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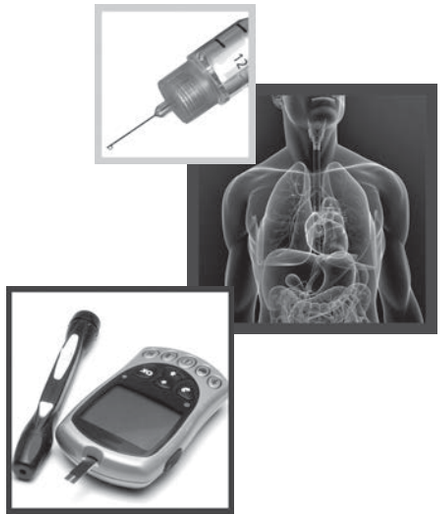

Biomarkers of diabetic retinopathy

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Practice Points

- Diabetic retinopathy (DR) should be seen as a retinal disease induced by chronic hyperglycemia corresponding to the nonproliferative DR stage, which in a few patients may progress to vision threatening complications, clinically significant macular edema and proliferative retinopathy.
- Several studies have provided evidence that good diabetic control is important to prevent disease progression, but whereas some patients develop vision-threatening diabetic complications despite good control, others escape the development of vision loss even with poor metabolic control.
- Characterization of different retinopathy phenotypes with different risks for vision-threatening DR complications opens the possibility of identifying genetic biomarkers that may help predict DR progression.
- Microaneurysm turnover has been validated in different retrospective and prospective studies and in studies from different centers as a prognostic biomarker of DR progression and the development of diabetic macular edema.
- Subclinical macular edema identified by spectral domain optical coherence tomography is also a candidate prognostic biomarker of development of diabetic clinically significant macular edema.
- Multifocal electroretinography detects early functional changes in the diabetic retina and is being tested in a large clinical trial as a potential prognostic biomarker of DR progression.
- Percentage central retinal thickness decrease from baseline measured by spectral domain optical coherence tomography appears, in a preliminary study, to be a predictive biomarker of the best corrected visual acuity response to anti-VEGF treatment in diabetic clinically significant macular edema.
- The availability of prognostic and predictive biomarkers of DR associated with generalized screening creates the conditions for preventive and personalized management of DR.

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SUMMARY Diabetic retinopathy (DR) progresses over time at different rates in different individuals, with only a limited number of patients developing significant vision loss owing to the two major vision-threatening complications of DR, clinically significant macular edema and proliferative retinopathy. Good metabolic control is important to prevent and delay disease progression, but whereas some patients develop vision-threatening DR complications and vision loss despite good control, others escape vision loss even with poor metabolic control. Microaneurysm turnover has been validated as a prognostic biomarker of development of clinically significant macular edema. Other good candidates for prognostic biomarkers of DR are subclinical macular edema identified by optical coherence tomography and multifocal electroretinography. Percentage retinal thickness decrease is also a good candidate for a predictive biomarker for visual acuity response to anti-VEGF therapy. Although other candidates for systemic biomarkers have been proposed, hemoglobin A1c remains the only confirmed systemic prognostic biomarkers of DR progression. The availability of prognostic and predictive biomarkers of DR associated with generalized screening creates the conditions for preventive and personalized management of DR.

Diabetic retinopathy (DR) is the leading cause of blindness among working-age adults in the US [1]. Vision loss related to DR is an important disability that threatens independence and can lead to depression, reduced mobility and reduced quality of life [2]. Its incidence is expected to increase significantly up to 2050 owing to the increase in the incidence of diabetes [3].

The Eye Diseases Prevalence Research Group classified DR into DR and vision-threatening DR, with DR defined as mild, moderate or severe DR; and vision-threatening DR defined as DR likely to result in vision loss in the absence of treatment (i.e., proliferative DR, clinically significant diabetic macular edema or both) [4].

This classification is important to address the issue of DR management in order to prevent vision loss and to identify which patients will progress to vision-threatening DR.

It is clear that systemic markers of diabetes, such as diabetes duration, poor glycemic control, increased blood pressure and lipid levels, are important factors, but they do not identify DR worsening [5], for example, some patients under good metabolic control may worsen more rapidly than some patients with poor metabolic control. These observations led to the identification of different phenotypes of DR progression based on specific changes in the retinal lesions [6].

In this review, we will focus on potential biomarkers of DR progression and therapeutic response that may be used in clinical practice.

It is, therefore, fundamental to identify the retinal lesions, their number and dynamics in the earlier stages of DR and correlate them with the progression of any stage of DR to vision-threatening DR [5].

