Diabetic retinopathy

Optimizing management strategies

Vivian Schreur

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Chance favors only the prepared mind
- Louis Pasteur, 1854

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General introduction

1. BACKGROUND OF DIABETIC RETINOPATHY

A brief history of diabetic retinopathy

One of the earliest references to the profession of ophthalmologist dates back to the first Babylonian dynasty in the region of Mesopotamia. It was around 1800 BC that King Hammurabi issued a law code, inscribed on a slab of stone stele, known as the Code of Hammurabi. Law 196 of this code stated that a surgeon successfully completing an eye operation earns 10 shekels from a freeman – the equivalent of an annual salary –, 5 shekels from a poor man, and 2 shekels from a slave. However, in the unfortunate event that the operation resulted in the loss of an eye of a freeman, the hands of the surgeon were to be cut off.[1] Since antiquity, eye care has fortunately forged ahead, and although the rewards for successful eye surgery no longer amount to an annual salary, neither are the consequences of surgical complications as harsh as in ancient Babylonia.

One of the great turning points in the field of ophthalmology was the development of a direct ophthalmoscope by Von Helmholtz in 1851. His device enabled observation of the retina, using a flickering candle as source of illumination.[1] For the first time, retinal alterations as a consequence of diabetes, first speculated upon by the French ophthalmologist Bouchardot in 1846, could now be observed in the living patient.[2] The ophthalmoscope found prompt clinical application, and in 1856, Jäger was the first to observe and describe diabetic macular changes. In a scrupulous average of 20 clinical sessions per patient, he drew paintings of the retina to compose one of the earliest ophthalmological atlases. One of the chapters of this atlas, called "Entzündung der Netzhaut bei Diabetes Mellitus" (in proper English: inflammation of the retina in diabetes mellitus) contained a description of a patient with diabetes with retinal hemorrhages and yellow-orange deposits.[3] His findings were received with skepticism. Markedly, it was Albrecht Von Graefe, one of the most influential ophthalmologists at that time, who claimed that there was no causal relationship between diabetes and retinal abnormalities.[4]

Diabetic retinal changes remained largely unacknowledged until Nettleship provided histological proof of cystoid 'degeneration of the macula' in patients with diabetes in 1872.[5] A few years later, Nettleship and McKenzie described these retinal alterations in more detail, including a thickening of all retinal layers, thickening of the retinal arteries with loss of muscle-cells, and minute aneurysms on the capillaries.[6] Despite these reports on diabetes-associated retinal abnormalities, it was still unclear whether these changes originated directly as a result of diabetes, or as a consequence of other conditions that may occur simultaneously, such as cardiovascular disease, hypertension and/or albuminuria. This was an ongoing

discussion in the first half of the 20th century, until Ballentyne in 1943 provided clinical and pathological proof of a causal relationship between diabetes and retinal changes.[7]

Since the Second World War, rapid progress has been made on the pathophysiology and management of diabetic retinopathy. Without pretending to be complete, Figure 1 provides a timeline of the milestones of diabetic retinopathy research in the last two centuries. The work of these pioneers was pivotal in research of diabetic retinopathy and through their discoveries, developments in diabetic eye care have moved on apace. We owe it to them that we now live in a time where we have multiple diagnostic and therapeutic options for a disease that has grown out to be one of the most important causes of vision loss.

Clinical features

Like nephropathy and polyneuropathy, diabetic retinopathy is a microvascular complication of diabetes mellitus (DM), and is in most patients characterized by a combination of ischemic and exudative changes.[8] One of the earliest signs of diabetic retinopathy and a hallmark of early-stage disease are microaneurysms, protrusions of the retinal capillary wall.

Retinal abnormalities associated with ischemic changes due to microvascular occlusion, include dot-blot hemorrhages (small ruptures of vulnerable retinal structures, such as microaneurysms and dilated capillaries), cotton wool spots (nerve fiber layer edema caused by localized retinal infarctions), venous beading (focal dilated segments of retinal venules), and intraretinal microvascular abnormalities (IRMAs, abnormal branching or dilation of existing capillaries) (Figure 2A). Eventually, the presence of severe ischemia will stimulate the release of vasoproliferative factors, such as vascular endothelial growth factors (VEGFs), resulting in endothelial cell proliferation, known as neovascularization. At that time non-proliferative diabetic retinopathy progresses into proliferative diabetic retinopathy, an important distinction as the latter is associated with a much higher risk of permanent visual impairment. Sudden vision loss can occur when these fragile new vessels rupture and cause a vitreous hemorrhage. Alternatively, contracting fibrovascular tissue can cause retinal distortion, tractional macular edema or even a tractional retinal detachment, which may result in irreversible vision loss.[8, 9]

