Chapter 2

Potential Imaging Biomarkers in the Development and Progression of Diabetic Retinopathy

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Additional information is available at the end of the chapter

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Abstract

Diabetic retinopathy (DR) is the most prevalent microvascular complication of diabetes and a leading cause of preventable blindness in the working-age population. However, due to a lack of suitable biomarkers, its prediction in asymptomatic patients is insufficient. Currently, DR is diagnosed at a stage when typical morphologic lesions become fundoscopically visible. Yet, chronically elevated blood glucose levels lead to characteristic alterations in retinal vessel caliber, blood flow, oxygen saturation, and the capillary network, which precede DR lesions. Furthermore, emerging evidence suggests that retinal neurodegenerative changes occur early in diabetes, initiating a disintegration of the retinal neurovascular unit prior to the appearance of microvasculopathy in DR. This chapter will discuss recent research achievements toward understanding the complexities of DR pathophysiology. It will focus on the nomination of potential imaging biomarkers for the prediction of DR development and progression using innovative structural, functional, and metabolic imaging techniques, including optical coherence tomography angiography (OCTA), retinal oximetry, ultra-wide field FA, and corneal confocal microscopy (CCM). Validation of these biomarkers would allow the identification of patients at high risk of developing DR and might initiate a swift move to early diagnosis and individualized care.

Keywords: diabetic retinopathy, biomarker, retinal blood flow, retinal oxygen saturation, retinal neurodegeneration, corneal confocal microscopy, ultra-wide field imaging, disorganization of the inner retinal layers, imaging, OCT, OCTA

1. Introduction: the role of biomarkers in disease prediction

The prevalence of diabetes mellitus is increasing worldwide. The International Diabetes Federation estimated that 415 million people had diabetes in 2015, 90% of whom were diagnosed with type 2 diabetes. With these trends continuing, 642 million patients with diabetes are expected by 2040 [1].

Patients with diabetes are at substantially increased risk of developing complications. In light of the cost of interventions implemented throughout the natural history of these complications, diabetes constitutes a tremendous clinical and public health burden that exceeds the resources of healthcare systems even in the most affluent countries. The International Diabetes Federation has reported that most countries spent 5–20% of their total healthcare budget on diabetes in 2015, which amounted to 673 billion US dollars in health expenditure worldwide. This figure is expected to increase to about 802 billion US dollars by 2040 [1].

The complications of diabetes are commonly divided into macrovascular complications including myocardial infarction, heart failure, and stroke, and microvascular complications including diabetic nephropathy, neuropathy, and retinopathy. Diabetic retinopathy (DR) is the most common microvascular complication of diabetes. The incidence of DR increases with the duration of diabetes. After 20 years, nearly all patients with type 1 diabetes and more than 60% of those with type 2 diabetes will develop signs of DR [2].

Current treatment guidelines target proliferative disease and macular edema, two sight threatening complications. The most common approaches are intravitreal injections of vascular endothelial growth factor (VEGF)-inhibiting agents or corticosteroids, laser treatments, and surgical interventions. These treatments are often sight saving, but are invasive and cost-prohibitive. Therefore, we need to shift our focus to targeting upstream events at earlier stages of non-proliferative DR (NPDR).

The major health economic burden caused by the increasing number of patients diagnosed with diabetes raises the need to identify patients at high risk of developing DR and sight threatening complications. Reliable biomarkers that help to predict the development and progression of the disease have to be defined.

A biomarker is traditionally defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [3]. In order to be useful in the prevention of DR, a biomarker should (1) non-invasively detect early preclinical disease before the first clinical signs of the disease appear, (2) be causally linked or be an indicator of a causal mechanism that leads to the development of the disease, and (3) be consistently and strongly associated with the disease [3, 4]. Suitable biomarkers should identify patients at low risk to defer DR screening intervals facilitating cost-effective management and optimized resource allocation. Furthermore, biomarkers should help to predict the progression of DR to the vision-threatening stage, and may forecast the response to different treatment modalities, facilitating individualized care [5]. Important factors for valid biomarkers are reproducibility and validity in different populations. Furthermore, their measurements must be quick, cost-effective, and applicable in daily clinical decision-making [5].

To date, many serum variables have been proposed to be associated with DR incidence and progression. According to the Diabetes Control and Complications Trial (DCCT), a median glycated hemoglobin (HbA1c) of 7.2% reduced DR incidence by 76% in patients with type 1 diabetes, and DR progression by 54% over a period of 6.5 years [6, 7]. Patients with type 2 diabetes had a 25% reduction of DR with good glycemic control [8]. Even though HbA1c remains the most widely accepted biomarker nowadays [5], the "Joslin 50-Year Medalist Study," which focused on the identification of endogenous protective factors in patients with a diabetes duration of at least 50 years (therefore named "Medalists") showed that longitudinal glycemic control was unrelated to diabetic complications. However, the presence of specific advanced glycation end products (AGEs) (plasma carboxyethyl-lysine and pentosidine) was strongly associated with the development of diabetic vasculopathy complications [9].

Cytokines from aqueous humor or vitreous sample have also been considered in the search for a DR biomarker. Increased levels of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), and nitric oxide (NO) are commonly found in DR. However, these biomarkers can only be assessed using invasive methods. As tears are more accessible than serum and intraocular fluids (i.e., vitreous or aqueous humor), research has also started to focus on the presence of potential markers in this body fluid. Candidate biomarkers in tear fluid include nerve growth factor (NGF), lipocalin-1, lactotransferrin, lysozyme C, lacritin, lipophilin A, immunoglobulin lambda chain, heat shock protein 27 (HSP 27), and tumor necrosis factor-α (TNF-α) [10].

Ocular imaging biomarkers would offer the advantage of gaining an insight in the actual pathologic evolution of DR non-invasively. The most important candidates for such biomarkers will be discussed in the following chapter.

2. Microvascular changes in diabetic retinopathy

2.1. Retinal vessel caliber

Diabetic retinopathy is diagnosed clinically by the presence of microaneurysms and small hemorrhages visualized during fundoscopy. Assessing the presence and number of microaneurysms as well as their rate of formation and disappearance has been suggested to be an appropriate marker of retinal vascular damage and therefore DR progression [11]. However, there are microvascular changes that have been shown to antecede fundoscopically visible lesions of DR including microaneurysms.

Within the last decades and with the implementation of specialized computer software systems, grading of retinal vessel diameters to document generalized vessel narrowing or widening has become increasingly sophisticated, objective, and reliable. Multiple population-based studies have used these systems to calculate retinal vascular caliber in terms of the central retinal artery and vein equivalent (CRAE, CRVE), which summarizes the average diameter of the internal lumen of the vessel, reflecting the visualized erythrocyte column [12]. In sum, the results of these studies provide evidence for an association between larger venular caliber and DR in patients with type 1 [11, 13] as well as type 2 diabetes [14, 15], therefore being consistent with clinical experience. However, reported findings on arteriolar caliber remain contradictory.

The population-based Multi-Ethnic Study of Atherosclerosis (MESA) study showed that arteriolar calibers are dilated in patients with diabetes [13], whereas other researchers claim that arteries tend to constrict in diabetes [14, 16].

The discrepancy between results of different studies may be due to differences between the study cohorts in demographic (e.g., distribution in age) and metabolic traits including blood glucose levels, duration of diabetes, and cardiovascular risk factors (such as hypertension/hyperlipidemia) as well as differences in sample size, follow-up period, and the methods applied. Before retinal vascular caliber assessment can be used as a biomarker in clinical practice, age-, sex-, body size-, and blood pressure-specific normative data are required.

2.2. Autoregulation of retinal vessel diameter

Besides a "static" measurement of retinal vessel diameter, "dynamic" changes in the diabetic retinal vasculature can be assessed too. The potential for an efficient diameter change in order to adjust blood flow according to changes in arterial blood pressure (pressure autoregulation) and retinal metabolism (metabolic autoregulation) is reduced in the early stages of DR [17]. Vasoactive molecules activate pericytes and smooth muscle cells to regulate the capillary diameter [18]. A dysfunction in pressure autoregulation of retinal arterioles implies that changes in the arterial blood pressure are directly transmitted to the retinal microcirculation [19]. The fact that pressure autoregulation decreases with increasing severity of DR highlights the destructive effect of arterial hypertension on the retinal microcirculation [17, 20].

Luminance flicker stimulation is an example to test the capability of retinal vessels to adapt perfusion to changes in retinal metabolism. Exposure to flickering light stimulates retinal neuronal cells to release local vasodilating metabolites, most importantly nitric oxide [21], which consequently leads to retinal vasodilatation. This results in an increase in retinal blood flow in healthy individuals [22]. Several studies have reported that the flicker light-induced vasodilation is reduced in patients with diabetes [17, 23, 24] and even in patients with prediabetes [25], being equivalent in magnitude to patients with manifest diabetes. Thus, monitoring retinal vascular reactivity may provide an early marker of autoregulation and endothelial dysfunction in the retinal microcirculation that clinicians could follow non-invasively.

2.3. Retinal blood flow

Besides measurement of retinal vessel caliber, numerous other techniques such as laser Doppler velocimetry, laser Doppler flowmetry (LDF), fluorescein angiography (FA), color Doppler, and Doppler optical coherence tomography (OCT) imaging have been proposed for quantifying retinal blood flow in patients with diabetes [26–30]. Contradicting results concerning retinal blood flow have been published. This may reflect the complexities of the pathological alterations that occur in the diabetic retina.