Definitions of biomarkers

Biomarkers have become the basis for preventive medicine, meaning medicine that recognizes 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 personalized medicine, treatments individually tailored to specific patients for highly efficient intervention in disease processes.

It is necessary to distinguish between disease-related and drug-related biomarkers [7]. Prognostic disease-related biomarkers indicate the progression of disease with or without treatment, whereas predictive biomarkers of therapeutic response help to assess the most likely response to a particular treatment type [8].

Chronic diseases often begin with an early, symptom-free phase. In such symptom-free patients there may be more or less probability of actually developing symptoms. In these cases, biomarkers help to identify high-risk individuals reliably and in a timely manner so that they can either be treated before onset of the disease or as soon as possible thereafter.

Many new biomarkers are being developed that involve imaging technology. Indeed, imaging biomarkers have interesting advantages. They are usually noninvasive, and they produce intuitive, multidimensional results. Yielding both qualitative and quantitative data, they are usually relatively comfortable for patients. When combined with other sources of information, they can be very useful to clinicians seeking to make a diagnosis.

Progression of DR

The initial stages of nonproliferative DR are characterized by the presence of microaneurysms

(MA), small hemorrhages, and indirect signs of vascular hyperpermeability and capillary closure, that is, respectively, hard and soft exudates. These alterations dominate the fundus picture in the initial stages of DR and are the only ones used for characterization of levels 10–43 of the generally accepted Early Treatment of Diabetic Retinopathy Study (ETDRS) classification of DR.

An abnormality of the blood–retinal barrier, demonstrated both by vitreous fluorometry and fluorescein angiography, is an early finding both in human and experimental diabetes [9,10]. The alteration of the blood–retinal barrier is well demonstrated by fluorescein leakage and has been shown to be one of the earliest findings in diabetic retinal disease. It is accepted that it leads to development of retinal edema.

On ophthalmoscopic examination and fundus photography, the formation of MAs, small hemorrhages and hard exudates are the initial changes that are identified. MAs may be counted and MA counting has been suggested as an appropriate marker of retinopathy progression [11,12].

It must be realized that MA formation and disappearance are dynamic processes. During a 2-year follow-up study of 24 Type 1 diabetic patients with mild background DR, using fluorescein angiography, Helstedt and Immonen [13] observed 395 new MAs and disappearance of 258 previously identified MAs.

The disappearance of MAs indicates capillary closure and progressive vascular regression. Therefore, to assess progression of DR, MA counting needs to take into account every newly developed MA identified in a new location as well as the disappearing MAs. To establish progression it is crucial to be able to compare exams performed at regular intervals. Fundus photography appears to be the ideal examination to identify changes and retinal disease progression because of its simplicity and noninvasive nature.

To date, DR is clinically identified by changes produced through either progressive vasoregression or abnormalities of the blood–retinal barrier, limiting the diagnostic and therapeutic focus to the vascular system. However, it has been established that DR involves the neuroglial as well as the vascular compartments [14,15]. Attempts have been made to identify functional changes of the retina that may precede MAs, such as blood flow changes but the results have been contradictory mainly because of technical problems and lack of reliable methodology. High resolution imaging with spectral domain optical coherence tomography (OCT) and multifocal electroretinography (mfERG) are promising procedures that are offering new perspectives for the evaluation of the early stages of DR.

Validation of biomarkers of DR progression must involve demonstration that these biomarkers are associated with vision loss, the most generally accepted clinical outcome. This is a major problem as it is well recognized that vision loss only occurs when approximately 50% of the macula neuronal component is damaged [16]. Vision loss is clearly a late development in retinal disease and what we need is to identify outcomes that can be recognized before vision loss is present.

Vision loss is directly associated with the two major complications of DR, clinically significant macular edema and proliferative DR, and does not occur before these complications develop. This concept is crucial. The only clinically meaningful outcomes, at present, are clinically significant macular edema and proliferative DR. they are generally associated with advanced stages of the retinopathy. There is, therefore, clear need to identify biomarkers of diabetic retinal disease progression that predict development of the late accepted clinically significant outcomes associated with vision loss.