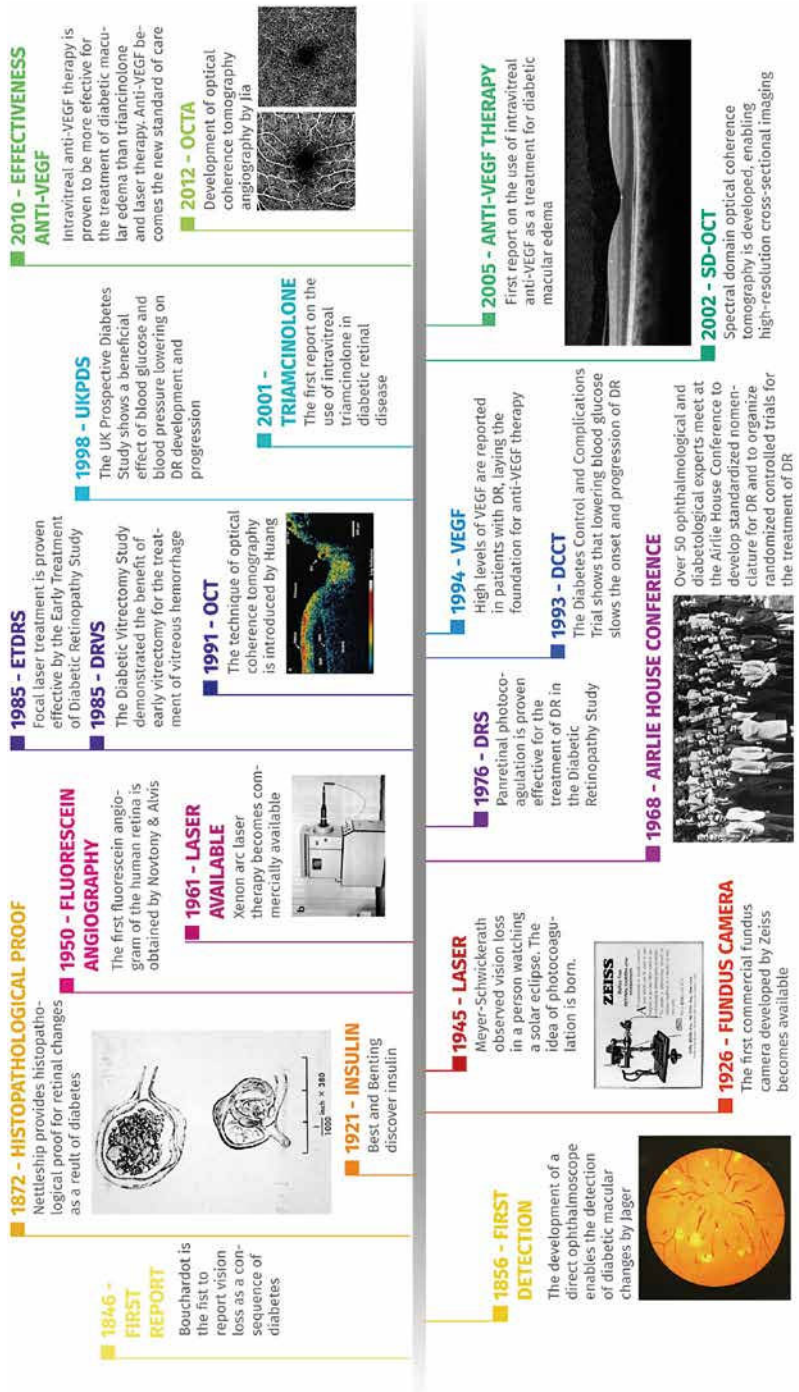


Figure 1 Historical timeline of major advances in diabetic eye care

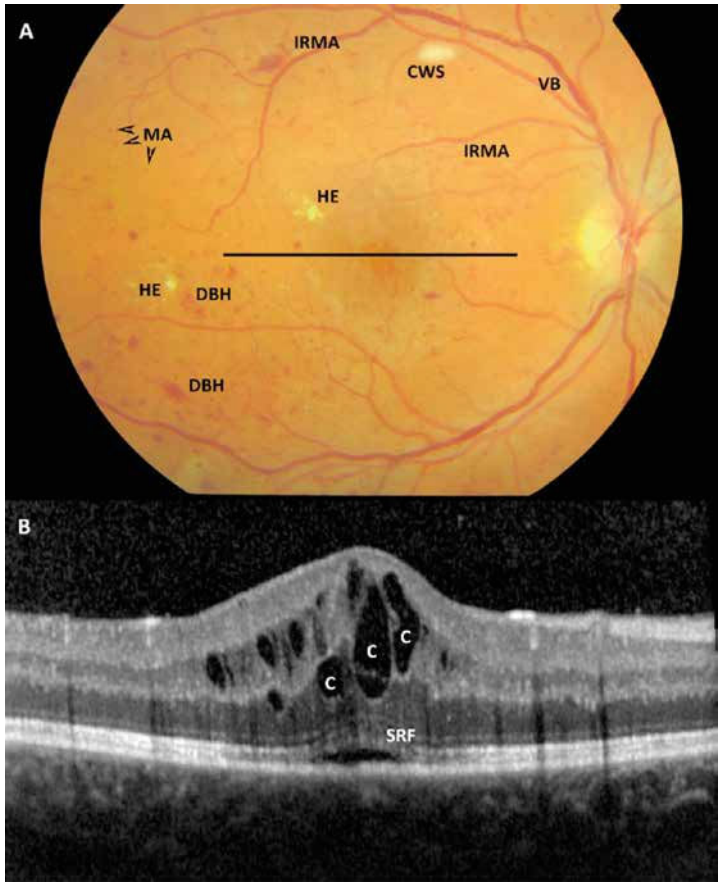


Figure 2 Clinical features of diabetic retinopathy. (**A**) Color fundus photograph showing intraretinal microvascular abnormality (IRMA), cotton wool spot (CWS), venous beading (VB), microaneurysms (MA), hard exudates (HE) and dot-blot hemorrhages (DBH). The horizontal line corresponds to the cross-sectional scan passing through the central fovea, shown on panel B. (**B**) Optical coherence tomography featuring multiple cysts (C) and subretinal fluid (SRF).

Exudative changes develop when the blood-retina barrier breaks down. Ophthalmoscopic findings in these cases include hard exudates (yellow-white lipoprotein deposits) and diabetic macular edema (DME) (Figure 2B). DME is an accumulation of fluid in the macula, the area in the eye with the highest turnover of nutrients. This is the area of the retina with the highest resolution, thus responsible for the visual acuity. Cysts and subretinal fluid are commonly detected, but a more diffuse form of retinal thickening can also be observed.[8, 9] Macular edema can develop

at all stages of diabetic retinopathy, and is the clinical feature that is most often associated with vision loss, especially in patients with type 2 DM.[10] Patients with DME experience a decline in visual acuity and/or metamorphopsia.