Most studies suggest that in patients without or with mild non-proliferative DR (NPDR), retinal blood flow is reduced [26, 27]. Evidence from animal studies in streptozotocin-treated rats also suggests decreased retinal blood flow in the very early stages of DR [31]. In more severe stages of NPDR, research has provided evidence that retinal blood flow increases above normal levels [28–30], which may arise from the increased demand caused by tissue hypoxia due to capillary basement membrane thickening and capillary occlusion [29, 32]. In proliferative disease, retinal blood flow is decreased again as measured with different techniques: Blair et al. used the dye dilution technique to measure the mean circulation time (MCT) calculated as the difference between the mean venous and arterial retinal passage times, which turned out to be statistically significantly longer in the eyes with proliferative DR (PDR) than in healthy eyes or eyes with NPDR [33]. Laser Doppler flowmetry (LDF), which measures blood flow at the optic nerve head (ONH), and color Doppler imaging, also showed a greater reduction in total retinal blood flow in patients with PDR than in patients with NPDR or healthy individuals [34, 35]. Recently, several groups have demonstrated the potential of Doppler OCT for assessing retinal blood flow in the diabetic eye. Doppler OCT can also detect volumetric blood flow and provide information about the structural anatomy. As shown with the techniques mentioned above, eyes with PDR had statistically significantly decreased retinal blood flow compared with normal eyes [36], especially those that had been treated with panretinal photocoagulation [28, 37, 38]. However, acute elevations in blood glucose can still trigger an increase in blood flow [26]. This finding suggests that the chronic hyperglycemic state in diabetes mellitus is associated with a reduction in retinal blood flow, but the retina still is able to respond to increased metabolic rates associated with acutely raised blood glucose by increasing retinal blood flow.

2.4. The capillary network

The structure of the retinal capillary network is unique. It has to feed one of the highest metabolically active tissues while limiting the extent of the vascular beds to a minimum in order to prevent optical interference to the photoreceptors [39]. The inner retina is perfused by four interconnected capillary plexi that include the peripapillary capillary plexus which is found in the retinal nerve fiber layer (RNFL) adjacent to the optic nerve head (ONH), the superficial capillary plexus in the ganglion cell layer (GCL), as well as an intermediate (ICP) and a deep capillary plexus (DCP), which are located at the two borders of the inner nuclear layer (INL) [40]. Currently most segmentation algorithms display the ICP and DCP as one capillary layer. The three vascular layers unite in the center of the macula to form a terminal capillary ring surrounding the foveal avascular zone (FAZ). The outer retina and the photoreceptors are dependent on blood supplied by diffusion from the choriocapillaris. The early changes in capillary architecture and perfusion in patients with diabetes have not yet been definitely established, as assessing the human retinal microvasculature *in vivo* is very difficult due to its small size and low optical contrast.

FA, introduced in 1961, has been the gold standard imaging technique for assessing the retinal capillary network [41]. The value of this imaging modality is undeniable, but so are its limitations. First, dye leakage and the superimposition of capillary beds from the different retinal layers into a single two-dimensional image hinder a proper differentiation between the superficial and deep capillary plexi [42]. Furthermore, FA is a time-consuming and invasive technique which does not render it optimal for DR screening or frequent longitudinal evaluation. In addition, intravenous fluorescein dye injections can occasionally cause adverse side effects, nausea/vomiting, urticaria and rarely, but critically, anaphylactic reactions in healthy people [43].

Optical coherence tomography angiography (OCTA) is a further advance in retinal microvascular evaluation and may represent a significant breakthrough in ophthalmic imaging, especially in diabetes care. Intravenous injection of extrinsic fluorescent dye is no longer required with this technology, but the perfused capillary architecture is non-invasively visualized with erythrocyte motion as an intrinsic contrast. A recent study has demonstrated that shorter acquisition times and a higher number of motion artifact-free images can be achieved using swept source technology [42].

Several features of early disruption of microvascular perfusion in the development and progression of DR have already been investigated and objectively quantified using OCTA. Diabetic macular ischemia, clinically defined as an enlargement and disruption of the foveal avascular zone (FAZ) and capillary dropout in adjacent parafoveal areas [44], is thought to have predictive potential for DR progression [45]. The considerable inter-subject variability in FAZ size even in healthy people and the large overlap in FAZ size between healthy individuals and patients with diabetes have to be considered though [46]. Hence, FAZ size alone was suggested to be a poor diagnostic variable [47], and qualitative FAZ assessment (e.g., with FAZ outline and regularity) may constitute a more reliable biomarker for the ischemic state of the macula in the diagnosis of DR, either complementary to or in place of a quantitative assessment [48].

OCTA is also reproducible for the measurement of vessel density in healthy eyes and eyes with DR. Compared with a healthy control group, patients with diabetes but without DR were shown to feature reduced parafoveal and perifoveal vessel density, and intercapillary areas increase as DR progresses [47, 49, 50]. A more consistent and severe decrease in vessel density has been observed in the superficial capillary network than in the deep plexus in most studies [51, 52]. Accordingly, mean vessel density in the superficial retinal layer, being highly inversely correlated to best-corrected visual acuity (BCVA), has already been proposed to be the best marker for a reliable differentiation between healthy eyes and those with DR [53]. Similarly, the total avascular area in the central 5.5-mm-diameter area was shown to distinguish eyes with DR from control eyes with 100% sensitivity and specificity. It was, therefore, suggested that total avascular area may be an excellent biomarker in the diagnosis of DR [47].

Compared with FA, where the edges of non-perfused areas appear fuzzy or cannot be detected at all, OCT angiograms clearly delimit the border between sparse-capillary areas and dense-capillary areas in most cases [52, 54]. Choi et al. also found impairment of flow in the choriocapillaris at all stages of DR, supporting the concept that choriocapillaris alterations may play a role in the pathogenesis of DR [55].

OCTA color-coded perfusion density mapping enhances areas of low capillary perfusion density in the SCP, DCP and the choriocapillaris in patients with diabetes. Additional trend analysis has shown a statistically significant decrease in capillary perfusion density values as DR progressed [56].

OCTA techniques have also been used to study the development and progression, as well as the treatment response of clinically visible signs of DR. Microaneurysms can be identified in OCTA, but with a significantly lower sensitivity compared with conventional FA [52]. Nevertheless, OCTA provides additional information about their originating capillary plexus. Significantly, more microaneurysms were found in the intermediate/deep capillary plexus than in the superficial one [54, 57]. Additionally, it has been proposed that OCTA is more useful to evaluate clinically active microaneurysms, which are a major cause of diabetic macular edema (DME) [58]. Intraretinal microvascular abnormalities (IRMA), on the other hand, were well detected by both FA and OCTA [54].

The significance of the individual evaluation of the integrity of the deep capillary plexus, impossible with FA alone, is further supported as macular outer retinal changes on spectral-domain OCT (SD-OCT) correspond to areas of capillary non-perfusion at the level of the DCP in patients with DR. The spectrum of outer retinal alterations encompassed different degrees of thinning of the outer nuclear layer (ONL), disruption of the photoreceptor lines, and focal photoreceptor layer thinning [59].

Diagnosis of retinal neovascularization on FA depends on identifying characteristic pathologic vessels with profuse leakage in late angiographic phases. With OCTA, spots of neovascularization that were not identified with FA were visualized as an abnormal flow signal above the inner limiting membrane, which may further help in the identification of patients requiring treatment [47, 55].

Certainly, there are limitations to the OCTA systems in their current state that have to be acknowledged including the incidence of motion artifacts and the relatively small field of view [41], but these can be improved with future development efforts [60]. In summary, OCTA enables the visualization of early microvascular perfusion abnormalities representing imminent DR development and simultaneous monitoring of the treatment response of pathognomonic lesions of DR. It could therefore provide clinicians and scientists in clinical trials with valuable and reliable biomarkers, using an imaging technology that is safely tolerated by patients.

2.5. Retinal oxygen supply

Capillary non-perfusion and tissue ischemia are well-known hallmarks of diabetic retinopathy. While FA provides information about the anatomic state of retinal vessels, changes in retinal oxygenation reflect metabolic dysfunction. Oxygen saturation (SO2) in retinal vessels is a direct measure of retinal oxygen metabolism [18].

Using retinal oximetry, retinal SO2 can be measured non-invasively in major retinal arterioles and venules. The retinal oximeter records fundus images reflected from the retina at two different wavelengths, one being sensitive to oxygen (600 nm), and one being insensitive to oxygen (570 nm). An inverse linear relation between the optical density ratio measured at the two wavelengths and SO2 is assumed. Retinal oxygen saturation can be presented numerically and as a color saturation map [61]. Low variability as well as high reproducibility and repeatability have been shown for retinal oximetry measurements in healthy individuals and in diseased retinas [62–64]. Furthermore, there have already been a number of approaches to compile normative databases for retinal oximetry measurements in Caucasian [61] and multiethnic populations [65], to set a basis for comparability for future clinical trials. Age is the most important factor that should be accounted for in the interpretation of retinal oximetry measurements. Beside age and ethnicity, other demographic factors do not seem to influence retinal oximetry results markedly [61, 65, 66]. Additionally, no statistically significant difference in SO2 levels between patients with type 1 and type 2 diabetes could be observed [61].

Oxygen saturation levels in retinal vessels seem to steadily increase with progressing severity of DR, even if it is not fully elucidated if both, arterioles and venules [67, 68], or solely venules are affected by this increase [69]. Compared with healthy individuals, the change in SO2 levels only becomes statistically significant at more advanced stages of severe NPDR or PDR. Some investigators support the concept that in earlier stages of DR, increased levels of SO2 are detected in retinal venules only, which stands for a decreasing oxygen extraction in these patients, whereas in patients with PDR, SO2 levels are also increased in retinal arterioles, resulting in unchanged levels of oxygen extraction [70].

The metabolic results reflected by retinal oximetry also seem to correlate with the extent of retinal ischemia measured in FA [67].