The fundus abnormalities seen in DR can conceptually be split into three categories – those findings resulting from leaking microvasculature (hemorrhages, lipid exudates, retinal edema; those findings resulting from structural damage to the microvasculature wall (MAs); and those findings resulting from ischemia with a subsequent overproduction of vascular growth factors (cotton-wool patches, intraretinal microvascular abnormalities, preretinal neovascularization, fibrous proliferation and vitreous hemorrhage) [17]. The severity of each of these findings has been classified and quantified based on the degree of retinal involvement. The Airlie House classification initially used only five photographic fields [18]. The Diabetic Retinopathy Study (DRS) created the modified Airlie House classification system and added more gradations of severity and used seven fields [19]. This classification was developed to classify DR progression to proliferative DR. Similarly, the ETDRS designed a classification to answer whether a strategy of earlier treatment with scatter photocoagulation to proliferative retinopathy was beneficial. Although complicated, the ETDRS

severity scale has become the *de facto* gold standard for grading retinopathy severity in clinical trials. Based on a patient's current ETDRS retinopathy level, one can predict the chance of developing high-risk proliferative retinopathy. However, a deficiency of the ETDRS classification becomes clear as it indicates that moderate nonproliferative DR (level 47) carries an 8.6% 1-year risk of developing high-risk proliferative retinopathy, the next step (level 53) carries a 45% of developing high-risk proliferative retinopathy. Therefore, a jump in a single numerical level of severity (47–53) results in a big jump in risk (8.6–45%) [20]. The Global Diabetic Retinopathy Project Group proposed another classification that was easier to use and included macular edema [21]. However, the final result did not translate into clinical practice, lost the advantages of the ETDRS classification to predict progression to proliferative retinopathy and the inclusion of macular edema was not helpful owing to the progressively increased availability of OCT, offering an objective evaluation of macular edema.

Prognostic disease-related biomarkers of DR

■ Systemic disease-related biomarkers

Serum disease-related biomarkers

The cornerstone of treating diabetes is to bring blood glucose levels to the lowest possible range without severe side effects.

In 1993, the Diabetes Control and Complications Trial demonstrated that intensive therapy lowered time-averaged blood glucose value (measured as hemoglobin A1c [HbA1c]) and significantly reduced development of microvascular complications in Type 1 diabetes. However, only 6.6% of the variation in risk of retinopathy was explained by the differences in treatment groups [22].

For Type 1 diabetes, despite the fact that glycemia is the major systemic risk factor for developing retinopathy, its overall contribution is only 11%, that is, 89% of the risk must be explained by other unknown factors [23]. Similarly, intensified glucose control has also been studied either alone or as a part of a multifactorial intervention in Type 2 diabetic patients. The first study to demonstrate an effect of glycemia on retinopathy progression was the UK Prospective Diabetes Study (UKPDS) [24]. This study showed that the overall effect of intensified treatment was modest, and that it took 6 years to see a difference between the conventional (mean HbA1c: 7.9%) and the intensive group (mean HbA1c: 7.0%).

In our clinical studies when considering the usual systemic parameters, such as lipid levels and blood pressure, only HbA1c values at baseline were consistently correlated with the development of the vision-threatening complications of diabetes such as clinically significant macular edema [25]. A number of observational and epidemiological studies have inconsistently reported an association between DR and elevated serum lipids [26]. Finally, reduction of blood pressure appears to be particularly beneficial for Type 2 diabetes but their effect has been attributed primarily in relation to the rates of development and progression of diabetic macular edema [27,28].

A variety of serum markers of inflammation and endothelial dysfunction have been proposed as biomarkers of DR. In a recent study, Muni *et al.* reanalyzed the Diabetes Control and Complications Trial cohort looking for increased levels of serum inflammatory markers and their correlation with DR progression [29]. After adjusting for known risk factors, increased levels of high-sensitivity C-reactive protein was the only serum marker tested that was associated with higher risk of incident clinically significant macular edema. Circulating levels of ICAM-1 were associated with the development of hard exudates.

Another recent publication looked at associations between C-reactive protein and von Willebrand factor with DR also in Type 1 diabetic patients, but their results were generally negative [30].

In their study they concluded that even though patients showed some degree of correlation between higher levels of high sensitive C-reactive protein and development of proliferative DR but this was not demonstrated in a multivariate analysis. No association was therefore found between von Willebrand factor and DR [30].

In summary, at present, the only validated systemic biomarker for the development of DR is HbA1c.

■ Genetic disease-related biomarkers

Several studies have provided evidence that good diabetes control is important to prevent progression of DR, but it is clear that some patients develop a rapidly progressing retinopathy despite good control, while others escape the development of severe retinopathy despite poor control.

The onset, intensity and progression of diabetic complications show large interindividual variations [31]. There is evidence from aggregation in families and specific ethnic groups, together with lack of serious complications in some diabetic patients with poor metabolic control that there is a genetic predisposition to develop some diabetic complications such as retinopathy [32].

Heritability has been estimated to be as high as 27% for DR and 52% for proliferative DR. Efforts to unravel the human genetics of DR have been undertaken using the candidate gene linkage approaches, and more recently, genome-wide association studies. A large number of putative genes and genetic variants have been reported in the literature and some of them exhibit consistent associations with DR (*ALR2*, *VEGF* and *RAGE* genes). However, these results have not been replicated in multiple populations, and, therefore, no genes have achieved widespread acceptance associated with high risk DR [33].

