field as we progress from treatment of VTDR to prevention of DR.

Non-proliferative diabetic retinopathy

Microaneurysms are the earliest visible microvascular abnormalities of DR on fundoscopy. These occur as a result of early histopathological changes in the retinal microvasculature in the form of thickening of the basement membrane, endothelial proliferation, pericyte loss and vascular smooth muscle cell dropout [3, 4]. These initial lesions are focal and are mainly located at the posterior pole of the retina but topographically the temporal retinal microvasculature is more prone to DR changes than the nasal counterparts [3, 5]. With progression of disease, the capillaries on the arterial side of the retinal circulation show increased vaso-regression and consequent capillary non-perfusion (CNP) [6]. The numbers of microaneurysms increase with wider areas of non-perfusion. Areas of CNP are seen to be crossed by remaining enlarged capillaries, which appear to act as arteriovenous shunts, receiving the blood directed from the surrounding closed capillary net [7]. The focus here is to prevent vaso-regression and progression of CNP.

However, it is worthwhile to take a step backward. Before microaneurysms become visible, the earliest alterations include the breakdown of the blood-retinal barrier (BRB) [8–10] and the impairment of neurovascular coupling [11, 12]. These preclinical retinopathy signs [13, 14] represent diabetes-related dysfunction of neurons, glia and the microvasculature before obvious clinical signs of vascular lesions [15]. Although pericyte damage has been implicated in causing loss of regulation of retinal vascular tone resulting in microaneurysms [16, 17], it is also of major relevance that retinal neurodegeneration participates in the development of early microvascular changes that occur in DR. Several studies have indicated that neurodegeneration is part of the disease pathway and represent early pathophysiological events of DR that may precede and therefore be linked to clinically detectable vascular alterations [18, 19]. Indeed, DR has been suggested a component

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of the generalised diabetic neuropathy, resulting in the impairment of the so-called retinal "neurovascular unit" that causing neuronal and vascular retinal abnormalities associated with the disease onset and progression [19]. As the Muller cells are interposed between the neuronal cells and the capillaries, the interactions between neurons, these glial cells and the microvasculature are also deranged resulting in several abnormal visual function tests prior to visible vascular changes [20]. Therefore, these preclinical retinopathy signs need to be validated further to be included in assessment of DR severity levels and prevention trials.

Currently, the stages of NPDR progress from mild, moderate, to severe NPDR based on the progression of retinal lesions from microaneurysms alone to co-existent small haemorrhages and indirect signs of vascular hyperpermeability and CNP, i.e., exudates and cotton wool spots respectively. These alterations are used for characterisation of the first four DR severity levels (Level 10 to 43). What remains to be fully understood is the mechanism involved in triggering these microvascular lesions. Hyperglycaemia appears to be sufficient to initiate the development of DR as revealed by the development of retinopathy in animals experimentally made hyperglycaemic [21–23].

In a 5-year longitudinal study which followed up 170 individuals with type 2 diabetes (T2D) with minimal DR on fundus examination using OCT image, it was found that there was a significant, progressive loss in the ganglion cell (GCL) and inner plexiform layers (IPL) (0.147 mm/year) that was independent of HbA1c, DR grade, progression of DR, or development of VTDR. In agreement with other studies that have reported progressive thinning of GCL+IPL in T2D individuals [19, 24, 25], it was confirmed that retinal neurodegenerative changes are a component of DR, and progress over the course of the retinopathy.

However, the observation that not all patients with poor metabolic control develop advanced stages of DR suggests that other factors, such as genetic predispositions, are likely to determine individual susceptibility to the disease. It is recognised that the duration of diabetes and the level of metabolic control condition the development of DR. However, these risk factors do not explain the great variability that characterises the evolution and rate of progression of retinopathy in different diabetic individuals. There is clearly individual variation in the presentation and course of DR. There are many patients who after many years with diabetes never develop sight-threatening retinal changes, maintaining good visual acuity. However, there are also other patients that after a short duration of diabetes, develop a rapidly progressing DR and some may not even respond to available treatments.