Pathogenesis

The pathogenesis of diabetic retinopathy is complex, involving numerous interrelated processes, and is to date not fully understood. The primary trigger seems to be chronic hyperglycemia, which sets off a chain of metabolic events. Multiple pathways, such as the polyol and hexosamine pathway, increased formation of advanced glycation end products (AGEs) and activation of protein kinase C, are found to induce the production of reactive oxygen species and upregulate various pro-inflammatory cells and cytokines.[9] The chronic presence of these substances stimulates the breakdown of the blood-retina barrier.[11] The blood-retina barrier acts as a physiological barrier that regulates the flow of proteins, ions and water flux into and out of the retina. It consists of an inner blood-retina barrier (the vascular endothelium) and an outer blood-retina barrier (the retinal pigment epithelium, (RPE)) (Figure 3).[12] In diabetic retinopathy, there are three important alterations in the blood-retina barrier: disruption of intercellular junctions, pericyte loss, and thickening of the basement membrane.[11]

Disruption of intercellular junctions: Endothelial cells form an adherent layer around the lumen and are connected through tight junctions and adhaerens junctions. Why these junctions are disturbed in diabetic retinopathy is not exactly known, but a decrease in occluding and vascular endothelial-cadherin levels are thought to play an important role in the loss of vascular integrity.[11] Thus allowing substances to freely flow across the blood-retina barrier, resulting in a disturbance of the retinal homeostasis.

Pericyte loss: The cells that are among the first to disappear are pericytes, which are hypothesized to have a contractile role similar to that of smooth muscle cells in larger vessels. Pericytes regulate the capillary blood flow and the loss of these cells causes microvascular dysfunction. Furthermore, microaneurysms originate at the weakened location in the capillary wall. In later stages of the disease, other retinal cells, such as endothelial cells are subject to apoptosis as well.

Thickening of the basement membrane: the basement membrane is a specialized type of extracellular matrix surrounding pericytes and endothelial cells. Besides providing structural support, the basement membrane acts as a filtration barrier and regulates cell proliferation and differentiation. Histological studies have repeatedly reported thickening of the basement membrane in diabetic retinopathy. Its role in the pathogenesis is, however, poorly understood.

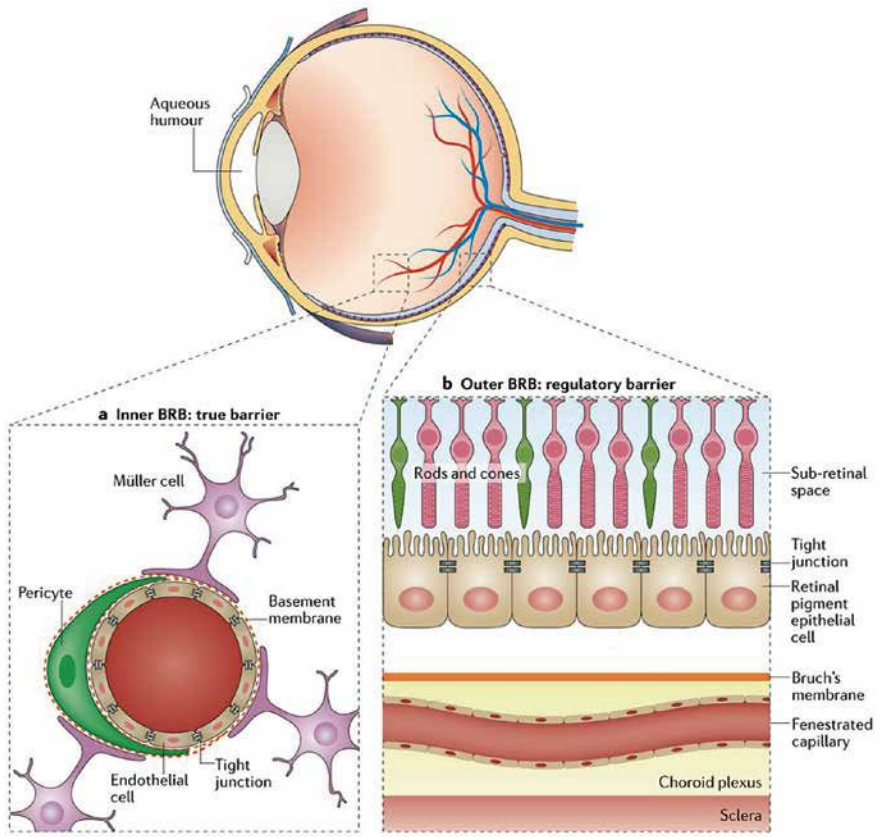


Figure 3 Schematic overview of the inner and outer blood-retina barriers. Adapted from Spadoni et al[13] with permission from Springer Nature.

The dysfunction of the retinal capillaries will eventually result in vascular occlusion and ischemia, which in turn stimulates the production of VEGFs and the growth of neovascularization. Conversely, breakdown of the blood-retina barrier leads to increased vascular permeability resulting in exudative changes, such as DME. The damage to the retinal microvasculature will in turn lead to low-grade inflammation of the retina, closing the vicious circle of the pathogenesis of diabetic retinopathy.