One of the major problems is associated with poor characterization of different retinopathy phenotypes. It is fundamental before embarking on a search for candidate genes to define clinical phenotypes characterized by specific patterns of severity and progression of DR. It is clear that it is necessary to first clearly identify the DR phenotypes that are associated with rapid progression of the retinopathy to severe forms of the disease, such as macular edema and proliferative retinopathy. Only then are studies on candidate genes worth pursuing, involving appropriately well-defined subgroups of patients [32]. This goal is now becoming possible with the results of our research group published for the first time in 2004 [29] and confirmed recently [34].

A case–control association study performed to identify genetic biomarkers that can predict DR progression in Type 2 diabetic patients showed particularly interesting results. A population of 307 Type 2 diabetic patients was classified in three different phenotypes of DR progression according to the findings of Nunes *et al.* [34]. Development of clinically significant macular edema was considered during a 2-year follow-up period of these patients. Eleven candidate genes were chosen based on literature searches using a knowledge-driven approach [35]. Single nucleotide polymorphisms described for these genes were filtered through bioinformatics tools and 177 were then genotyped in the 307 patients using the TaqMan Open Array Genotyping Platform. Statistically significant genotype distributions between the different DR phenotypes and the occurrence of clinically significant macular edema were found for single nucleotide polymorphisms in *AKR1B1*, *ICAM1*, *NOS1*, *NOS3*, *PPARGC1A* and *VEGFA*. Larger studies are now needed to further establish the set of genetic markers that are associated with different phenotypes of DR progression and may help predict development of vision-threatening DR complications.

■ Organ-specific disease-related biomarkers

MA turnover

MAs and hemorrhages identified as red dots are the initial changes seen on ophthalmoscopic examination. They may be counted on fundus photography and red dot counting has been suggested as an appropriate marker of retinopathy progression [36,37].

MA formation and their disappearance are dynamic processes.

The disappearance of a MA is not a reversible process and indicates vessel closure and progressive vascular damage. Meaningful MA counting must, therefore, consider every newly developed MAs identified in a new location but also the disappearing ones.

MA disappearance is most probably due to thrombotic phenomena leading to progressive remodeling of the retinal vasculature in diabetes [38]. The MA rates of formation and disappearance are, therefore, good candidates as markers of retinal vascular remodeling and may be good indicators of retinopathy progression.

MA counting on fundus photographies and MA counting on fluorescein angiography have been previously proposed as predictive indicators for progression of DR [39–41]. Recently developed software, RetmarkerDR (Critical Health SA, Coimbra, Portugal), allows for the identification of the exact location of each red dot in successive fundus photographs performed in each eye **(Figure 1)** [42]. Identification of the exact location of an individual red dot is considered particularly important because new MAs develop only once in a specific location, and their disappearance leaves mainly remnants of basement membrane in their place [10,43].

Our studies showed that, even in the early stages of DR, there is an active MA turnover. In fact, most MAs show a lifetime of less than 1 year, with rates of formation and disappearance varying between patients, confirming previous reports [44].