It is highly relevant that, the fundus abnormalities seen in DR can conceptually be split into three major categories: (i) findings resulting from leaking microvasculature (haemorrhages, exudates, retinal oedema); (ii) observations resulting from progressive structural damage to the retina, represented by neurodegeneration; and (iii) those secondary to CNP and ischaemia, with a subsequent overproduction of vascular growth factors (including intraretinal neovascular abnormalities, preretinal neovascularization, fibrous proliferation and vitreous haemorrhage) [26, 27].

Our group has also identified three patterns of DR progression or phenotypes [27, 28]. Phenotype A includes eyes with reversible and relatively low levels of abnormal fluorescein leakage, a slow rate of microaneurysm formation and a normal foveal avascular zone. This group appeared to represent eyes presenting slowly progressing retinal disease. Phenotype B includes eyes with persistently high leakage values, indicating a dominant alteration in the BRB, relatively low rates of microaneurysm accumulation and a normal foveal avascular zone. This group appears to be a 'wet' form of diabetic retinopathy. Phenotype C includes eyes with variable and reversible leakage, increased microaneurysm formation and definite capillary nonperfusion [27, 28].

Interestingly, we also found that neurodegenerative changes are characterised by distinct profiles in these different retinopathy phenotypes. Phenotype A showed the presence of neurodegeneration but over the 5-year follow-up period its value remained relatively stable. On the other hand, phenotypes B and C showed a more rapid progression in the thinning of GCL-IPL. It is particularly interesting to note that although the neurodegenerative changes of these two phenotypes showed similar rates of progression, phenotype C was associated with failure in the retinal microvascular response and progression of CNP [29].

The identification of three major phenotypes of DR allows an integrated perspective of DR progression. Chronic hyperglycaemia induces generalised cell damage to the retina involving the entire retinal neurovascular unit but causing different degrees of damage in different cells in different individuals. Some patients develop generalised low-grade vascular, neuronal and glial damage which manifests as a slow-progressing neuropathy along with a slow-progressing vascular damage (phenotype A). Other patients develop breakdown of the BRB with resulting retinal oedema, possibly associated with the neuroglial damage and an active inflammatory repair process (phenotype B). Finally, another group of patients, either due to an abnormal accumulation of vascular endothelial growth factor (VEGF) or other angiogenic factors associated with rapidly developing hypoxia undergo induction of an abnormal interaction between endothelial and blood cells, possibly due to specific genetic characteristics; they show signs of active microvascular disease and more rapid progression to VTDR (phenotype C). The response to anti-VEGF treatment may also allow us to identify phenotypes based on treatment response. As about 50% of patients treated with anti-VEGF agents show persistent fluid by the end of two years of treatment, non-VEGF pathways may play a role. These include steroidal and non-steroidal anti-inflammatory agents, inhibitors of the Kinin-Kallikrein system, renin-angiotensin system, angiopoietin-Tie2 and anti-integrin [30]. Activating the *Wnt*-signalling pathway may be an option both for neuroprotection as well as to target retinal ischaemia [30]. Anti-oxidants and targeting mitochondrial dysfunction may play a role in early DR too [31]. Gene therapies targeting VEGF and non-VEGF pathways remain exploratory at this time. Further work is required in assessing the contribution of each pathway in the pathogenesis of DR and its complications so that patients could be better stratified and precise treatment options may be made available.

Overview of DR progression. Currently, our understanding is that diabetic retinal disease follows a sequence of events that is initiated by neurodegenerative changes resulting directly from hyperglycaemia. These neurodegenerative changes are best identified by progressive thinning of the GCL+IPL detected by OCT examination. In one-third of individuals with T2D the microvascular response to the neurodegenerative changes fails and CNP develops, involving initially the central superficial retinal capillary plexus [27, 32]. Eventually, the microvascular disease involves the deep capillary plexus leading to increasing capillary closure, which is compensated by the development of thoroughfare channels that cross the areas of closed capillaries [33]. These enlarged vessels extend to the midperiphery and periphery of the retina and contribute to the progression of the CNP to the entire retina causing increased ischaemia of the retina, with thinning of the GCL+IPL in the midperiphery and periphery of the retina [34]. The development of predominantly peripheral lesions (PPL) namely, haemorrhages, microaneurysms, intraretinal microvascular abnormalities, venous beading, and neovascularization outside than within the ETDRS 7 standard photographic fields, is a direct result of the increasing ischaemia, progressively involving the entire retina, quadrant by quadrant, and leading to the

development of PDR.