At first, the findings of increased oxygen saturation levels in patients with diabetes with or without DR seem to conflict with the traditional concept of DR being an ischemic disease. However, this observation can be explained by at least three mechanisms: (1) capillary non-perfusion and shunting (2) thickening of the basement membrane of capillary vessel walls, and (3) greater affinity of hemoglobin for oxygen [71]. Capillary non-perfusion in conjunction with the formation of shunt vessel is already known from histologic studies in the diabetic retina. In capillary shunting, while some vessels dilate, others constrict, leading to blood flow bypassing parts of the capillary network. Blood is then transported faster through these dilated preferential channels, resulting in a shortened arterio-venous passage time and therefore a reduced oxygen extraction time [72]. Further, with thickening of the capillary basement membranes, inevitably, oxygen diffusion from the blood to the retinal tissue is hindered as the transport distance increases [73]. All these mechanisms lead to a maldistribution of oxygen. Oxygen cannot be delivered to the retinal cells in these ischemic areas, which makes venular blood relatively hyperoxic and retinal tissue relatively hypoxic. As a compensatory response, oxygen demand will increase, and more blood will be directed to the tissue. Therefore, oxygenation in arterioles increases too [68].

Intraocular injections of substances inhibiting the production of vascular endothelial growth factor (VEGF), as well as laser treatment and vitrectomy are therapeutic for complications in advanced DR and all of them influence retinal oxygen metabolism.

The vitreous cavities of patients with PDR who have undergone vitrectomy have lower oxygen tension than those who do not have diabetes [74]. Anti-VEGF injections can reduce diabetic macular edema and retinal neovascularization leading to a gain in visual acuity in patients with diabetic maculopathy and/or PDR. The introduction of this treatment modality has considerably improved the visual rehabilitation for patients with DR, but still, some patients respond better to the treatment than others. Interestingly, a recent study indicates that together with arterial blood pressure, SO2 in retinal arterioles may predict visual acuity and central retinal thickness (CRT) in patients with diabetic macular edema after anti-VEGF treatment [75]. Retinal laser treatment destroys retinal tissue and therefore reduces oxygen consumption in treated retinal areas, which in turn reduces hypoxia and the subsequent production of VEGF [76]. The effects of this treatment can be detected with retinal oximetry. A slight increase in SO2 in retinal venules and unchanged SO2 in retinal arterioles was measured immediately after treatment in patients with diabetic maculopathy and patients with PDR, resulting in reduced oxygen extraction. Three months after treatment, arteriolar and venular SO2 were both increased, but arteriovenous SO2 difference was unchanged compared with pretreatment levels [77]. A more recent study in patients with treatment-naive PDR suggested that pre-laser retinal SO2 was not able to predict immediate post-treatment activity of neovascularization, but post-treatment changes in SO2 were closely linked to disease activity 3 months after photocoagulation. Each 1% increase in retinal venular SO2 was independently associated with a 30% higher risk of increased PDR activity despite laser treatment. This implies that if photocoagulation is successfully performed, the amount of the hypoxic retinal tissue is decreased. In the adjacent vital retinal tissue, oxygen is extracted efficiently from retinal arteries, which lowers the venous SO2 and the arteriovenous SO2 levels [78]. Therefore, investigation of oxygen supply may be a potential non-invasive marker of angiogenic disease activity in the monitoring of the treatment response in DR. Prospective studies are under way to further validate retinal oximetry as a biomarker in DR.

3. The identification of lesions in the retinal periphery

Increasing evidence from research suggests that the first lesions in DR develop in the periphery of the retina and that these lesions are potentially associated with DR progression [79, 80]. The gold standard for determining the severity of DR is the extended modified Airlie House classification, which was first used in the Early Treatment Diabetic Retinopathy Study (ETDRS) in 1991 [81]. This rigorously standardized grading scale comprises 13 distinct levels, ranging from the absence of DR to the most severe manifestations of the disease localized in the central posterior 90° of the retina, representing approximately 30% of the entire retinal surface. The ETDRS grading scale is an established measure of disease activity and predictive of the risk of DR progression and visual loss over time [82]. However, due to imaging limitations, a systematic assessment of the retinal periphery was not feasible when the original ETDRS criteria were created. Therefore, the presence of pathologic features outside the 7-fields of ETDRS photography was not accounted for in this grading scale. With the advent of commercially available high-resolution ultrawide-field (UWF) scanning laser ophthalmoscopes, peripheral retinal lesions within and outside the area of the 7-standard ETDRS fields can now be evaluated [83]. Instead of 30° captured by a single ETDRS photo, these UWF imaging systems cover up to 200° in a single image, representing approximately 82% of the retinal area. Combining low-powered green (532 nm) and red (633 nm) laser light, a composite color image with a resolution of 14 μm can be acquired in just a quarter of a second. The high-resolution scanning laser ophthalmoscopy UWF technique allows improved imaging through media opacities such as cataracts, and images can even be acquired without pupillary mydriasis.

There are a number of examples in the literature showing that UWF imaging is comparable to conventional retinal imaging techniques for DR grading. In these studies, images were evaluated for the presence of predominantly peripheral lesions (PPLs), defined as lesions with more than 50% of the lesion located outside one of the ETDRS fields. Compared with eyes without PPL, it is estimated that eyes with PPL at baseline have a 3.2-fold increased risk of a 2-step or more DR progression and a 4.7-fold increased risk for progression to PDR over 4 years, independent of baseline DR severity and HbA1c levels [84].

Identification of DR lesions with non-mydriatic UWF imaging has been compared with standard non-mydriatic multifield fundus photography (NMFP) in large population-based DR teleophthalmology programs. Determining the risk for DR progression associated with an individual's retinal findings in imaging is fundamental in such programs for appropriate risk assessment as well as timing of screening intervals. Ungradable images generally result in referral for comprehensive eye examination because the severity of DR cannot be ascertained. The efficiency of DR teleophthalmology programs could be improved by reducing the unnecessary referrals due to ungradable images, which would lead to considerable savings in logistical complexities, travel arrangements, and time burdens for patients and the healthcare system [83]. UWF imaging can reduce the ungradable image rate by 71% and image evaluation time by 28% compared with NMFP [85]. UWF imaging additionally resulted in a more severe DR level in 9–15% of eyes [84, 86]. Non-mydriatic UWF images were shown to compare favorably with dilated ETDRS photography in determining DR severity, and discrepancies between ETDRS and UWF images were found to be mostly attributable to hemorrhages or microaneurysms [83, 87]. Silva et al. suggested that approximately one third of lesions including hemorrhages, microaneurysms, IRMA, and neovascularization were found predominantly outside the ETDRS fields, being more frequent in temporal than nasal fields [83]. Furthermore, UWF imaging substantially increases the identification of peripheral non-diabetic lesions such as lattice and other retinal degenerations, retinal tears and holes, and choroidal lesions [88]. The utility of UWF imaging has also been demonstrated in comparison with conventional slit-lamp biomicroscopy in a "real-life" clinical setting [89], and in comparison with the gold standard dilated fundus examination with scleral indentation, where Optomap showed high specificity and moderate sensitivity for lesions posterior to the equator, but low sensitivity for lesions anterior to the equator [90]. It was even proposed that assessing of UWF combined with OCT images allows more eyes with higher grades of DR to be detected than in a clinical examination alone or combined with imaging in a clinical setting [91].

The ETDRS extensively evaluated FA but did not provide evidence for a substantially improved ability to predict subsequent DR progression applying this technique. However, due to the limited field of view, traditional FA may miss major areas of peripheral capillary non-perfusion and neovascularization. The advent of UWF FA has provided the opportunity to visualize both the central and peripheral retina in a single examination [92]. Sim et al.

evaluated the association between peripheral retinal ischemia of UWF FA images and central ischemia in DR, and observed a moderate correlation between the peripheral ischemic index and FAZ area, as well as peripheral leakage index and FAZ area in eyes which have not been treated with laser yet [44]. Similarly, 3.9 times more non-perfusion, 1.9 times more neovascularization, and 3.8 times more panretinal photocoagulation scars could be detected in UWF FA compared with the 7-standard field ETDRS images [93]. An increase in retinal non-perfusion was associated with worsening DR [94]. As peripheral non-perfusion probably underlies the development of PPL [80], the identification of PPL may be a potential surrogate marker for estimating the location and extent of peripheral non-perfusion [94].

Current study results assessing the value of UWF FA in eyes with diabetic macular edema (DME) are still contradictory [28, 93, 94].

Besides the paramount advantages of incorporating UWF imaging into the diagnosis and management of DR, certain limitations including low portability and the need for extensive imager training to obtain high quality images must be acknowledged [95]. UWF imaging systems are still expensive but their cost is likely to decrease over time with further technological innovations and market competitions.

In summary, peripheral lesions identified in UWF imaging may substantially alter the risk of DR onset, progression and outcome. Currently a new DR severity grading scale will be established combining clinical with imaging information from UWF photographs and angiograms. A large longitudinal multicenter study sponsored by the Diabetic Retinopathy Clinical Research Network (DRCR.net) has been designed to assess the relation between baseline variables on UWF color fundus photographs and UWF FA with long-term DR outcomes [95].

4. Disorganization of the retinal inner layers for diabetic macular edema prediction

Diabetic macular edema (DME) is one of the most vision-threatening manifestations of DR, affecting almost 30% of patients with a duration of diabetes mellitus of more than 20 years [96].