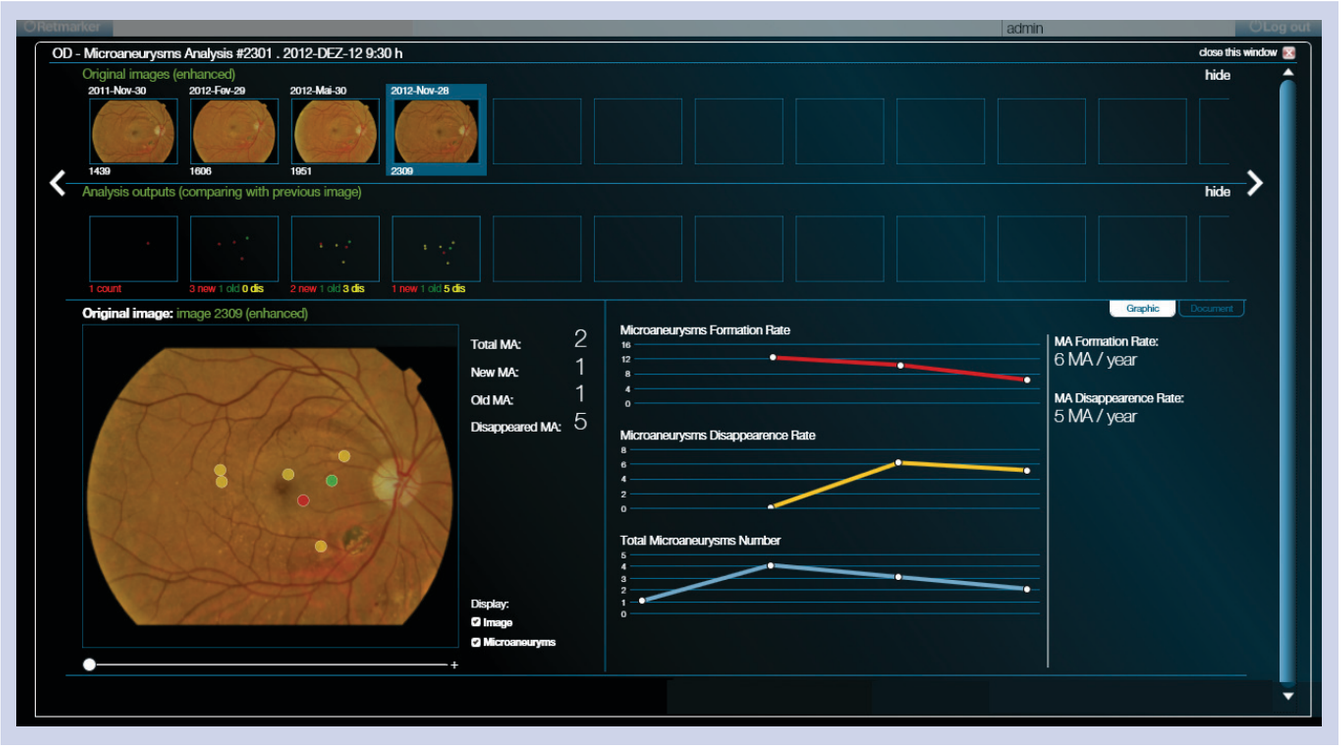

Figure 1. The RetmarkerDR software automatically calculates microaneurysm formation and disappearance rates and as a result, microaneurysm turnover.

Using the RetmarkerDR, we analyzed retrospectively 113 Type 2 diabetic patients with mild-to-moderate nonproliferative DR, followed up for 10 years (2 years as controls in DR clinical trials, and for the following 8 years by usual care) [45]. During this period, 17 out of the 113 patients (15.0%) developed clinically significant macular edema needing treatment.

A MA formation rate of at least 2 MAs/year was found in 12 out of the 17 eyes that developed clinically significant macular edema (70.6%), whereas this was only found in eight of the 96 eyes that did not develop clinically significant macular edema during the 10-year follow-up period (8.3%).

This study showed that in the initial stages of DR, higher MA turnover obtained from color fundus photography is a good indicator of retinopathy activity and development of clinically significant macular edema.

Our results have been confirmed by a different group in Munich that used also RetmarkerDR [46]. In their study, 160 eyes followed for 5 years were analyzed (CALDIRET study). Forty nine eyes developed clinically significant macular edema over the study period. An increased MA formation rate was confirmed to be associated with the development of clinically significant macular edema. A MA formation rate higher than 2 per year was present in 71.4% of the eyes that developed clinically significant macular edema, while eyes that did not develop clinically significant macular edema showed a MA formation rate lower than 2 per year (68.5% of the cases). This study confirmed our results in which 70.6% of the eyes that developed clinically significant macular edema showed a MA formation rate higher than 2.

More recently, we performed a prospective, observational study to follow-up eyes/patients with mild nonproliferative DR during a 2-year period [25].

Four hundred and ten patients with diagnosed adult-onset Type 2 diabetes, aged 40–75 years, with mild nonproliferative DR (levels 20 and 35 of ETDRS were included. The field 2 color fundus images were subjected to automated MA analysis using RetmarkerDR. RetmarkerDR allows for the computation of the MA turnover for each eye, that is, the sum of the MA formation and disappearance rates.

Of the 410 eyes/patients that entered the study, 348 were considered for analysis because they reached either the study end point, clinically significant macular edema, or performed the last visit (V24).

Of these 348 eyes/patients, 322 attended the last visit of follow-up without developing clinically significant macular edema, whereas 26 were diagnosed during the 2-year period of follow-up as having clinically significant macular edema.

MA turnover was 11.2 ± 11.2 in the 26 eyes/patients that developed clinically significant macular edema and 5.0 ± 5.2 in the remaining 322 eyes ($p < 0.001$). The MA turnover showed a high predictiveness for clinically significant macular edema with a receiving operating characteristics (ROC) area of 0.695 for a MA turnover cutoff of 9 or more, a sensitivity of 57.7% and a specificity of 81.2% was achieved (i.e., 79.4% of the eyes are correctly classified).