Simultaneously with the occurrence of the CNP, there is a breakdown of the BRB resulting in abnormal accumulation of fluid and retinal oedema. This alteration occurs independently of the progressive capillary closure and may be predominant in a small percentage of individuals with T2D. It is possible to identify eyes with retinal oedema and little capillary closure or the occurrence of both together. The association of breakdown of the BRB and capillary closure may explain the development of clinically significant DMO and vision loss.

Retinal imaging for DR

Colour fundus photography: The ETDRS group classified eyes with DR as mild, moderate, severe, very severe NPDR and early and high-risk PDR [1, 35, 36]. The DR severity and progression is graded with the modified Airlie House Classification using seven 30-degree field stereoscopic photography established in the ETDRS study, capturing around 35% of total retinal area [37–41]. A scoring system known as the Diabetic Retinopathy Severity Score (DRSS) was developed which scores each eye from 10 (no retinopathy) to 85 (advanced PDR). A progression of DR was defined as a 2 or 3 step change in the DRSS as studied in the Diabetes Control and Complications Trial and United Kingdom Prospective Diabetes Study [40, 41]. The ETDRS-DRSS is the standard for DR grading across clinical trials. The Diabetic Retinopathy Clinical Research Network (DRCR.net) has recently demonstrated that 200° ultra-wide-field imaging (UWFI) showing only the ETDRS fields of view is equivalent to the original DR severity grading based on 7-field photography [42]. The incorporation of UWFI to DR classification is awaited.

OCTA: Fundus fluorescein angiography (FFA) has been the main method used to examine retinal circulation, although it involves the intravenous injection of the fluorescein dye, until the recent introduction of non-invasive OCT angiography (OCTA). OCTA allows visualisation of the retinal circulation at different depths without injecting any dye. OCTA is unable to demonstrate BRB breakdown and vascular leakage, but some approaches may offer this complementary information [43]. Vessel density is a quantitative metric of capillary closure obtained with OCTA that has shown good correlation with severity of DR and its progression. Durbin et al [32] showed that VD measured in the superficial capillary plexus can discriminate healthy from DR eyes. The regional distribution of retinal capillary changes detected by OCTA appear to be particularly relevant in staging DR. Swept source OCTA enables visualisation of the retinal vasculature over larger fields of view allowing identification of the different ETDRS stages of DR [34]. In summary, OCTA metrics of CNP obtained in a non-invasive manner with repeated examinations may be more informative than systemic risk markers for predicting DR progression and are expected to impact management of DR [44].

OCT: Optical coherence tomography (OCT) is particularly relevant in DR imaging because it offers, in a non-invasive manner, metrics of central retinal thickness (objective quantification of macular oedema) and of thinning of the ganglion cell plus inner plexiform layers (objective quantification of neurogenerative changes). These metrics are of major relevance to follow the progression of macular oedema and to identify the degree of retinal neurodegenerative changes in the most initial stages of the retinopathy, and later as the result of the capillary closure and ischaemia. Currently, studies on improved characterisation of macular oedema using novel OCT-derived leakage parameters to locate areas of breakdown of the BRB is underway [45, 46]. In later stages, disorganisation of inner retinal layers (DRIL) is a poor prognostic marker of treatment response [47].

Biomarkers of NPDR progression. Biomarkers have become the basis for preventive medicine, i.e., medicine that recognises diseases or the risk of disease early and takes specific countermeasures to prevent the development of disease. Biomarkers are also seen as the key to personalised medicine, treatments individually tailored to specific patients for highly efficient intervention in disease processes.

It is necessary to distinguish between prognostic, disease-related, and predictive, drug-related, biomarkers [48, 49]. Prognostic markers show the progression of disease with or without treatment whereas predictive biomarkers help to assess the most likely response to a particular treatment type. At present, the only validated systemic biomarker for development of DR is HbA1c.