Some pathological alterations in diabetes cannot be visualized by ophthalmoscopy, as a growing body of evidence suggests that besides vascular changes, a neurodegenerative component is also present. The retina is a neurocircuit and in diabetic retinopathy, impairment of the retinal function has been demonstrated by abnormal

electroretinographic responses and delayed dark adaptation.[14, 15] Neurosensory alterations as a consequence of diabetes include biochemical defects, such as apoptosis of neural cells, impaired metabolism of the neurotransmitter glutamate, and reactive gliosis.[8] Activated retinal glial cells, including Müller cells, astrocytes and microglial cells, contribute to the inflammatory response through the production of cytokines and vascular endothelial growth factor VEGFs.[16, 17]

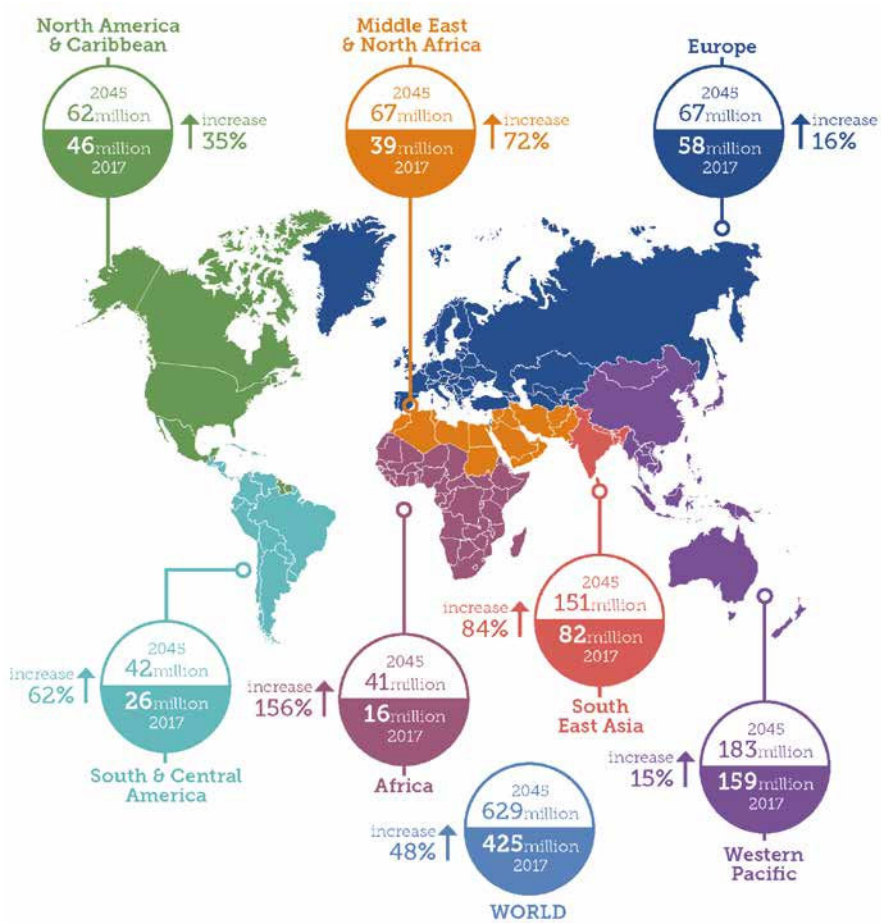


Figure 4 Number of people with diabetes worldwide and per region in 2017 and 20145 (20-79 years). Reprinted from the Diabetes Atlas, eighth edition 2017, by the International Diabetes Federation.[18]

Epidemiology

The number of people suffering from DM has increased rapidly over the past decades and has currently reached epidemic proportions. Worldwide, an estimated 425 million people were living with DM in 2017, and this number is expected to rise to 629 million by 2045 (Figure 4).[18] Type 2 DM (based on insulin resistance) makes up for about 90% of these people, while the majority of the other 10% suffers from Type 1 DM (based on insulin insufficiency). The diabetes epidemic can be largely attributed to increased longevity and lifestyle factors resulting in overweight, such as poor dietary habits and physical inactivity.

Diabetic retinopathy is present in about a third of all people with DM. A third of this group suffers from vision-threatening diabetic retinopathy.[19, 20] In 2015, moderate or severe vision loss was present in an estimated 0.6% of all patients with DM, which may seem relatively low, but still accounts for a total of 2.6 million people.[21] Although the absolute number of people suffering from vision loss due to diabetic retinopathy is expected to rise due to the diabetes epidemic, the incidence of vision loss as a consequence of diabetic retinopathy is currently on a decline, particularly in developed countries.[22-25] This achievement reflects considerable improvements in diabetes care, such as improved devices to measure blood-glucose levels and to administer insulin, increased awareness of the need for intensive glucose and blood pressure control, the introduction of (free) screening programs, and the expansion of the therapeutic arsenal.[8, 26, 27] In low to middle income countries, where four out of five people with DM are living, these improvements remain to be implemented, and this will be a major challenge for the oncoming years.[27]

The sharp increase in the prevalence of diabetes is obviously accompanied by an increase in diabetic retinopathy-related healthcare costs. Besides directly related costs, such as the costs of outpatient visits, image acquisition and treatment, there are indirect costs. These are difficult to calculate but can have a large impact on society. Examples are costs related to sick leave, disability and early retirement. Several studies addressed the economic burden of diabetic retinopathy, but the estimations vary considerably between countries, depending on the healthcare organization and resource availability. As determined in German, Canadian and Swedish populations, the annual costs for patients with no or mild diabetic retinopathy amount €26 per patient (which seems rather little in our experience), and may rise up to €257-468 for a patient with proliferative disease, and €216-681 for a patient with maculopathy.[28-30] Most likely, these calculations vastly underestimate the financial burden of DME in current clinical practice, because they were conducted before the widespread implementation of anti-VEGF therapy. Therapy of diabetic retinopathy is not only chronic but also has become much

more expensive (anti-VEGFs versus laser) over the last decade. In a more recent Spanish study, the annual costs for patients with diabetic macular edema treated by anti-VEGFs were found to be a ten-fold of those for patients treated by focal laser.[31]

Risk factors

Diabetic retinopathy is a multifactorial disease, meaning that multiple risk factors are involved. The most important modifiable risk factors of the development and progression of diabetic retinopathy are hyperglycemia and hypertension, and to a lesser extent dyslipidemia and obesity. Non-modifiable risk factors include DM duration, puberty, pregnancy, and heritability.[9] For diabetic retinopathy progression, the presence of diabetic retinopathy is a major risk factor.[32] Knowledge of the risk factors for diabetic retinopathy is of essential importance to identify patients at high risk of developing vision loss.