Eyes with a MA turnover higher than 9 during the initial 6 month period showed a higher risk for clinically significant macular edema development than eyes with a lower MA turnover (odds ratio: 5.886; 95% CI: 2.503–13.844).

The MA turnover predictive values for clinically significant macular edema development were as follows: for the period of 2 years of follow-up, the positive predictive value was 20%, and the negative predictive value was 96%, showing that a low MA turnover value predicts slow disease progression and indicates that development of clinically significant macular edema is unlikely.

Multivariate analysis also showed that MA turnover is predictive of clinically significant macular edema independently of the HbA1c values.

MA turnover has, therefore, been validated in retrospective and prospective studies performed in independent centers as a prognostic biomarker of DR progression and development of its most frequent vision-threatening DR complication, clinically significant macular edema.

Subclinical macular edema

The clinical evaluation of macular edema is characterized by its subjectivity. Direct and indirect ophthalmoscopy may reveal nothing but an alteration of the foveal reflexes. Slit lamp biomicroscopy demonstrate changes in retinal thickness in the macular area but it is dependent on the observer. Macular edema is now well identified using OCT. Measurement of retinal thickness by OCT is reliable and an increase in retinal thickness defines macular edema [47–51].

OCT provided new insights into morphological changes of the retina in DR and diabetic macular edema. It showed that macular edema may assume different morphologic patterns [52,53].

OCT images of diabetic macular edema depict the presence of low intraretinal reflectivity, due to fluid accumulation in the extracellular space of the retina. The process begins as increased thickening with a sponge-like appearance of the retinal layers, showing an increase in the extracellular spaces, later advancing to the typical image of cystoid spaces [54,55]. In addition, a quantitative characterization of macular edema became feasible, as determined by measurements of retinal thickness and volume. Our research group has now shown that it is possible to measure the blood–retinal barrier alterations with OCT, a noninvasive procedure without the need for the intravenous injection of fluorescein [56].

OCT has improved investigators' ability to follow macular edema changes, allowing for detection of increased retinal thickness, that is, edema, even before clinical detection by slit-lamp examination. The term subclinical diabetic macular edema has been proposed to describe these early stages of macular edema [51]. Nevertheless, there are few data in the literature regarding the natural history of eyes with subclinical macular edema. Browning *et al.* found progression to clinically significant macular edema in 48 out of 153 eyes with subclinical macular edema, over a median follow-up period of 14 months [57]. More recently, the Diabetic Retinopathy Clinical Research Network (DRCR-net) showed that one-quarter to one-half of these eyes will progress to clinically significant macular edema within 2 years of its identification [58]. Our group designed a prospective study to follow patients with Type 2 diabetes and mild nonproliferative DR, during 2 years, with repeated clinical and OCT examinations. Eyes with subclinical macular edema at baseline were identified and rate of progression to clinically significant macular edema investigated. We also analyzed the systemic and ocular features that may be associated with progression to clinically significant macular edema, using the definition of subclinical macular edema proposed by the DRCR.net (i.e., absence of edema involving the center of the fovea on slit-lamp examination, and a center point thickness measurement on Stratus OCT of ≥225 µm and <299 µm; **Figure 2**). Four hundred and ten eyes from 410 subjects with Type 2 diabetes and mild nonproliferative DR (ETDRS levels 20–35) were included in this prospective study and identified for subclinical macular edema. The study group comprised 259 men (63.2%)

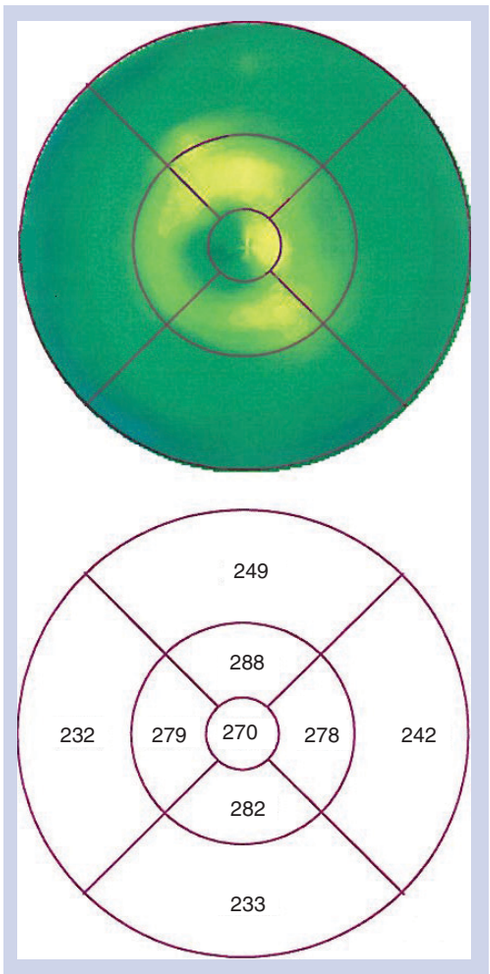

Figure 2. Optical coherence tomography identification of subclinical macular edema.