Candidates for ocular imaging biomarkers of DR progression are retinal vessel density determined by OCTA, as an indicator of CNP and ischaemia; central retinal thickness, determined by OCT as an indicator of macular oedema and thinning of the GCL+IPL as an indicator of retinal neurodegenerative changes. Ocular imaging biomarkers have been shown to be more reliable than systemic markers to identify DR progression [43].

Studies involving larger numbers of well-phenotyped patients with DR are now needed to establish the set of genetic markers that may help predict DR severity progression and development of VTDR in type 2 diabetic patients.

Proliferative diabetic retinopathy

Natural history studies have shown progression rates from no DR to PDR utilising ETDRS DR severity levels over four to six years, with eyes with more severe DR likely to progress to PDR faster than those from mild and moderate NPDR to severe NPDR [50]. Two-step change in ETDRS severity level is the most preferred trial end-point to evaluate the worsening or improvement of DR [51–53]. The eligibility criteria for a DR prevention trial depends on the type of intervention. For example, the UKPDS trials evaluated the effect of oral medication to control the risk factors of DR and so it is appropriate that all people with type 2 diabetes with or without DR were included. However, if the agent requires frequent intravitreal therapy to prevent progression of DR, it would be more appropriate for eyes with moderately severe to severe NPDR. Several studies on anti-VEGF therapy for DMO have shown that a proportion of eyes show reversal of severity levels of DR on colour photographs. This has triggered the debate on whether this regression is a sufficient marker of disease reversal.

PDR and relation to VEGF. Hyperglycaemia induces several pathways and VEGF is the most potent cytokine that causes both vascular leakage as well as proliferation [43, 53].

Current treatment approach for PDR and DMO. Since the 1960s, panretinal photocoagulation has been the mainstay of treatment for PDR, supported by data from the Diabetic Retinopathy Study (DRS) [43]. However, DRS also showed that 50% of eyes with PDR would still progress to severe vision loss even after panretinal photocoagulation [43]. PDR causes regression of neovascularisation, reduction of vitreous haemorrhage and prevents tractional retinal detachment [54]. Vitreous VEGF levels also decreases after PRP. However, PRP has several limitations, including permanent loss of peripheral field of vision, night blindness, worsening of macular oedema, vitreous haemorrhage, uveal effusion and transient loss of central vision [37]. Moreover, it requires clear media and good patient cooperation. Therefore, there is a need for new treatment that can reverse CNP in the diabetic retina without destroying retinal tissue.

For DMO, although the landmark ETDRS showed that macular laser is effective in reducing the risk of moderate visual loss by 50%, intravitreal anti-VEGF therapy has become the standard of care with multiple randomised clinical trials demonstrating an improvement in visual outcome [55, 56]. Currently, research on

increasing durability of anti-VEGF agents and alternate agents for non-responders to anti-VEGF agents are being investigated.

Retinal imaging for PDR

Ultra-wide-field imaging: Compared to conventional ETDRS photography, UWFI of the retina captures approximately three times more retinal area and provides more information about the peripheral retina [57–59]. UWFI has enabled identification of PPL, which are more severe outside than within the ETDRS 7 standard photographic fields [60]. The addition of PPL to current ETDRS-DRSS severity levels aids identification of fast progressors. These eyes have larger areas of CNP on angiography and the locations of PPL are found to correspond to areas of non-perfusion [60]. The DRCR.net compared DRSS grades with UWFI in DR eyes in protocol AA and observed reasonable agreement [42]. A total of 41% eyes were also found to have PPL, and in 12.5% eyes with PPL, when the presence of PPL was combined with the DRSS, these eyes had a 1-step worse level of DR compared to that graded according to DRSS [42]. This again reiterates the importance of detection of peripheral lesions using UWFI for DR grading. This finding also highlights the need for a new classification for DR.

Retinal perfusion

Fluorescein angiography: Although FFA can visualise CNP, CNP was not included in the grading of ETDRS DR severity level [61]. Areas of CNP are conventionally measured in disc areas and so subtle areas of CNPs are often missed [62]. Recent studies using binarization have used angiographic characteristics such as darker choroid or pruning of vessels for denoting CNP [63–65]. Efforts are ongoing for automated detection of CNP on FA, but the variabilities due to subjective assessment of differences in grayscale are challenging [63].