The landmark Diabetes Control and Complications Trial (DCCT) showed that with intensive insulin treatment, the development of diabetic retinopathy could be reduced by an astounding 76%, and the progression of the retinopathy slowed by 54% in patients with type 1 diabetes, compared with conventional therapy.[33] It is important to realize that this reduction was accomplished against a group of well-regulated diabetes patients. The reduction would even be higher when compared to a group were diabetes control was lacking. The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated the benefit of tight blood glucose control also for patients with type 2 diabetes.[34] This study also addressed the effect of blood pressure on diabetic retinopathy, reporting that the incidence was strongly associated with higher blood pressure. Patients assigned to tight blood pressure control had a 34% reduction in progression of retinopathy, and a 47% reduced risk of visual acuity loss by three lines or more of the early treatment of diabetic retinopathy study (ETDRS) chart.[35, 36] A relationship between dyslipidemia and diabetic retinopathy, especially with exudative abnormalities, such as hard exudates and macular edema, has repeatedly been reported,[37, 38] although this does not hold true in all studies.[39] Research on the influence of body mass index (BMI) on diabetic retinopathy has also yielded conflicting results.[40]

DM duration is one of the strongest predictors for the development and progression of retinopathy. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that the prevalence was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years DM duration in patients with early-onset DM.[41] In addition, the WESDR provided evidence for puberty as a risk factor for diabetic retinopathy, demonstrating that in postmenarchal females, the risk of retinopathy was increased by 30% when compared to premenarchal females.[42] Pregnancy was

also identified as an independent risk factor for worsening of diabetic retinopathy, requiring temporary intensified ophthalmologic surveillance.[43, 44] Furthermore, genetic risk factors are hypothesized to play an important role, Heritability rates are estimated to be as high as 27% for diabetic retinopathy in general, and 52% for the proliferative variant.[45, 46] To date, however, attempts to identify risk alleles through candidate gene association studies and whole genome sequencing studies have had limited success, and results could not be replicated in different cohorts.[47]

Despite our current knowledge on the risk factors of diabetic retinopathy, there is substantial variation in development and progression of diabetic retinopathy among patients with similar clinical characteristics at baseline.[19, 48] While some patients will develop retinopathy regardless of excellent control of metabolic risk factors, some poorly controlled patients somehow escape loss of vision. This suggests that other, unknown risk factors are involved. This is further substantiated by the fact that in type 1 DM patients with very long duration of diabetes (>50 years), 43% was found to remain free from proliferative diabetic retinopathy.[49] Even more interesting, in these patients glycemic control, generally recognized as the most important risk factor, did not significantly influence the development of microvascular complications.[49, 50] To be able to distinguish patients at low risk from those at high risk, further research should be directed towards the identification of new risk factors and protective factors. These risk factors may be searched for in different areas, including, serum measurements, imaging characteristics, and (epi)genetic markers.

2. DIAGNOSTIC OPTIONS

Ophthalmoscopy and slit lamp biomicroscopy have long been the standard screening method for diabetic retinopathy and are generally performed by an ophthalmologist or trained optometrist after pupillary dilation. Despite advances in imaging modalities, this remains an important diagnostic tool in the evaluation of diabetic retinopathy, especially in countries with limited resource availability.[51]

Color fundus photography (CFP) is employed in ophthalmological clinical practice since 1926, when the Carl Zeiss Company produced the first commercial fundus camera, and is now a cornerstone in detection and monitoring of diabetic retinopathy.[2] Standard seven-field color fundus photography according to the ETDRS protocol can be used to detect retinal changes, such as microaneurysms, hemorrhages, hard exudates, cotton wool spots, venous beading, and IRMA. At the Airlie House Conference in 1968, a group of experts developed a standardized

classification of diabetic retinopathy, and this gold standard for diabetic retinopathy has been used for grading for many years.[52] In 2003, a practical and simplified version of the severity scale was developed for daily clinical practice (Table 1).[53]

Widefield photography (WFP) offers the possibility to image a larger area than conventional CFP in a single image. This may allow for better visualization of peripheral changes, but this technology remains to be further investigated before it can be fully employed for screening purposes.[54]

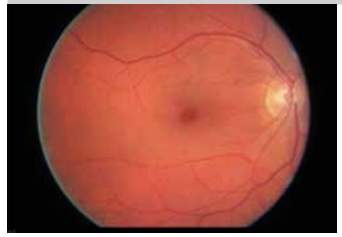
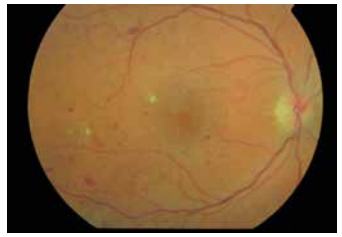
Fluorescein angiography (FA) uses the contrast agent fluorescein to visualize the retinal circulation, and was first performed in human by Novotny & Alvis in 1961.[55] In diabetic retinal disease, FA allows detection of ischemic areas, requiring laser treatment, and can aid in the distinction between neovascularization and IRMA (Figure 5). In addition, macular edema and sources of leakage can be detected, such as microaneurysms, therefore guiding in focal laser treatment or anti-VEGF therapy.[56, 57]

Optical coherence tomography (OCT) is a technique based on interferometry that non-invasively captures cross-section images of the retina. It was introduced by Huang et al. in 1991, became commercially available in 1996, and has since found widespread application in detection and monitoring of DME.[58] The development of high-resolution spectral domain OCT in 2002 enables even more detailed evaluation of retinal morphology.[59] Retinal thickness, most commonly quantified in the central 1 mm around the fovea, has become an important biomarker for treatment indication and response.[60] Retinal changes such as cysts, subretinal fluid, hard exudates and retinal layer disruption can be readily distinguished on OCT (Figure 6).[61]

Optical coherence tomography angiography (OCTA) uses signals of multiple OCT-scans to construct three-dimensional images of the retinal blood flow, based on motion contrast. The development of this technique by Jia in 2012 is one of the most recent advances in the field ophthalmological imaging.[62] Although many abnormalities can be appreciated in patients with diabetic retinopathy, such as microaneurysms and areas of nonperfusion (Figure 7),[63] its routine application in diabetic eye care remains to be investigated.