and 151 women (36.8%) with a mean (± standard deviation [SD]) age of 61.2 ± 8.3 years (range: 40–78). Mean (± SD) diabetes duration and mean (± SD) level of HbA1c were, respectively, 10.0 ± 5.0 years and 7.9 ± 1.5%. The study showed that eyes with subclinical macular edema at baseline have a 3.686-fold increased risk to progress to clinically significant macular edema within a period of 2 years after its detection. Two previous studies have reported the natural course of eyes with subclinical macular edema. In the DRCR.net study [58] an estimated proportion of 38% of the eyes with subclinical macular edema met primary study criteria by 2 years. Browning *et al.* [57] using a different definition of subclinical macular edema found a rate of conversion of 31.4% over a period of follow-up with a median of 14 years. These values are higher than the rate of 18.7% found by our group. These independent studies, however, confirm that subclinical macular edema in a diabetic patient is a good candidate for a prognostic biomarker for the development of clinically significant macular edema.

Implicit time delay in mfERG

Functional abnormalities of the retina and vision can occur before clinical signs of retinopathy appear in diabetes [15]. The psychophysical and 'conventional' electrophysiological studies of visual function in individuals in diabetes demonstrate that functional alterations may be present even in the absence of vascular changes assessed by retinal photography. The implication of these findings is that, in addition to retinopathy-associated vision loss, diabetes may induce changes in vision function that are not secondary to vascular damage.

The mfERG has been proposed as capable of detecting early functional changes, to provide an index of retinal status, and to predict not only which eyes but also which retinal locations will develop new retinopathy signs in the near future [59]. The implicit time in mfERG (elapsed time from the stimulus to P1 peak) is spatially associated with retinopathy, correlates with retinopathy severity and is a predictor for the development of retinopathy over a 1-year [60,61], and 3-year period [62]. In addition, implicit time has greater sensitivity and less intersubject variability than other mfERG functional parameters such as b-wave amplitude and oscillatory potentials also proposed as being altered in early stages of DR [59].

Implicit time abnormalities of mfERG were shown to be locally associated with retinopathy changes and related to severity of the retinopathy. In the areas with retinopathy changes, approximately 49% of the local mfERGs had abnormal implicit times, whereas only 20% of the local implicit times were abnormal in areas that did not have signs of retinopathy. Bearse *et al.* stated that abnormally delayed local mfERG implicit times often precede the appearance of retinopathy in corresponding local retinal patches [59]. Harrison *et al.* concluded, in a study authored by the same group, that together mfERG, systolic blood pressure and sex are good predictors of localized edema in patients with DR [63].

Furthermore, it should be noted that the mfERG P1 component is generated primarily by bipolar cells [64], which lie within the inner nuclear layer of the retina. Thus, the neurons

primarily involved in generating the index of retinal function that we are investigating lie in the same intraretinal location as the vascular cells that are implicated in DR lesions. For all these reasons this parameter stands out as a candidate biomarker of functional changes related to neurodegeneration in the eye of diabetic patients. An EU-funded multinational clinical trial, EUROCONDOR, is looking at the potential role of mfERG as a predictor of DR progression [15].

Predictive biomarkers of therapeutic response

■ Degree of decrease of OCT central retinal thickness

Anti-VEGF drugs have shown their efficacy in treating diabetic clinically significant macular edema. Approval of ranibizumab by the regulatory agencies to treat visual impairment caused by diabetic macular edema fulfills the previously unmet medical need for a treatment that can improve visual acuity in these patients. Many patients have shown an improvement of best corrected visual acuity (BCVA) with significantly superior benefit over standard-of-care photocoagulation in patients with visual impairment due to diabetic macular edema. These results are sustained and the intravitreal administration is generally well tolerated and with minimal ocular or systemic adverse events [65–67].

Although the effectiveness of intravitreal anti-VEGF has been well demonstrated, not every patient responds to treatment with BCVA improvement. It would be of major interest to identify a predictive biomarker of optimal or suboptimal response to treatment. A series of reports have suggested that different OCT morphological patterns are associated with different responses to treatment.