OCTA: The OCTA allows non-invasive detection of CNP. Current OCTA devices focus on the macula mainly and use metrics such as vessel density, perfusion index, foveal avascular zone area, fractal dimension, etc. [66–68] to quantify CNP more objectively than FA. Moreover, OCTA may detect vascular abnormalities such as microaneurysms in the deep capillary plexus which is not visible on FA, and identify DR lesions in patients who have normal fundus on ophthalmoscopy [69, 70]. A useful application of OCTA is the characterisation of diabetic macular ischaemia (DMI) [71]. Wide-field OCTA may provide more information up to the mid-peripheral retina [34]. These features have to be validated well before incorporating into a DR classification system.

Ultra-wide-field angiography: Recently, ultra-wide-field FA (UWFA) has become a practical alternative to standard field FA as it avoids the need for repeated imaging of the retinal peripheral fields and also allows visualisation of a wider field [72]. Although wide-field OCTA may help in monitoring neovascularisation in DR eyes undergoing PRP, it cannot detect changes in leakage; however, it may detect peripheral CNP better than FA along with individual layer segmentation, and this area of research needs to be prioritised [73]. Leakage of retinal vasculature suggests recent active disease and a combination of FA and OCTA may be required to assess whether PRP has adequately stabilised eyes with PDR [74].

Artificial intelligence in retinal imaging: Artificial intelligence systems have been shown to achieve near-human capabilities for the detection of DR from colour fundus photographs in efficiency and accuracy. While most systems have focused on the AI-based grading of DR as referable or non-referable DR from a screening point of view, recently a lot of focus has been given to deep-learning-based grading of DR into ETDRS or similar stages along with DMO [75, 76]. Few other groups have tried identifying the different DR lesions by classifying them into red and yellow lesions [77]. However, there is a gap in knowledge regarding AI systems, especially with the detection of location of lesions to identify eyes with PPL.

Since around 50% of DR lesions may be present in the retinal periphery [60, 78], detection of these PPLs with UWFI may become the standard of care in future, and automated analysis of UWF images may ease quantification of such lesions, and delineate their relation to prediction of DR severity and progression [60]. However, there is a need for further scientific studies to ascertain real-life replication of the accuracy of these automatic systems as quality of retinal images captured determine the applicability of these systems [79].

Automated detection of CNP areas may prove to be a reliable and consistent method of grading compared to human observers, but has remained relatively less explored [80–82]. Detection algorithms targeting vessel leaks in malarial retinopathy has shown sensitivity in the range of 80-95% and may be developed for DR as well [83]. One platform has evaluated the objective automated quantification of microaneurysms and segmentation of leakage areas on UWFA images and has observed significant consistency of detection and comparability to human graders [84, 85]. Some authors have evaluated a semi-automated platform using automated detection of leakage index, ischaemic index and microaneurysm count in UWFA images, followed by correction by expert reader and found significant relationship with the same indices and DR severity [86].

A recent study on UWFA images achieved an AUC of 0.82 using a fully convolutional deep-learning model of automatic grid assessment of topographical distribution of CNP. There may be significant issues with UWFA images related to eye movement, poor alignment, small pupil or artefacts due to eyelashes, and hence the boundary of the gradable retina must be identified to maximise output towards a successful prediction of DR severity. With improvements in techniques of grading, quantification and categorisation of CNP and determining its relation to DR severity, it can be useful as an end-point in clinical trials, especially as we move towards treatment for prevention or reversal of non-perfusion.

Impact of anti-VEGF agents on retinal non-perfusion. Peripheral CNP correlate with DR and DMO severity [87, 88]. The threshold of retinal CNP at which PDR develops is not clearly understood. A study from the UK analysed images from two randomised clinical trials (RDP study and CLARITY study) and found that eyes with more than 107.3-disc area of CNP were at maximal risk of developing PDR, and eyes having neovascularization of the disc tended to have the largest areas of CNP [89].