Despite the large number of diagnostic options, it is unlikely, and more importantly not necessary, that a diabetic patient will undergo all these examinations during a single evaluation of his or her retinal condition. The essential information that is needed for clinical decision making can often be derived from a smaller number of

Table 1 International clinical diabetic retinopathy severity scale according to Wilkinson et al[53]

	Severity level	Findings on ophthalmoscopy/color fundus photography
	No apparent retinopathy	No abnormalities
	Mild non-proliferative diabetic retinopathy	Microaneurysms only
	Moderate non-proliferative diabetic retinopathy	More than just microaneurysms, but less than severe non-proliferative diabetic retinopathy
	Severe non-proliferative diabetic retinopathy	Any of the following, also known as the 4-2-1 rule (and no signs of proliferative diabetic retinopathy): - More than 20 hemorrhages in each of 4 quadrants - Definite venous beading in 2+ quadrant - Prominent IRMA in 1+ quadrant
	Proliferative diabetic retinopathy	One or more of the following: - Neovascularization - Vitreous/preretinal hemorrhage

imaging modalities. The healthcare costs are already staggering and clinicians have an obligation make effective means of available resources. This also applies to a research setting, where not all imaging modalities are required to evaluate DR status. However, the use of a standardized set of imaging techniques and a uniform grading protocol is key for replicating results. In case of, for example, epidemiologic and genetic studies into diabetic retinopathy, replication and comparison of results is critical to obtain a larger body of evidence. The lack of phenotype standardization of diabetic retinal disease is currently a large obstacle for these studies. Future work should therefore address the use of a minimal set of imaging requirements informative enough to establish a patient's phenotype, in an affordable and timely fashion.

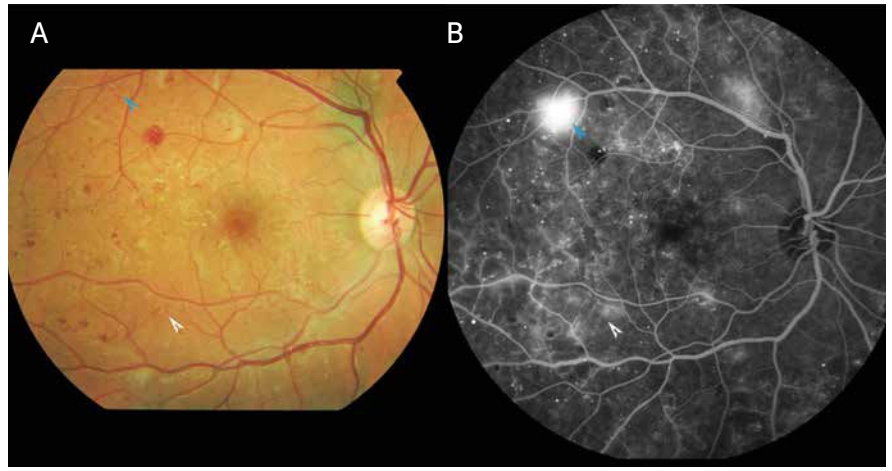


Figure 5 A patient with proliferative diabetic retinopathy visible on (**A**) color fundus photography and (**B**) fluorescein angiography (right). Microaneurysms a represented by small hyperfluorescent dots, and ischemia translates as hypofluorescent areas on fluorescein angiography. Blue arrowheads indicate a retinal neovascularization that is more readily visible in fluorescein angiography as an enlarging hyperfluorescence spot caused by leakage. White arrowheads indicate an intraretinal microvascular abnormality, that cannot be distinguished from neovascularization on color fundus photography, but can be differentiated from frank neovascularization on fluorescein angiography because it does not leak.