Elevated levels of vascular endothelial growth factor (VEGF) are a major contributor to retinal microvascular dysfunction and the development of DME. VEGF interferes with tight junctions of the vascular endothelium, leading to a breakdown of the blood retinal barrier and consequently leakage into the retinal tissue [97]. Therefore, repetitive intraocular injections of anti-VEGF agents are a first-line therapy among the currently available treatments for DME. These injections have demonstrated efficiency in reducing macular thickness and improving best-corrected visual acuity (BCVA) [98]. However, while beneficial for some patients, others do not respond to intraocular drug injections. Furthermore, the resolution of DME may not be followed by a recovery in visual function. To date, no reliable methods exist to determine which individuals with DME will or will not respond to available treatments. The implementation of predictive biomarkers would guarantee an efficient therapeutic selection to identify patients with a limited prognosis of visual recovery despite ongoing therapeutic actions, where early visual disability support instead of burdensome treatment schedules may be warranted. SD-OCT provides high-resolution imaging of the retinal structure and allows insight into the pathogenesis of DME *in vivo*. Central retinal thickness (CRT) measured with OCT is commonly used in the evaluation and management of DME. However, CRT only explains 27% of the variation in visual acuity [99]. Various other OCT measures have been studied, but none of these measures has been consistently demonstrated to account for visual outcomes in patients with DME, and most of these studies were conducted retrospectively in mixed treatment cohorts. Examples of these measures include the integrity of the ellipsoid zone (EZ) (formerly described as the inner segment/outer segment photoreceptor junction) [100, 101], the integrity of the external limiting membrane [101, 102], the visibility of the cone outer segment tips (COST) [103], as well as the presence of subretinal fluid [104] and hyperreflective foci [105, 106].

Furthermore, intraretinal cystoid fluid has been named as a predictor of poor response to anti-VEGF treatment in a prospective study [101], as well as in two post hoc analyses [107, 108] in large datasets of patients with DME using a machine-learning approach. Recently, disorganization of the retinal inner layers (DRIL) has been suggested to be a valid predictive biomarker for visual outcomes in patients with DME. DRIL was defined as the inability to distinguish boundaries between any two of the inner retinal layers (including the ganglion cell-inner plexiform layer (GCIPL) complex, the inner nuclear layer, and the outer plexiform layer) in >50% of the foveal 1-mm zone [103]. DRIL in the central millimeter is strongly associated with visual acuity in eyes with center-involving DME. Resolving DRIL seemed to be a good indicator of subsequent visual improvement [109]. In addition, the presence and extent of DRIL before treatment are correlated with BCVA outcomes to anti-VEGF therapy after the loading dose of ranibizumab in treatment naive patients with DME [101]. Similarly, patients with DME showed gain in visual acuity if DRIL resolved compared with non-resolvers, whose visual acuity worsened. This correlation between DRIL and visual acuity could not be substantiated for eyes with macular edema due to other causes [110]. Additionally, it is well known that approximately 55% of patients with DME have co-existent macular capillary non-perfusion [111], which may be masked angiographically by leakage from the edema. Macular capillary non-perfusion hinders efficient transport of oxygen and nutrients to the inner retinal layers, which in turn compromises inner retinal integrity and may therefore lead to the appearance of DRIL in OCT scans. This concept has been substantiated by a recent study reporting 84.4% sensitivity and 100% specificity of DRIL in detecting angiographic evidence of capillary non-perfusion in the macula [112].

The exact mechanisms of DRIL affecting VA have yet to be determined, but their correlation in eyes with DME is plausible as DRIL may represent an interruption in anatomic structures within these inner retinal layers including axons and nuclei of bipolar, amacrine, and/or horizontal cells, and therefore a disruption in the visual pathway from photoreceptors to retinal ganglion cells.

These data suggest that DRIL is a robust biomarker of visual acuity in eyes with present or resolved DME, correlating better with visual acuity than other OCT measures including CRT. Future multicenter longitudinal studies have to validate the predictive potential of DRIL by prospectively collecting data on the visual outcome of patients with DME, with additional studies to clarify the histologic equivalent accompanying the appearance of DRIL in SD-OCT [103].

5. Diabetic retinopathy as a neurodegenerative disease

5.1. The neurovascular unit

Fundoscopic clinical examination of patients with DR reveals pathognomonic features including hard exudates, hemorrhages, microaneurysms, and cotton wool spots. However, it does not reveal the complex organization of the neurosensory retina. Similar to other tissues throughout the central nervous system, neurons, glia, microglia, and blood vessels are organized into neurovascular units that work interdependently in close coordination in the retina [113].

The complex interconnections in the neurovascular unit prompted early anatomists to call this tissue the retina, literally a network of cells [114]. The capillary networks of the inner retina are in close contact with neurons of the inner nuclear and ganglion cell layer. These capillaries consist of a basal lamina with a single layer of adherent endothelial cells surrounded by pericytes, glial, and microglial cells on the external surface. Microglia interact directly with retinal pericytes and are intimately associated with retinal neurons [115].

This intimate physical contact and functional integration are essential for vision and facilitate physiologic adaptation in response to varying conditions. Neuronal activity evokes localized reactions including vasodilation and increased blood flow to meet the energy demands of neuronal signal transduction and transmission [114]. In addition to the coordination of metabolic demand, close signaling interdependence manifests itself in the blood-retinal barrier, which controls the flux of fluids and metabolites into the retinal tissue [116].

The diabetic environment causes the neurovascular unit to disintegrate both in early and late DR with the physiology of the neurovascular unit being similarly altered as it is in diseases of the brain such as stroke [117], Alzheimer's, and Parkinson's diseases [118]. Although DR has traditionally been considered merely a microvascular diabetic complication, recent studies support the concept that retinal neurodegeneration precedes and contributes to the formation of microvascular abnormalities in DR. These findings suggest that DR should at least be considered a combined neuro-vascular degeneration [113].

5.2. Retinal neurodegeneration

Signs of neurodegeneration were not visible in fundus examination in the era of the ETDRS. Therefore, these changes did not contribute to the characterization or diagnosis of the disease. However, retinal neurodegeneration has widely been accepted as part of DR over the last decades.

These abnormalities in retinal neural tissue lead to well-studied functional changes that typically precede the clinical diagnosis of DR, and in some cases occur even prior to the diagnosis of diabetes. Neurofunctional impairment becomes apparent as a dysfunction in dark adaption [119], abnormal contrast sensitivity [120], and altered microperimetry [105], as well as electroretinogram (ERG) results. The electroretinogram (ERG) is one of the most effective diagnostic tools in this context, with the oscillatory potential implicit time being the most consistent and widely reported aspect of the ERG that changes early in DR [121]. A delay in implicit time in multifocal ERG (mfERG) has been shown to be highly predictive (86% sensitivity and 84% specificity) of new retinopathy development at specific locations over 3 years in patients with early stages of DR at baseline [122, 123]. The European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) trial currently tests mfERG for its use and potential in DR prediction. However, while ERG is a very sensitive technique to detect neurofunctional deficits, it is also a quite burdensome and time-consuming examination.

Anatomical evaluation of retinal neurodegeneration has become possible with the implementation of SD-OCT. In OCT, the most useful measure for identifying diabetes-induced neurodegeneration is the thickness-reduction of the retinal nerve fiber layer (RNFL) and the ganglion cell complex, consisting of the ganglion cell layer (GCL) and the inner plexiform layer (IPL). Retinal ganglion cells (RGCs) are the retinal neurons in which the apoptotic process related to diabetes is first detected [124]. An impaired integrity of these cells compromises information processing and the transmission of visual signals to the brain. The damage primarily affects the RGC's nuclei and dendrites, as shown by a diffuse thinning of the combined retinal ganglion cell-inner plexiform layer (GCIPL). Secondarily, their axons are affected too, as indicated by a reduction of the retinal nerve fiber layer (RNFL) thickness [125]. A significant thinning of the GCIPL complex alone [126] or in combination with thinning of the RNFL has already been shown in patients with type 1 diabetes even without any fundoscopically manifest signs of DR [127, 128]. A longitudinal analysis in patients with type 1 diabetes depicted an average progressive thickness loss of 0.25 μm/year and 0.29 μm/year in the RNFL and the GCL + IPL, respectively, over a 4-year follow-up period in patients with no or minimal DR, independent of age, sex and even Hb1Ac. Intriguingly, the extent of thickness loss was similar to that of patients with severe glaucoma [129]. Research results are also consistent in finding reduced RNFL and GCIPL thicknesses in patients with type 2 diabetes [130–132].

Further, relation between structural signs of diabetic retinal neurodegeneration and functional deficits has been investigated thoroughly. Reduced GCIPL complex thickness has been shown to significantly correlate with impaired visual function assessed by contrast sensitivity and pattern ERG amplitudes in patients with diabetes without DR [131]. In patients with type 1 diabetes and no or minimal DR, GCL thickness was an important predictor of loss of macular visual function measured by the Rarebit perimetry [133].

Research has also started to focus on the temporal and causal relationship of neurogenic and vascular changes in DR. Preliminary results of the EUROCONDOR study suggest that in patients with no or mild DR, retinal vessel caliber is independently associated with structural changes of the neuroretina. Specifically, CRAE was statistically significantly associated with macular GCL thickness and CRVE with RNFL thickness at the optic disc [134]. An association of venular dilatation and thinning of the RNFL along with deficits in the ERG was detected in adolescents with type 2 diabetes, showing that the structural changes are accompanied by early vascular dysfunction [135].

The mechanisms behind this neurodegeneration are not completely clear. Increased apoptosis in neuronal tissue may be caused by chronic hyperglycemia, when neuronal cells experience up to 4-fold increase in glucose uptake. If hyperglycemia is prolonged, nerves are damaged [136]. Additionally, glucose and glutamate accumulation in the extracellular space, increased oxidative stress, inflammation and imbalance in the production of neuroprotective factors are other factors thought to be involved in the development of neurodegeneration in the setting of DR [137]. Apoptosis of the retinal ganglion cells also tends to be accompanied by reactive changes in macroglial cells, known as "reactive gliosis." Apart from astrocytes, the predominant type of macroglia is the Müller cell, which is unique to the retina. One of the most prominent characteristics of reactive gliosis is that Müller cells overexpress glial acidic fibrillary protein (GFAP), which is considered a sensitive indicator of central nervous system injury, and is normally only expressed by retinal astrocytes [138]. Müller cells span the entire retina, surround all blood vessels, and produce molecules that contribute to the modulation of blood flow and vascular permeability. In addition, they are essential for the survival of neurons. Therefore, glial cells, and especially Müller cells, are thought to play a key role in the pathogenesis of both retinal microangiopathy and neurodegeneration. Unfortunately, Müller cells can currently not be imaged *in vivo.*

Because neurons cannot be replaced, DR becomes irreversible with continuous disease progression. The identification of biomarkers that predict the development of neurodegeneration as well as mediators in the cross talk between neurodegeneration and microangiopathy is crucial for the development of new therapeutic strategies in DR. Safe and effective neuroprotective agents could possibly prevent neuronal apoptosis and vision loss but also impede the impairment of neurovascular coupling. Consequently, microvascular impairment and clinically apparent DR could be delayed. Evidence from the numerous studies mentioned above suggests that diabetic retinal neurodegeneration most likely precedes the microvasculopathy of DR. Functional examinations, like mfERG as well as structural evaluation of the inner retinal layers with SD-OCT may permit an early detection of the disease. However, further longitudinal studies are required to clarify the precise temporal relation between neurodegeneration and the microvascular alterations of DR.