Relatively few studies have evaluated the change in CNP with the use of anti-VEGF agents. The RECOVERY study evaluated treatment-naive PDR eyes without DMO with extensive CNP areas undergoing aflibercept therapy and found that a monthly dosing of aflibercept may reduce progression of CNP areas better than 3-monthly dosing, with an improvement of DRSS scores [90]. Some studies have evaluated UWFA in DR [91, 92]. The PERMEATE study observed a significant reduction of leakage index 12 months after anti-VEGF therapy [93]. Bonnin et al observed an improvement in DRSS scores 3 months after anti-VEGF therapy with a reduction in capillary leakage, with no significant change in perfusion status [39, 94]. Novel agents are required to improve or prevent the progression of CNP.

Clinical outcomes of Anti-VEGF therapy in DR. Over the past years, several trials have been designed and conducted for the treatment of DMO using anti-VEGF drugs. Post-hoc analysis of these trials have revealed that irrespective of DMO, the DR status of patients also improves in these patients with anti-VEGF therapy [50, 95]. As previously discussed, there is no evidence that the anti-angiogenic and anti-permeability functions of VEGF blockade

aid the restoration or normalisation of the retinal vasculature and potentially reopen the non-perfused retinal vasculature in PDR [61, 96]. However, the improvement in DR severity is maximum in patients with moderate to severe NPDR (DRSS levels 47/53) and this can be rapid and well-sustained over the years, irrespective of baseline systemic risk factor levels [97].

Post-hoc analysis of trials on anti-VEGF agents for treating DMO: The DRCR.net protocol T was designed to study the outcomes of the three different anti-VEGF drugs, aflibercept, ranibizumab and bevacizumab in DMO. The post-hoc analysis of the study which aimed to determine the DR regression in the three treatment groups [98] found that the DRSS scores improved with all three drugs, and more significantly after aflibercept treatment. These findings are similar to previous studies which also detected DR severity improvement with repeated anti-VEGF injections in DMO, even in cases where fixed monthly dosing was not followed [55, 56,99–106].

Seventy percent (70%) of PDR eyes had a sustained improvement over 2 years, and a significant proportion of eyes that did not improve in the first year, did so on follow-up, suggesting that continued treatment despite initial delay may still be beneficial. A worsening of PDR was observed in 17.2-26.4% of eyes, with vitreous haemorrhage being the most common manifestation of progression. In NPDR eyes, 22.1-37.7% showed improvement in disease severity, especially after aflibercept and ranibizumab treatment, with higher improvement rates in advanced stages of NPDR [98]. Higher improvement rates were observed in eyes which received more injections, similar across all three drug groups. Worsening of NPDR was seen in 7.1–10.2% eyes, with 2.4% progressing to PDR.

Similarly, the RISE and RIDE trials evaluated the effects of intravitreal ranibizumab in patients of DMO and found that 35.7–38.5% of eyes showed 2-step or more improvement in DRSS scores [55]. Advanced severity stages of NPDR eyes experienced 2-step or more DRSS improvement in more than 75% cases. Treated eyes progressed to PDR at lesser rates than the sham group and needed PRP less frequently. A peculiar observation was that eyes which had baseline DRSS scores of ≤43 (mild or moderate NPDR) had more stable DR than eyes which had improved to <43 (mild or moderate NPDR) with repeated treatment. Hence, although a more severe DR stage at baseline may have higher DRSS improvement, it seems to be more unstable and prone to worsening on intermittent treatment. The RISE/RIDE studies had an open label extension phase in which eyes received ranibizumab in PRN dosing [101]. Eyes which had initially showed DR severity improvement were maintained in almost 70% patients in the PRN phase, with visual improvements irrespective of change in DR severity. Moreover, regardless of baseline DRSS, all patients received an average of 4–5 injections during the PRN phase, suggesting that long-term monitoring and maintenance dosing may be important for continued DRSS stability.

Another important finding of the RISE/RIDE studies was that although they did not measure retinal reperfusion rates, they observed a delayed rate of progression of CNP with ranibizumab.