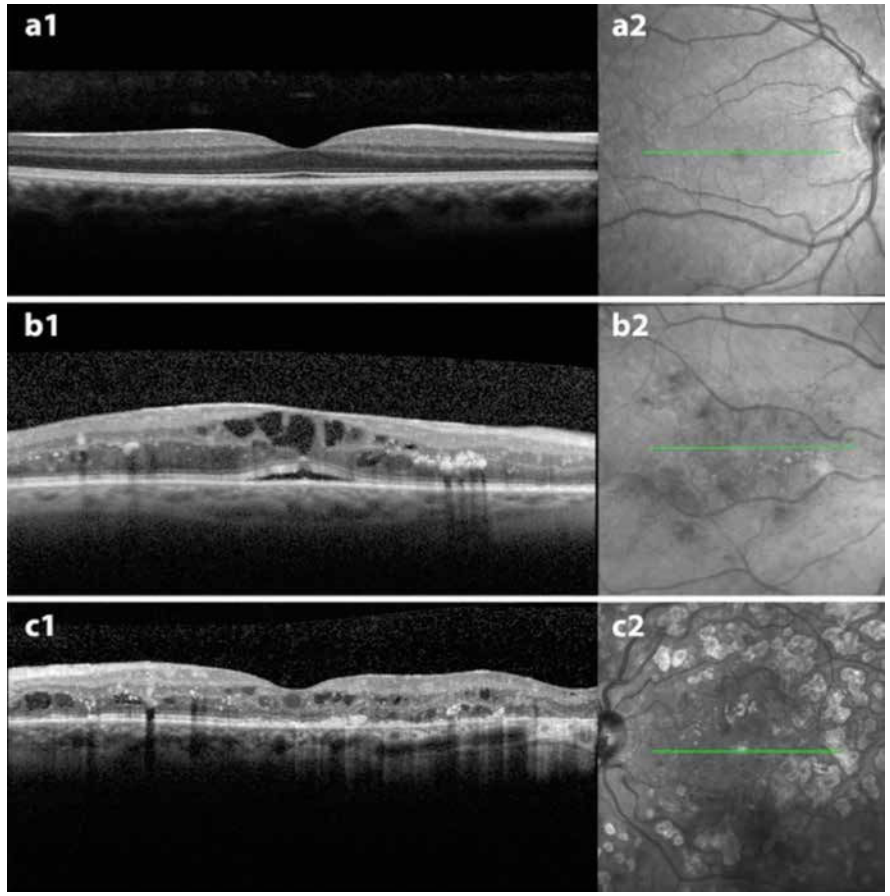


Figure 6 Optical coherence tomography (**a1**-**c1**) with the corresponding infrared image (**a2**-**c2**) of: a healthy person (**a1**, **a2**); a person with cystoid diabetic macular edema, with concurrent subretinal fluid, and hard exudates, shown as hyperreflective material in the outer retinal layers (**b1**, **b2**); a person with long-standing diabetic macular edema as can be recognized by the reflectivity of the cystic fluid, disorganization of the retinal inner layers, and disruption of the external limiting membrane and the photoreceptor layer (**c1**, **c2**). Green line indicates the cross-section of the displayed B-scan.

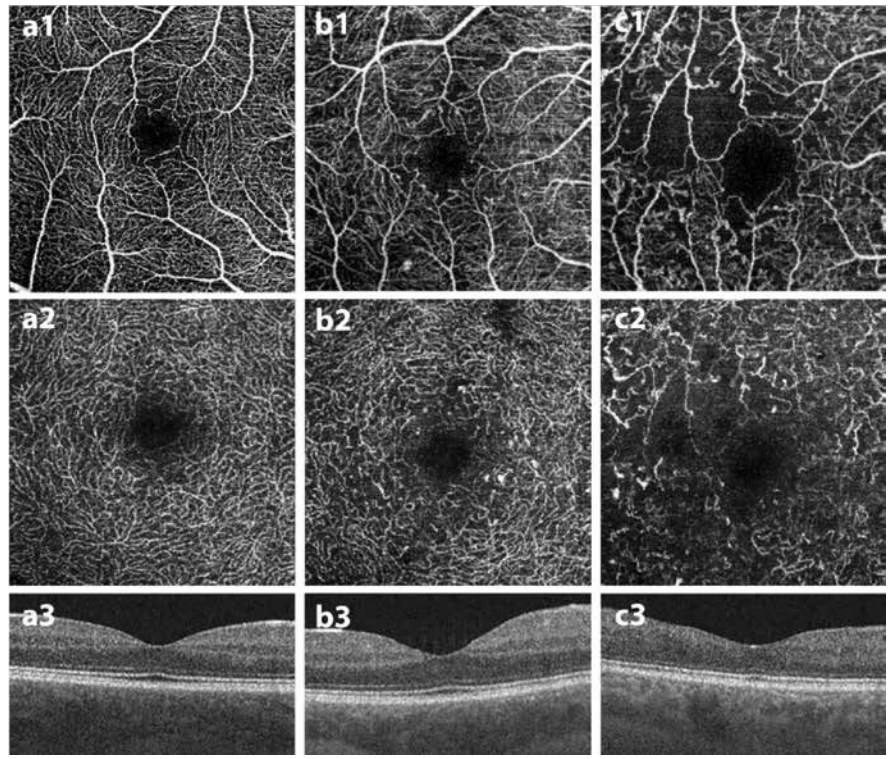


Figure 7 Optical coherence tomography angiography of: a healthy person, showing the superficial (**a1**) and deep (**a2**) capillary plexus, with its consecutive B-scan (**a3**); a person with moderate diabetic retinopathy, showing an irregular foveal avascular zone, minor capillary dropout and a microaneurysm in the superficial plexus (**b1**), multiple microaneurysms and capillary dropout in the deep capillary plexus (**b2**), and its consecutive B-scan (**b3**); and a person with severe diabetic retinopathy, showing severe macular ischemia, and the presence of microaneurysms in both the superficial (**c1**) and deep (**c2**) capillary plexus, and small cystic changes of the retinal inner layers on the consecutive B-scan (**c3**).

3. SCREENING

In the vast majority of the patients, diabetic retinopathy does not cause any symptoms until the later stages of the disease. By that time, treatment may not fully restore the loss in visual function. In case of DME, early proliferative retinopathy and tractional detachement threatening the macula, proper intervention with anti-VEGFs, laser photocoagulation or surgery before the point of no return, can prevent

irredeemable visual loss in many cases. This is also the rationale for screening programs aimed at identifying patients at risk of developing sight-threatening complications. The International Council of Ophthalmology currently recommends a screening vision examination and a retinal examination, performed by appropriately trained personnel. The retinal examination should include either (1) direct or indirect ophthalmoscopy or slit-lamp biomicroscopic examination of the retina or (2) 30° to widefield, mono- or stereoscopic, and dilated or undilated (with a non-mydriatic camera) color retinal fundus photography with or without accompanying OCT.[64] The detection of sight-threatening retinopathy is an indication for the start of treatment and is defined as follows: pre-proliferative diabetic retinopathy according to the 4-2-1 rule, proliferative diabetic retinopathy, or macular edema. In patients with type 2 DM, eye screening should start shortly after the initial diagnosis of diabetes, as unnoticed hyperglycemia may have been present for some time, already causing retinal microvascular damage. In type 1 DM, the sudden onset of diabetes allows a longer interval to the first screening, and is often initiated after 5 years.