5.3. Neurodegeneration outside the retina

Neurodegenerative changes occur outside the retina too. The cornea is one of the most densely innervated structures of the human body. A rich network of sensory nerves, known as the subbasal nerve plexus (SNP), derives from the ophthalmic division of the trigeminal nerve and lies between the corneal epithelium and Bowman's membrane [139]. This layer can be visualized with corneal confocal microscopy (CCM), a highly reproducible [140] in vivo imaging technique that provides diagnostic efficiency comparable to that of intra-epidermal nerve fiber density (IENFD) assessment [141, 142]. IENFD is the current gold standard for evaluating small nerve fiber damage, but is invasive, time-consuming and requires significant laboratory expertise. Evaluation of small fiber neuropathy is essential, as they constitute 70–90% of peripheral nerves and are preferentially involved in the development of diabetic peripheral neuropathy (DPN). DPN affects at least 50% of patients with diabetes mellitus and is the main initiating factor for foot ulceration and subsequent lower extremity amputation [143]. Unfortunately, to date, the guidelines for DPN mainly advocate electrophysiology besides clinical symptom testing, which is sensitive only for the detection of large fiber damage [144]. CCM could potentially serve as a non-invasive, objective biomarker for identifying small fiber damage and making an early diagnosis of DPN. The main changes in SNP morphology detected in patients with diabetes include a decrease in corneal nerve fiber density (CNFD), defined as the total number of major nerves per mm^2; corneal nerve fiber length (CNFL), defined as the total length of all nerve fibers and branches (mm/mm^2); and corneal nerve branch density (CNBD), defined as the number of branches emanating from major nerves per mm^2 [145]. Previous studies have evaluated the relationship between SNP morphology and the development and progression of DR. SNP impairment appears to progress in parallel with DR and could even be demonstrated in patients with diabetes without DR [146–149]. This finding would support the concept that besides neuronal loss in the retina, corneal neurodegeneration might precede the development of visible microangiopathy in DR too.

Even though recent studies indicate that inner retinal layer thinning representing retinal neurodegeneration is associated with DPN, the direct relation between SNP morphology and variables of retinal neurodegeneration has not yet been clarified. Eventually, CCM has the potential to be a surrogate for an early diagnosis of and an early biomarker for DR and DPN that could identify those at risk.

6. Conclusions

Diabetes mellitus is clearly a major health problem in an increasingly aging population worldwide. Diabetic retinopathy is a complex complication of this disease, which is influenced by a range of local and systemic factors. Potential non-invasive biomarkers derived from innovative imaging modalities as introduced above offer precious information about the morphologic as well as functional state of the diabetic retina, which is not detectable on routine clinical examination. These promising biomarkers may allow personalized medicine with treatment schedules tailored to patients' individual needs. Furthermore, as the population principally affected by DR comprises working-age individuals, understanding of the pathophysiology of the disease and developing appropriate therapy are essential to halt decrease in productivity and an increasing need for social support. Besides this significant economic benefit, the final validation of these biomarkers in prospective studies is expected to contribute decisively to the designing of clinical trials to identify new drug candidates that may prevent DR in the initial disease stages. Finally, and most importantly, this could result in a dramatic quality-of-life improvement for patients with diabetes and their families.

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References

[1] Herman WH. The global burden of diabetes: An overview. In: Dagogo-Jack S, editor. Diabetes Mellitus in Developing Countries and Underserved Communities. Cham: Springer International Publishing; 2017. pp. 1-5

[2] Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, et al. Retinopathy in diabetes. Diabetes Care. 2004;**27**(Suppl 1):S84-S87

[3] Steyerberg EW, Pencina MJ, Lingsma HF, Kattan MW, Vickers AJ, Van Calster B. Assessing the incremental value of diagnostic and prognostic markers: A review and illustration. European Journal of Clinical Investigation. 2012;**42**:216-228

[4] Ikram MK, Cheung CY, Lorenzi M, Klein R, Jones TLZ, Wong TY. Retinal vascular caliber as a biomarker for diabetes microvascular complications. Diabetes Care. 2013;**36**: 750-759

[5] Ting DSW, Tan GSW, Agrawal R, Yanagi Y, Sie NM, Wong CW, et al. Optical coherence tomographic angiography in type 2 diabetes and diabetic retinopathy. JAMA Ophthalmology. 2017;**135**:306-312

[6] Lachin JM, Genuth S, Cleary P, Davis MD, Nathan DM. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The New England Journal of Medicine. 2000;**342**:381-389

[7] Diabetes Control and Complications Trial Research Group. Progression of retinopathy with intensive versus conventional treatment in the Diabetes Control and Complications Trial. Ophthalmology. 1995;**102**:647-661

[8] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet (London, England). 1998;**352**: 837-853

[9] Sun JK, Keenan HA, Cavallerano JD, Asztalos BF, Schaefer EJ, Sell DR, et al. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: The joslin 50-year medalist study. Diabetes Care. 2011;**34**(4):968-974

[10] Hagan S, Martin E, Enriquez-de-Salamanca A. Tear fluid biomarkers in ocular and systemic disease: Potential use for predictive, preventive and personalised medicine. The EPMA Journal. 2016;**7**:15

[11] Klein R, Klein BEK, Moss SE, Wong TY, Hubbard L, Cruickshanks KJ, et al. The relation of retinal vessel caliber to the incidence and progression of diabetic retinopathy: XIX: The Wisconsin epidemiologic study of diabetic retinopathy. (Chicago, IL: 1960). Archives of Ophthalmology. 2004;**122**:76-83

[12] Hubbard LD, Brothers RJ, King WN, Clegg LX, Klein R, Cooper LS, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the atherosclerosis risk in communities study. Ophthalmology. 1999;**106**:2269-2280

[13] Tien YW, Islam FMA, Klein R, Klein BEK, Cotch MF, Castro C, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: The multi-ethnic study of atherosclerosis (MESA). Investigative Ophthalmology and Visual Science. 2006;**47**:2341-2350

[14] Klein R, Klein BEK, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes. XXI: The Wisconsin epidemiologic study of diabetic retinopathy. Ophthalmology. 2007;**114**:1884-1892

[15] Yang X, Deng Y, Gu H, Ren X, Lim A, Snellingen T, et al. Relationship of retinal vascular calibre and diabetic retinopathy in Chinese patients with type 2 diabetes mellitus: the Desheng Diabetic Eye Study. Br J Ophthalmol. 2016 Oct;**100**(10):1359-65. DOI: 10.1136/bjophthalmol-2014-306078. Epub 2016 Jan 28

[16] Dong Y, Lin L, Yan H, Fu Y, Zong Y, Yuan Y, et al. Shifts in retinal vessel diameter and oxygen saturation in Chinese type 2 diabetes mellitus patients. BMC Ophthalmology. 2016;**16**:43

[17] Bek T. Diameter Changes of Retinal Vessels in Diabetic Retinopathy. Curr Diab Rep. 2017 Aug 8;**17**(10):82. DOI: 10.1007/s11892-017-0909-9

[18] Hardarson SH, Harris A, Karlsson RA, Halldorsson GH, Kagemann L, Rechtman E, et al. Automatic retinal oximetry. Investigative Opthalmology and Visual Science. 2006;**47**:5011

[19] Kohner EM, Patel V, Rassam SM. Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. Diabetes. 1995;**44**:603-607

[20] Frederiksen CA, Jeppesen P, Knudsen ST, Poulsen PL, Mogensen CE, Bek T. The blood pressure-induced diameter response of retinal arterioles decreases with increasing diabetic maculopathy. Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. 2006;**244**:1255-1261

[21] Schmetterer L, Findl O, Fasching P, Ferber W, Strenn K, Breiteneder H, et al. Nitric oxide and ocular blood flow in patients with IDDM. Diabetes. 1997;**46**:653-658

[22] Michelson G, Patzelt A, Harazny J. Flickering light increases retinal blood flow. Retina (Philadelphia, PA). 2002;**22**:336-343

[23] Garhofer G, Zawinka C, Resch H, Kothy P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. The British Journal of Ophthalmology. 2004;**88**:887-891

[24] Pemp B, Garhofer G, Weigert G, Karl K, Resch H, Wolzt M, et al. Reduced retinal vessel response to flicker stimulation but not to exogenous nitric oxide in type 1 diabetes. Investigative Ophthalmology and Visual Science. 2009;**50**:4029-4032

[25] Lott MEJ, Slocomb JE, Shivkumar V, Smith B, Quillen D, Gabbay RA, et al. Impaired retinal vasodilator responses in prediabetes and type 2 diabetes. Acta Ophthalmologica. 2013;**91**:1-18

[26] Bursell SE, Clermont AC, Kinsley BT, Simonson DC, Aiello LM, Wolpert HA. Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. Investigative Ophthalmology and Visual Science. 1996;**37**:886-897