Our research group performed a retrospective study of 59 eyes with diabetic clinically significant macular edema treated with intravitreal injections of ranibizumab for a period of 6 months in a clinical practice setting [68]. Analysis of central retinal thickness values obtained with spectral domain OCT showed that the degree of central retinal thickness decrease correlated well with BCVA improvement. Twenty six eyes that showed a decrease of central retinal thickness >20% improved their BCVA by 10.3 ± 13.00 letters, whereas the other 33 eyes that responded to treatment with a central retinal thickness decrease <20% had a BCVA improvement of only 1.8 ± 7.2 letters ($p < 0.001$). In this study, the higher the percentage decrease in central retinal thickness from baseline the better the BCVA response.

Percentage central retinal thickness decrease from baseline measured by spectral domain OCT appears, therefore, to be a good candidate for a predictive biomarker of the BCVA response to intravitreal anti-VEGF treatment in diabetic clinically significant macular edema. These findings were registered in a clinical practice setting but need to be confirmed in a larger number of patients.

Conclusion

Studies such as the Diabetes Control and Complications Trial (DCCT), the UKPDS and the ETDRS validated methods now considered standard in treating DR when it occurs, that is, tight control of blood glucose levels to prevent retinopathy and laser photocoagulation to halt progression after development of clinically significant macular edema or proliferative retinopathy. However, despite the aim of tight blood glucose control and the use of retinal photocoagulation and new intravitreal drugs, blindness still occurs. Identification of different DR progression in different patients and response to therapies targeted at the earliest stages of retinal disease remain a priority for eye research. To achieve this goal it is urgent to identify biomarkers of disease progression and of therapeutic response. Candidates for biomarkers of DR are the object of this review **(Table 1)**.

MA turnover on fundus photographs, taking into account their exact, specific location in the eye fundus has the potential to become an extremely valuable biomarker of the overall progression of diabetic retinal vascular disease. MA turnover rate appears to be a direct indication of the progression of retinal vascular damage and activity of disease.

Subclinical macular edema measured by quantifying changes in retinal thickness with OCT is another promising biomarker of progression to clinically significant macular edema. The measurements are reliable, and changes in retinal thickness are a direct indication of macular edema and breakdown of the blood–retinal barrier. Another promising biomarker is mfERG. A large clinical trial now in progress will examine its use and potential. Testing these promising prognostic biomarkers and their final validation is expected to contribute decisively to the design of clinical trials evaluating the efficacy of new drugs capable to halt DR in the initial

Table 1. Candidates for biomarkers of diabetic retinopathy.

Biomarker	Type of biomarker	Subtype	Clinical outcome
Prognostic disease-related biomarkers	Systemic	Serum [24,25]	DR progression
		Genetic [35]	
	Organ specific	MA turnover [25,45,46]	CSME
		Subclinical macular edema [51,57,58]	
		mfERG [59,60]	
Predictive biomarkers of therapeutic response	Organ specific	Decrease of OCT central retinal thickness [68]	Anti-VEGF treatment of CSME

CSME: Clinically significant macular edema; DR: Diabetic retinopathy; MA: Microaneurysm; mfERG: Multifocal electroretinography; OCT: Optical coherence tomography.

stages of the disease. Other potential organ-specific biomarkers of disease progression such as hemodynamic changes [69,70] are still far away from clinical application, and longitudinal data is required.

Considering therapeutic response to DR vision-threatening complications, percentage central retinal thickness decrease measured by spectral domain OCT appears to be a predictive biomarker of the BCVA response to anti-VEGF treatment in diabetic macular edema.

Finally, HbA1c remains the only confirmed systemic prognostic biomarker of DR progression.

Future perspective

Biomarkers can be used to diagnose disease risk, presence of the disease in an individual or to tailor treatment for the disease in an individual.

Biomarkers have become the basis for preventive medicine, meaning medicine that recognizes risk of disease early and may open opportunities to prevent progression of the disease. They are the key to personalized treatments tailored to specific patients.

DR is a disease of the retinal neurovascular unit occurring in the well-recognized multifactorial environment of diabetes. It is a complex disease with individual characteristics in each patient depending on the predominant disease mechanism involved and the target cell of the neurovascular unit predominantly affected.

There is a wide range of retinal damage in diabetes and the progression of DR varies between different patients with different risks for development of vision-threatening complications.

An individual approach to management of DR based on prognostic and predictive biomarkers can be envisioned in the near future. Therefore, major developments in the personalized management of DR are expected in the next 5 years. These will involve generalized cost-effective screening, identification of the eyes that show disease activity and are at risk for progression to vision-threatening DR, and more timely treatment before development of irreversible vision loss.

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