The VISTA and VIVID trials evaluated the role of aflibercept in DMO eyes, and found that almost a third of eyes showed 2-step or more improvement in DRSS scores with monthly dosing of aflibercept [107]. These effects were well maintained through the 148 weeks of follow-up.

The results of these studies indicate that the visible regression of DR on colour photographs with anti-VEGF agents is dose-dependent. However, the minimum interval of anti-VEGF dosing required to sustain the initial improvement attained over the lifetime of the patient remains unclear.

Prospective clinical trials in PDR: A randomised clinical trial, DRCR Protocol S compared the efficacy of intravitreal ranibizumab alone versus PRP for treatment-naive PDR eyes [108]. Ranibizumab-treated eyes had better visual acuity outcomes related to DR progression than PRP, probably explained by the lower prevalence of new-onset DMO. Although lesser PDR-associated events were seen in eyes treated with ranibizumab and fewer eyes underwent vitrectomy, a proportion of eyes still progressed to complications in spite of continuous treatment. This suggests the probable need of starting early anti-VEGF therapy and the need for aggressive therapy [109].

In eyes with PDR along with vitreous haemorrhage, anti-VEGF treatment may be used to treat the underlying DR, although DRCR Protocol H did not show any significant difference in vitrectomy rates between ranibizumab-injected and sham-treated eyes [110].

The CLARITY trial, a non-inferiority trial that compared aflibercept and PRP in active PDR eyes [111], showed that regression of neovascularization was significantly higher with aflibercept than PRP-treated eyes. Moreover, aflibercept led to higher improvements in DRSS and less incidence of eyes requiring vitrectomy. However, post hoc analysis of this trial showed that disc neovascularisation was more resistant to aflibercept therapy compared to new vessels elsewhere [112]. This observation may suggest that anti-VEGF may be less responsive beyond a certain total area of CNP that leads to disc neovascularisation or there may be other angiogenic agents contributing to disc neovascularisation compared to retinal new vessels.

The PROTEUS trial compared PRP against PRP with additional ranibizumab for regression of neovascularization area in eyes with high-risk PDR over 1 year [113]. The study found that 44% eyes receiving combined treatment showed complete regression of neovascularization as compared to 25% in PRP alone group. This approach is likely to provide a more sustainable effect than anti-VEGF monotherapy for PDR.

The PRIDE study compared the effects of PRP alone versus ranibizumab alone versus PRP-ranibizumab combined therapy in PDR in terms of change of neovascularization area over 12 months [114]. The study found that at month 3, 67% of patients in both the ranibizumab alone group and combined groups showed complete resolution of leakage from neovascularisations; while at the end of the 12-month study period, the maximum percentage of patients showing complete resolution of leakage from neovascularisation was in the ranibizumab alone group, along with a better improvement in visual acuity.

However, compliance with repeated anti-VEGF therapy and the cost implications are restrictive. Therefore, longer-acting agents are awaited. Newer agents that prevent the progression of CNP are also awaited.

Prospective clinical trials in NPDR: In the DRCR.net Protocol W trial, eyes with moderate to severe NPDR without DMO were randomised to receive aflibercept and sham [115]. The study observed that the risk of developing VTDR, both PDR and DMO, over 2 years, was lesser with aflibercept. Similarly, the PANORAMA trial is an ongoing clinical trial evaluating the role of aflibercept versus sham in severe NPDR without DMO in improving DRSS over a 2-year period. At week 24, 58% of eyes receiving aflibercept achieved two-step or more improvement in DRSS compared to sham, irrespective of the dosing schedule [116]. Also, aflibercept-treated eyes had an almost 80% reduction in chances of development of VTDR of DR compared to sham. These trials show that DRSS improvement can occur in eyes without DMO treated with aflibercept.

Diabetic macular oedema

Anti-VEGF agents have become the mainstay of treatment for visual impairment due to centre-involving DMO, with approximately 50% of patients improving by two lines of visual acuity within two years. DMO can be associated with any DR severity level and progresses irrespective of DR severity. Moreover, visual outcomes in DMO do not significantly differ in eyes grouped into different DR severity levels by DRSS score.