[27] Nagaoka T, Sato E, Takahashi A, Yokota H, Sogawa K, Yoshida A. Impaired retinal circulation in patients with type 2 diabetes mellitus: Retinal laser Doppler Velocimetry Study. Investigative Ophthalmology and Visual Science. 2010;**51**:6729-6734

[28] Patel V, Rassam S, Newsom R, Wiek J, Kohner E. Retinal blood flow in diabetic retinopathy. British Medical Journal (Clinical Research Edition). 1992;**305**:678-683

[29] Kohner EM, Hamilton AM, Saunders SJ, Sutcliffe BA, Bulpitt CJ. The retinal blood flow in diabetes. Diabetologia. 1975;**11**:27-33

[30] Grunwald JE, Riva CE, Sinclair SH, Brucker AJ, Petrig BL. Laser Doppler velocimetry study of retinal circulation in diabetes mellitus. Archives of Ophthalmology (Chicago, IL: 1960). 1986;**104**:991-996

[31] Takagi C, Bursell SE, Lin YW, Takagi H, Duh E, Jiang Z, et al. Regulation of retinal hemodynamics in diabetic rats by increased expression and action of endothelin-1. Investigative Ophthalmology and Visual Science. 1996;**37**:2504-2518

[32] Dahl-Jorgensen K. Diabetic microangiopathy. Acta Paediatrica (Oslo, Norway: 1992) Supplement. 1998;**425**:31-34

[33] Blair NP, Feke GT, Morales-Stoppello J, Riva CE, Goger DG, Collas G, et al. Prolongation of the retinal mean circulation time in diabetes. Archives of Ophthalmology (Chicago, IL: 1960). 1982;**100**:764-768

[34] Cuypers MH, Kasanardjo JS, Polak BC. Retinal blood flow changes in diabetic retinopathy measured with the Heidelberg scanning laser Doppler flowmeter. Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. 2000;**238**:935-941

[35] Goebel W, Lieb WE, Ho A, Sergott RC, Farhoumand R, Grehn F. Color Doppler imaging: A new technique to assess orbital blood flow in patients with diabetic retinopathy. Investigative Ophthalmology and Visual Science. 1995;**36**:864-870

[36] Pechauer AD, Hwang TS, Hagag AM, Liu L, Tan O, Zhang X, et al. Assessing total retinal blood flow in diabetic retinopathy using multiplane en face Doppler optical coherence tomography. Br J Ophthalmol. 2017 May 11. pii: bjophthalmol-2016-310042. DOI: 10.1136/bjophthalmol-2016-310042

[37] Grunwald JE, DuPont J, Riva CE. Retinal haemodynamics in patients with early diabetes mellitus. The British Journal of Ophthalmology. 1996;**80**:327-331

[38] Wang Y, Fawzi AA, Varma R, Sadun AA, Zhang X, Tan O, et al. Pilot study of optical coherence tomography measurement of retinal blood flow in retinal and optic nerve diseases. Investigative Ophthalmology and Visual Science. 2011;**52**:840-845

[39] PK Y, Balaratnasingam C, Cringle SJ, Mcallister IL, Provis J, Dao-Yi Y. Microstructure and network organization of the microvasculature in the human macula. Investigative Ophthalmology and Visual Science. 2010;**51**:6735-6743

[40] Campbell JP, Zhang M, Hwang TS, Bailey ST, Wilson DJ, Jia Y, et al. Detailed vascular anatomy of the human retina by projection-resolved optical coherence tomography angiography. Scientific Reports. 2017;**7**:42201

[41] Spaide RF, Klancnik JM, Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. JAMA Ophthalmology. 2015;**133**:45-50

[42] de Carlo TE, Romano A, Waheed NK, Duker JS. A review of optical coherence tomography angiography (OCTA). International Journal of Retina and Vitreous. 2015;**1**:5

[43] Kwiterovich KA, Maguire MG, Murphy RP, Schachat AP, Bressler NM, Bressler SB, et al. Frequency of adverse systemic reactions after fluorescein angiography. Results of a prospective study. Ophthalmology. 1991;**98**(7):1139-1142

[44] Sim DA, Keane PA, Rajendram R, Karampelas M, Selvam S, Powner MB, et al. Patterns of peripheral retinal and central macula ischemia in diabetic retinopathy as evaluated by ultra-widefield fluorescein angiography. American Journal of Ophthalmology. 2014 Jul;**158**(1):144-153.e1. DOI: 10.1016/j.ajo.2014.03.009. Epub 2014 Apr 4

[45] Sim DA, Keane PA, Zarranz-Ventura J, Bunce CV, Fruttiger M, Patel PJ, et al. Predictive factors for the progression of diabetic macular ischemia. American Journal of Ophthalmology. 2013;**156**:684-92.e1

[46] Tam J, Martin JA, Roorda A. Noninvasive visualization and analysis of parafoveal capillaries in humans. Investigative Ophthalmology and Visual Science. 2010;**51**:1691-1698

[47] Hwang TS, Gao SS, Liu L, Lauer AK, Bailey ST, Flaxel CJ, et al. Automated quantification of capillary nonperfusion using optical coherence tomography angiography in diabetic retinopathy. JAMA Ophthalmology. 2016;**97239**:1

[48] Conrath J, Giorgi R, Raccah D, Ridings B. Foveal avascular zone in diabetic retinopathy: Quantitative vs qualitative assessment. Eye (London, England). 2005;**19**:322-326

[49] Lin AD, Lee AY, Zhang Q, Rezaei KA, Kinyoun J, Wang RK, et al. Association between OCT-based microangiography perfusion indices and diabetic retinopathy severity. Br

J Ophthalmol. 2017 Jul;**101**(7):960-964. DOI: 10.1136/bjophthalmol-2016-309514. Epub 2016 Nov 15

[50] Dimitrova G, Chihara E, Takahashi H, Amano H, Okazaki K. Quantitative retinal optical coherence tomography angiography in patients with diabetes without diabetic retinopathy. Investigative Ophthalmology and Visual Science. 2017;**58**:190-196

[51] Al-Sheikh M, Akil H, Pfau M, Sadda SR. Swept-source OCT angiography imaging of the Foveal avascular zone and macular capillary network density in diabetic retinopathy. Investigative Opthalmology and Visual Science. 2016;**57**:3907

[52] Ishibazawa A, Nagaoka T, Takahashi A, Omae T, Tani T, Sogawa K, et al. Optical coherence tomography angiography in diabetic retinopathy: A prospective pilot study. American Journal of Ophthalmology. 2015;**160**:35-44.e1

[53] Durbin MK, An L, Shemonski ND, Soares M, Santos T, Lopes M, et al. Quantification of retinal microvascular density in optical coherence tomographic angiography images in diabetic retinopathy. JAMA Ophthalmology. 2017;**135**:370-376

[54] Couturier A, Bonnin S, Erginay aLI, Massin P, Gaudric A, Tadayoni R. Capillary Plexus Anomalies in Diabetic Retinopathy on Optical Coherence Tomography. Retina. 2015 Nov;**35**(11):2384-91. DOI: 10.1097/IAE.0000000000000859

[55] Choi W, Waheed NK, Moult EM, Adhi M, Lee B, De Carlo T, Jayaraman V, Baumal CR, Duker JS, Fujimoto JG. Ultrahigh speed swept source optical coherence tomography angiography of retinal and choriocapillaris alterations in diabetic patients with and without retinopathy. Retina. 2017 Jan;**37**(1):11-21. DOI: 10.1097/IAE.0000000000001250

[56] Agemy Sa, Scripsema NK, Shah CM, Gentile RC, Hsiao Y-s, Zhou Q. Retinal Vascular Perfusion Density Mapping Using Optical Coherence Tomography Angiography in Normals and Diabetic Retinopathy Patients. Retina. 2015 Nov;**35**(11):2353-63. DOI: 10.1097/IAE.0000000000000862

[57] Salz Da, de Carlo TE, Adhi M, Moult E, Choi W, Baumal CR, et al. Select features of diabetic retinopathy on swept-source optical coherence tomographic angiography compared with fluorescein angiography and normal eyes. JAMA Ophthalmology. 2016:1-7

[58] Akihiro Ishibazawa TM, Shimizu K, Young-Seok Song AY. Evaluation of microaneurysms in diabetic retinopathy using different types of optical coherence tomography angiography. The Association for Research in Vision and Ophthalmology Conference, Baltimore. 2017. Poster A0091abstract

[59] Scarinci F, Nesper PL, Fawzi AA. Deep retinal capillary nonperfusion is associated with photoreceptor disruption in diabetic macular ischemia. American Journal of Ophthalmology. 2016;**168**:129-138

[60] Mauricio J, Barros BD, Leonardo D, Isaac C, Avila M. Diabetic retinopathy and OCT angiography : clinical findings and future perspectives. International Journal of Retina and Vitreous. Int J Retina Vitreous. 2017 Mar 13;**3**:14. DOI: 10.1186/s40942-017-0062-2. eCollection 2017

[61] Geirsdottir A, Palsson O, Hardarson SH, Olafsdottir OB, Kristjansdottir JV, Stefansson E. Retinal vessel oxygen saturation in healthy individuals. Investigative Ophthalmology and Visual Science. 2012;**53**:5433-5442

[62] Yip WF, Siantar R, Perera SA, Milastuti N, Ho KK, Tan B, et al. Reliability and determinants of retinal vessel oximetry measurements in healthy eyes. Investigative Ophthalmology and Visual Science. 2014;**55**:7104-7110

[63] Turksever C, Orgul S, Todorova MG. Reproducibility of retinal oximetry measurements in healthy and diseased retinas. Acta Ophthalmologica. 2015;**93**:e439-e445

[64] Palsson O, Geirsdottir A, Hardarson SH, Olafsdottir OB, Kristjansdottir JV, Stefánsson E. Retinal oximetry images must be standardized: A methodological analysis. Investigative Ophthalmology and Visual Science. 2012;**53**:1729-1733

[65] Jani P, Mwanza J-C, Billow K, Waters A, Moyer S, Garg S. Normative values and predictors of retinal oxygen saturation. Retina. 2014;**34**:394-401

[66] Akil H, Falavarjani K, Sadda S, Sadun A. Optical coherence tomography angiography of the optic disc: An overview. Journal of Ophthalmic and Vision Research. 2017;**12**:98

[67] Guduru A, Martz TG, Waters A, Kshirsagar AV, Garg S. Oxygen saturation of retinal vessels in all stages of diabetic retinopathy and correlation to ultra-wide field fluorescein angiography. Investigative Ophthalmology and Visual Science. 2016;**57**:5278-5284

[68] Khoobehi B, Firn K, Thompson H, Reinoso M, Beach J. Retinal arterial and venous oxygen saturation is altered in diabetic patients. Investigative Ophthalmology and Visual Science. 2013;**54**:7103-7106

[69] Hardarson SH, Stefansson E. Retinal oxygen saturation is altered in diabetic retinopathy. British Journal of Ophthalmology. 2012;**96**:560-563

[70] Jørgensen CM, Hardarson SH, Bek T. The oxygen saturation in retinal vessels from diabetic patients depends on the severity and type of vision-threatening retinopathy. Acta Ophthalmologica. 2014;**92**:34-39

[71] Hardarson SH. Retinal oximetry. Acta Ophthalmologica. 2013;**91**:1-47

[72] COGAN DG, KUWABARA T. Capillary shunts in the pathogenesis of diabetic retinopathy. Diabetes. 1963;**12**:293-300

[73] Ashton N. Vascular basement membrane changes in diabetic retinopathy. Montgomery lecture, 1973. The British Journal of Ophthalmology. 1974;**58**:344-366

[74] Holekamp NM, Shui Y-B, Beebe D. Lower intraocular oxygen tension in diabetic patients: Possible contribution to decreased incidence of nuclear sclerotic cataract. American Journal of Ophthalmology. 2006;**141**:1027-1032

[75] Jørgensen CM. Lack of differences in the regional variation of oxygen saturation in larger retinal vessels in diabetic maculopathy and proliferative diabetic retinopathy. British Journal of Ophthalmology. 2016. No pagination

[76] Stefansson E, Machemer R, de Juan EJ, McCuen BWn, Peterson J. Retinal oxygenation and laser treatment in patients with diabetic retinopathy. American Journal of Ophthalmology. 1992;**113**:36-38

[77] Jørgensen C, Bek T. Increasing oxygen saturation in larger retinal vessels after photocoagulation for diabetic retinopathy. Investigative Ophthalmology and Visual Science. 2014;**55**:5365-5369

[78] Torp TL, Kawasaki R, Wong TY, Peto T, Grauslund J. Changes in retinal venular oxygen saturation predict activity of proliferative diabetic retinopathy 3 months after panretinal photocoagulation. Br J Ophthalmol. 2017 Aug 1. pii: bjophthalmol-2017-310576. DOI: 10.1136/bjophthalmol-2017-310576

[79] Shimizu K, Kobayashi Y, Muraoka K. Midperipheral fundus involvement in diabetic retinopathy. Ophthalmology. 1981;**88**:601-612

[80] Niki T, Muraoka K, Shimizu K. Distribution of capillary nonperfusion in early-stage diabetic retinopathy. Ophthalmology. 1984;**91**:1431-1439

[81] Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs – An extension of the modified Airlie House classification. ETDRS Report Number 10. Ophthalmology. 1991;**98**:786-806

[82] Early Treatment Diabetic Retinopathy Study Research Group. Treatment techniques and clinical guidelines for photocoagulation of diabetic macular edema. Early Treatment Diabetic Retinopathy Study Report Number 2. Ophthalmology. 1987;**94**:761-774

[83] Silva PS, Cavallerano JD, Sun JK, Soliman AZ, Aiello LM, Aiello LP. Peripheral lesions identified by mydriatic ultrawide field imaging: Distribution and potential impact on diabetic retinopathy severity. Ophthalmology. 2013;**120**:2587-2595

[84] Silva PS, Cavallerano JD, Haddad NMN, Kwak H, Dyer KH, Omar AF, et al. Peripheral lesions identified on ultrawide field imaging predict increased risk of diabetic retinopathy progression over 4 years. Ophthalmology. 2015;**122**:949-956

[85] Silva PS, Cavallerano JD, Tolls D, Omar A, Thakore K, Patel B, et al. Potential efficiency benefits of nonmydriatic ultrawide field retinal imaging in an ocular telehealth diabetic retinopathy program. Diabetes Care. 2014;**37**:50-55

[86] Price LD, Au S, Chong NV. Optomap ultrawide field imaging identifies additional retinal abnormalities in patients with diabetic retinopathy. Clinical Ophthalmology. 2015;**9**:527-531

[87] Silva PS, Cavallerano JD, Sun JK, Noble J, Aiello LM. Nonmydriatic ultrawide field retinal imaging compared with dilated standard 7-field 35-mm photography and retinal specialist examination for evaluation of diabetic retinopathy. Am J Ophthalmol. 2012 Sep;**154**(3):549-559.e2. DOI: 10.1016/j.ajo.2012.03.019. Epub 2012 May 23

[88] Silva PS, Cavallerano JD, Haddad NM, Tolls D, Thakore K, Patel B, et al. Comparison of nondiabetic retinal findings identified with nonmydriatic fundus photography vs ultrawide field imaging in an ocular telehealth program. JAMA Ophthalmology. 2016;**134**:330-334

[89] Purbrick RMJ, Izadi S, Gupta A, Victor Chong N. Comparison of optomap ultrawide-field imaging versus slit-lamp biomicroscopy for assessment of diabetic retinopathy in a real-life clinic. Clinical Ophthalmology. 2014;**8**:1413-1417

[90] Mackenzie PJ, Russell M, Ma PE, Isbister CM, Maberley DL. Sensitivity and specificity of the optos optomap for detecting peripheral retinal lesions. Retina (Philadelphia, PA). 2007;**27**:1119-1124

[91] Manjunath V, Papastavrou V, Steel DHW, Menon G, Taylor R, Peto T, et al. Wide-field imaging and OCT vs clinical evaluation of patients referred from diabetic retinopathy screening. Eye (London, England). 2015;**29**:416-423

[92] Nagiel A, Lalane RA, Sadda SR, Schwartz SD. Ultra-widefield fundus imaging: A review of clinical applications and future trends. Retina (Philadelphia, PA). 2016;**36**:660-678

[93] Wessel MM, Aaker GD, Parlitsis G, Cho M, D'Amico DJ, Kiss S. Ultra-wide-field angiography improves the detection and classification of diabetic retinopathy. Retina (Philadelphia, PA). 2012;**32**:785-791

[94] Silva PS, Dela Cruz AJ, Ledesma MG, van Hemert J, Radwan A, Cavallerano JD, et al. Diabetic retinopathy severity and peripheral lesions are associated with nonperfusion on Ultrawide field angiography. Ophthalmology 2015:1-8

[95] Sun JK, Aiello LP. The future of ultrawide field imaging for diabetic retinopathy pondering the retinal periphery. JAMA Ophthalmology. 2016;**134**:247-248

[96] Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. Ophthalmology. 1984;**91**:1464-1474

[97] Grant MB, Afzal A, Spoerri P, Pan H, Shaw LC, Mames RN. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opinion on Investigational Drugs. 2004;**13**:1275-1293

[98] Salam A, DaCosta J, Sivaprasad S. Anti-vascular endothelial growth factor agents for diabetic maculopathy. The British Journal of Ophthalmology. 2010;**94**:821-826

[99] Browning DJ, Glassman AR, Aiello LP, Beck RW, Brown DM, Fong DS, et al. Relationship between optical coherence tomography-measured central retinal thickness and visual acuity in diabetic macular edema. Ophthalmology. 2007;**114**:525-536

[100] Maheshwary AS, Oster SF, Yuson RMS, Cheng L, Mojana F, Freeman WR. The association between percent disruption of the photoreceptor inner segment-outer segment junction and visual acuity in diabetic macular edema. American Journal of Ophthalmology. 2010;**150**:63-7.e1

[101] Santos AR, Costa MÂ, Schwartz C, Alves D, Figueira J, Silva R, Cunha-Vaz JG. Optical coherence tomography baseline predictors for initial best-corrected visual acuity response to intravitreal anti-vascular endothelial growth factor treatment in eyes with diabetic macular edema: The chartres Study. Retina. 2017 May 2. DOI: 10.1097/IAE.0000000000001687

[102] Ito S-i, Miyamoto N, Ishida K, Kurimoto Y. Association between external limiting membrane status and visual acuity in diabetic macular oedema. The British Journal of Ophthalmology. 2013;**97**:228-232

[103] Sun JK, Lin MM, Lammer J, Prager S, Sarangi R, Silva PS, et al. Disorganization of the Retinal Inner Layers as a Predictor of Visual Acuity in Eyes With Center-Involved Diabetic Macular Edema. JAMA Ophthalmol. 2014 Nov;**132**(11):1309-16. DOI: 10.1001/jamaophthalmol.2014.2350

[104] Deak GG, Bolz M, Ritter M, Prager S, Benesch T, Schmidt-Erfurth U. A systematic correlation between morphology and functional alterations in diabetic macular edema. Investigative Ophthalmology and Visual Science. 2010;**51**:6710-6714

[105] Deak GG, Bolz M, Kriechbaum K, Prager S, Mylonas G, Scholda C, et al. Effect of retinal photocoagulation on intraretinal lipid exudates in diabetic macular edema documented by optical coherence tomography. Ophthalmology. 2010;**117**:773-779

[106] Bolz M, Schmidt-Erfurth U, Deak G, Mylonas G, Kriechbaum K, Scholda C. Optical coherence tomographic hyperreflective foci: A morphologic sign of lipid extravasation in diabetic macular edema. Ophthalmology. 2009;**116**:914-920