However, suboptimal response to these agents remains a problem. The modest correlation of visual acuity change with a reduction in central subfield thickness shows that factors other than visible fluid are responsible for changes in visual acuity in DMO [117]. Non-invasive OCTA has helped delineate the retinal perfusion in all vascular layers of the retina and has helped to better define DMI. DMI may be an additional cause of vision loss along with DMO, however, the pathogenesis is less understood. The prevalence and characterisation of DMI with and without visual acuity loss have not been not extensively studied [118]. DMI prevalence increases with DR severity. However, the current DR grading does not include DMI. Moreover, both DR grades and DMI do not correlate with visual acuity changes. Further studies are needed to understand the relationship between DR severity, DMO and DMI, which may help develop treatment strategies. Some proposed therapies for DMI have targeted neurodegeneration, and pathological neovascularisation to reverse the ischaemia. It needs to be seen how DMI finds a place in a newer DR classification with a focus on treatment modalities and prognostication.

Way ahead

Need for a reclassification of DR. The ETDRS-DRSS was developed for research and is essentially too complex to be administered in a clinical setting. The original DRSS was established based on the seven-field photography system, which only evaluates vascular changes in approximately 30% of the retina. At present, we have additional imaging tools at hand namely, UWFI, OCTA and UWFA. Newer DR classifications incorporating pre-retinopathy signs and peripheral retinal lesions are awaited. Further research is required to validate some of these signs to be incorporated into clinical practice. More importantly, patients may not perceive any symptoms until the development of later stages of DR.

Ambiguity of DRSS as a severity marker. Since the DRSS was standardised for treatment-naive eyes, the utility of the same scoring system in monitoring eyes which have received treatment in the form of PRP or anti-VEGF has been long debated. Observations from clinical trials reinforce that the only factor significantly associated with 2-step or more DRSS improvement in all treatment groups was baseline DRSS over long-term follow-up [119]. In the same context, an eye with moderate to severe NPDR may be more responsive to DRSS improvement than an eye with mild NPDR at baseline, despite being more unstable. Hence, when the moderate to severe NPDR eye improves to mild NPDR stage, this 'induced' state is quite different from a 'treatment-naive' mild NPDR eye of the same DRSS [120, 121]. Hence, the DRSS fails to adequately describe this post-treatment underlying state in DR eyes. On the contrary, a UWFA at this point might help in evaluating the peripheral ischaemia, which in turn might help predict the natural course and further treatment response in the 'induced' and 'treatment-naive' eyes. Artificial intelligence-based standardisation of UWF images may further help in achieving a uniform criterion for treatment purposes and also for monitoring eyes in clinical trials.

Capillary non-perfusion. There is paucity of literature evaluating UWFA or wide-field OCTA in characterisation of peripheral ischaemia in DR and how it changes with anti-VEGF or any other treatment. Measurement of CNP needs to be validated before it can be used in randomised clinical trials investigating drugs for preventing progression of CNP. There is also limited literature regarding the frequency of dosing of anti-VEGF agents to maintain DR severity improvements. Long-term follow-up studies using non-invasive imaging techniques such as OCTA is required to evaluate changes in CNP. In this regard, UW-OCTA holds promise, since almost 24% of patients may have recurrent neovascularisation even after receiving multiple injections [122]. Multiple PDR trials have evaluated neovascularization status based on clinical examination or photography alone, leading to variability in assessment of neovascularization and this may be avoided with the use of UW-OCTA [123].

Prevention of DMO and PDR. Research on the treatment of DR has advanced significantly. However, there is a lag in the discovery of robust sustainable easily administered preventive options for the development and progression of DR. There is also a need to explore anti-fibrotic options to prevent or treat advanced PDR. Although anti-VEGF agents have revolutionised our management options for DMO and PDR, a proportion of patients with DMO respond sub-optimally to these agents. Most may be due to inadequate treatment, but novel agents with better durability, less frequent administration and perhaps inhibiting VEGF and non-VEGF pathways are awaited.

A new agent, faricimab, which targets the *Ang*-2 and VEGF-A pathway, might be useful to target both VEGF and non-VEGF pathways, to increase the durability of treatment in retinal diseases including DMO [124, 125]

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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