[107] Sophie R, Lu N, Campochiaro PA. Predictors of functional and anatomic outcomes in patients with diabetic macular edema treated with ranibizumab. Ophthalmology. 2015;**122**:1395-1401

[108] Gerendas BS, Prager S, Deak G, Simader C, Lammer J, Waldstein SM, et al. Predictive imaging biomarkers relevant for functional and anatomical outcomes during ranibizumab therapy of diabetic macular oedema. Br J Ophthalmol. 2017 Jul 19. pii: bjophthalmol-2017-310483. DOI: 10.1136/bjophthalmol-2017-310483

[109] Sun JK, Radwan SH, Soliman AZ, Lammer J, Lin MM, Prager SG, et al. Neural Retinal Disorganization as a Robust Marker of Visual Acuity in Current and Resolved Diabetic Macular Edema. Diabetes. 2015 Jul;**64**(7):2560-70. DOI: 10.2337/db14-0782. Epub 2015 Jan 29

[110] Radwan SH, Soliman AZ, Tokarev J, Zhang L, van Kuijk FJ, Koozekanani DD. Association of Disorganization of Retinal Inner Layers With Vision After Resolution of Center-Involved Diabetic Macular Edema. JAMA Ophthalmol. 2015 Jul;**133**(7):820-5. DOI: 10.1001/jamaophthalmol.2015.0972

[111] Sim DA, Keane PA, Zarranz-Ventura J, Fung S, Powner MB, Platteau E, et al. The effects of macular ischemia on visual acuity in diabetic retinopathy. Investigative Ophthalmology and Visual Science. 2013;**54**:2353-2360

[112] Nicholson L, Ramu J, Triantafyllopoulou I, Patrao NV, Comyn O, Hykin P, Sivaprasad S. Diagnostic accuracy of disorganization of the retinal inner layers in detecting macular capillary non-perfusion in diabetic retinopathy. Clin Exp Ophthalmol. 2015 Nov;**43**(8):735-41. DOI: 10.1111/ceo.12557. Epub 2015 Jun 24

[113] Gardner TW, Davila JR. The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. Graefe's Archive for Clinical and Experimental Ophthalmology. Graefes Arch Clin Exp Ophthalmol. 2017 Jan;**255**(1):1-6. DOI: 10.1007/s00417-016-3548-y. Epub 2016 Nov 10

[114] Stem MS, Gardner TW. Neurodegeneration in the pathogenesis of diabetic retinopathy: Molecular mechanisms and therapeutic implications. Current Medicinal Chemistry. 2013;**20**:3241-3250

[115] Klaassen I, Van Noorden CJF, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. Progress in Retinal and Eye Research. 2013;**34**:19-48

[116] Cunha-vaz J, Ribeiro L, Lobo C. Progress in retinal and eye research phenotypes and biomarkers of diabetic retinopathy. Progress in Retinal and Eye Research. 2014;**41**:90-111

[117] del Zoppo GJ. The neurovascular unit in the setting of stroke. Journal of Internal Medicine. 2010;**267**:156-171

[118] Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 2008;**57**:178-201

[119] Drasdo N, Chiti Z, Owens DR, North RV. Effect of darkness on inner retinal hypoxia in diabetes. Lancet (London, England). 2002;**359**:2251-2253

[120] Gualtieri M, Bandeira M, Hamer RD, Damico FM, Moura ALA, Ventura DF. Contrast sensitivity mediated by inferred magno- and parvocellular pathways in type 2 diabetics with and without nonproliferative retinopathy. Investigative Ophthalmology and Visual Science. 2011;**52**:1151-1155

[121] Tzekov R, Arden GB. The electroretinogram in diabetic retinopathy. Survey of Ophthalmology. 1999;**44**:53-60

[122] Han Y, Bearse MAJ, Schneck ME, Barez S, Jacobsen CH, Adams AJ. Multifocal electroretinogram delays predict sites of subsequent diabetic retinopathy. Investigative Ophthalmology and Visual Science. 2004;**45**:948-954

[123] Harrison WW, Bearse MAJ, Ng JS, Jewell NP, Barez S, Burger D, et al. Multifocal electroretinograms predict onset of diabetic retinopathy in adult patients with diabetes. Investigative Ophthalmology and Visual Science. 2011;**52**:772-777

[124] Kern TS, Barber AJ. Retinal ganglion cells in diabetes. The Journal of Physiology. 2008;**586**:4401-4408

[125] Barber AJ. A new view of diabetic retinopathy: A neurodegenerative disease of the eye. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2003;**27**:283-290

[126] Chen Y, Li J, Yan Y, Shen X. Diabetic macular morphology changes may occur in the early stage of diabetes. BMC Ophthalmology. 2016;**16**:12

[127] van Dijk HW, Verbraak FD, Kok PHB, Garvin MK, Sonka M, Lee K, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. Investigative Ophthalmology and Visual Science. 2010;**51**:3660-3665

[128] Araszkiewicz A, Zozulińska-Ziółkiewicz D, Meller M, Bernardczyk-Meller J, Piłaciński S, Rogowicz-Frontczak A, et al. Neurodegeneration of the retina in type 1 diabetic patients. Polskie Archiwum Medycyny Wewnetrznej. 2012;**122**:464-470

[129] Sohn EH, van Dijk HW, Jiao C, Kok PHB, Jeong W, Demirkaya N, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci U S A. 2016 May 10;**113**(19):E2655-64. DOI: 10.1073/pnas.1522014113. Epub 2016 Apr 25

[130] Verma A, Raman R, Vaitheeswaran K, Pal SS, Laxmi G, Gupta M, et al. Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy? Ophthalmic Research. 2011;**47**:202-207

[131] Zhu T, Ma J, Li Y, Zhang Z. Association between retinal neuronal degeneration and visual function impairment in type 2 diabetic patients without diabetic retinopathy. Science China Life Sciences. 2015;**58**:550-555

[132] Chhablani J, Sharma A, Goud A, Peguda HK, Rao HL, Begum VU, et al. Neurodegeneration in type 2 diabetes: Evidence from spectral-domain optical coherence tomography. Investigative Opthalmology and Visual Science. 2015;**56**:6333

[133] van Dijk HW, Verbraak FD, Stehouwer M, Kok PH, Garvin MK, Sonka M, DeVries JH, Schlingemann RO, Abràmoff MD. Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. Vision Res. 2011 Jan 28;**51**(2):224-8. DOI: 10.1016/j.visres.2010.08.024. Epub 2010 Aug 27

[134] Frydkjaer-Olsen U, Soegaard Hansen R, Simó R, Cunha-Vaz J, Peto T, Grauslund J; EUROCONDOR. Correlation between Retinal Vessel Calibre and Neurodegeneration in Patients with Type 2 Diabetes Mellitus in the European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR). Ophthalmic Res. 2016;**56**(1):10-6. DOI: 10.1159/000444396. Epub 2016 Apr 8

[135] Bearse MA, Adams AJ, Han Y, Schneck ME, Ng J, Bronson-Castain K, et al. A multifocal electroretinogram model predicting the development of diabetic retinopathy. Progress in Retinal and Eye Research. 2006;**25**:425-448

[136] Srinivasan S, Pritchard N, Sampson GP, Edwards K, Vagenas D, Russell AW, et al. Focal loss volume of ganglion cell complex in diabetic neuropathy. Clin Exp Optom. 2016 Nov;**99**(6):526-534. DOI: 10.1111/cxo.12379. Epub 2016 Mar 29

[137] Simó R, Sundstrom JM, Antonetti DA. Ocular anti-VEGF therapy for diabetic retinopathy: The role of VEGF in the pathogenesis of diabetic retinopathy. Diabetes Care. 2014;**37**:893-899

[138] Simó R, Hernández C; European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR). Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. Trends Endocrinol Metab. 2014 Jan;**25**(1):23-33. DOI: 10.1016/j.tem.2013.09.005. Epub 2013 Nov 1

[139] Ma A-A, Fares U, Suleman H, Lowe J, Dua HS. Architecture and distribution of human corneal nerves. The British Journal of Ophthalmology. 2010;**94**:784-789

[140] Hertz P, Bril V, Orszag A, Ahmed A, Ng E, Nwe P, et al. Reproducibility of in vivo corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. Diabetic Medicine. 2011;**28**:1253-1260

[141] Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, et al. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes. 2014;**63**:2454-2463

[142] Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, et al. Small nerve Fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: Comparing corneal confocal microscopy with Intraepidermal nerve Fiber density. Diabetes Care. 2015;**6**:1138-1144

[143] The global burden of diabetic foot disease. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. Lancet. 2005 Nov 12;**366**(9498):1719-24

[144] Kaku M, Vinik A, Simpson DM. Pathways in the diagnosis and management of diabetic polyneuropathy. Current Diabetes Reports. 2015;**15**:1-16

[145] Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, et al. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes Care. 2013;**36**:3646-3651

[146] Nitoda E, Kallinikos P, Pallikaris A, Moschandrea J, Amoiridis G, Ganotakis ES, et al. Correlation of diabetic retinopathy and corneal neuropathy using confocal microscopy. Current Eye Research. 2012;**37**:898-906

[147] Bitirgen G, Ozkagnici A, Malik RA, Kerimoglu H. Corneal nerve fibre damage precedes diabetic retinopathy in patients with type 2 diabetes mellitus. Diabetic Medicine. 2014;**31**:431-438

[148] Zhivov A, Winter K, Hovakimyan M, Peschel S, Harder V, Schober H-C, et al. Imaging and quantification of subbasal nerve plexus in healthy volunteers and diabetic patients with or without retinopathy. PLoS One. 2013;**8**:e52157

[149] Messmer EM, Schmid-Tannwald C, Zapp D, Kampik A. Vivo confocal microscopy of corneal small fiber damage in diabetes mellitus. Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. 2010;**248**:1307-1312