Better glycemic control and early intervention for additional risk factors like hypertension and hypercholesterolemia have reduced the incidence and progression rate of diabetic retinopathy

In the absence of pathology, annual screening has long been the standard of care. However, better glycemic control and early intervention for additional risk factors like hypertension and hypercholesterolemia has resulted in reduced incidence and progression rates of diabetic retinopathy, allowing longer screening intervals for those at low risk.[65-67] This could substantially cut costs, and reduce the demand for eye care that is already stretched to its limits. Screening protocols in for example the USA, Sweden and The Netherlands have therefore adopted intervals of two or three years in those at low risk.[68-70] In an attempt to further improve cost-effectiveness and reduce the burden of diabetic eye screening, models have been developed that calculate individual screening intervals based on known risk factors, such as diabetes duration, glycemic control and blood pressure.[71, 72] However, before such a model can be applied in clinical practice, its predictive performance should be empirically evaluated in datasets that were not used to develop the model, a process known as external validation. Further research should point out whether these models that calculate personalized screening intervals are useful in different populations.

4. THERAPEUTIC OPTIONS

Prevention

It cannot be stressed enough that prevention is the backbone of all interventions that aim to minimize morbidity associated with diabetic retinopathy. The importance of metabolic control was laid out by previously discussed land mark trials, such as the DCCT and UKPDS, that provided strong evidence that tight glycemic and blood pressure control, reduce the risk of diabetic retinopathy development and progression. Good glycemic control is generally defined as a glycated hemoglobin A1c level of below 53 mmol/mol or 7%, while blood pressure should be targeted to be below 140/90 mmHg.[73, 74]

Panretinal photocoagulation

After the Second World War, in 1945, the 25-year old Gerhard Meyer-Schwickerath, had an epiphany after learning that one of his students received a macular burn whilst watching a solar eclipse: he hypothesized that retinal disease could be treated by targeting the sun's rays onto his patients retinas on the roof of his clinic.[75] After the development of commercial, non-weather dependent photocoagulation systems, the Diabetic Retinopathy Study (DRS) provided indisputable evidence for the effectiveness of panretinal photocoagulation for the treatment of diabetic retinopathy in 1976.[76, 77] This was followed by the recommendation of the ETDRS group that panretinal photocoagulation should not be performed in all stages of diabetic retinopathy, but only in severe non-proliferative diabetic retinopathy (4-2-1 rule), or in proliferative diabetic retinopathy.[78, 79]

The aim of panretinal photocoagulation is to improve peripheral oxygenation and remove the trigger for VEGF-production and the consequent growth of neovascularization. Laser light is converted to therman energy by the tissue, in order to denaturate proteins that in turn will result in cell death of the retinal pigment epithelium and adjacent photoreceptors. The photoreceptors have a high oxygen turnover, and destroying them is an effective way of reducing the retinal oxygen consumption. With a single panretinal photocoagulation treatment with 1200-1500 burns, oxygen consumption can be reduced by 20%.[80, 81] Retinal cell death due to laser treatment may result in adverse effects, such as constriction of the peripheral visual field and difficulties with color vision, contrast sensitivity and light-dark adaptation have been reported as adverse effects.[82] Additionally, panretinal photocoagulation may provoke DME, or worsen edema in patients with pre-existing DME.[83]

Focal photocoagulation

Where PRP is applied to reduce ischemic areas in the peripheral retina, focal photocoagulation focuses on the treatment of leakage, mainly in the macular area. Using focal laser, hard exudates and sources of leakage, such as microaneurysms can be targeted, and it is hypothesized that these lesions can directly be occluded by photocoagulation.[84] Diffuse macular edema was generally treated with grid laser photocoagulation, placing numerous burns around the fovea in an attempt to relieve retinal hypoxia restore the blood retinal barrier, but this technique has largely been replaced by the administration of intravitreal agents.[84] Although the use of focal laser treatment has diminished since the introduction of intravitreal medication, it remains an important therapeutic option in DME refractive to anti-VEGF or in case of discrete sources of leakage outside of the foveal area.

Corticosteroids

After recognizing an inflammatory component in the pathogenesis of various retinal eye diseases, the use of corticosteroids was successfully tested in patients with age-related macular degeneration in 1995, followed by patients with DME in 2001.[85, 86] The use of triamcinolone was compared to focal/grid laser therapy in a randomized controlled trial by the Retinopathy Clinical Research Network (DRCRN) Protocol B study. Short-term results were in favor of triamcinolone, after 1 year however, there were no significant differences between the groups, and after 2 years, mean visual acuity was better in the laser group.[87, 88] In addition, side effects such as elevation of intraocular pressure of ≥ 10 mmHg and cataract formation as a result of corticosteroids therapy are common.[87] Therefore, treatment is currently reserved for selected, particularly pseudophakic patients with persistent and refractory DME.

Anti-VEGFs

In 1994, Aiello was the first to demonstrate that hypoxic retinal tissue produces elevated levels of vascular endothelial growth factor in ocular fluid, laying the foundation for the development of anti-VEGF therapy.[89] The first anti-VEGF therapy investigated in humans was pegaptanib (Macugen), and was found to be more effective than sham injections. This soon led to the development of more potent anti-VEGF agents, including ranibizumab and aflibercept.[90] Bevacizumab was originally developed for cancer therapy, and was never approved by the Food and Drug Administration (FDA) for intraocular use, in contrast to ranibizumab and aflibercept. Bevacizumab is widely used for off-label treatment for DME using approximately 1/500th of the dosage used for colon carcinome. This significantly cuts costs, as a bevacizumab injection costs about €25, while aflibercept and ranibizumab cost in the vicinity of €800 - 1000 per injection, respectively. The

DRCRN Protocol T study, comparing the three agents head-to-head-to-head found no clinically relevant difference overall. Subgroup analysis, however, showed that in patients with low baseline visual acuity (Snellen visual acuity of 20/50 or less) aflibercept was significantly more effective than ranibizumab and bevacizumab, although ranibizumab has caught up with aflibercept in the 2-year results.[91, 92]

The functional and anatomical superiority of anti-VEGF over focal laser treatment prompted a paradigm shift for the management of center-involving DME. However, individual treatment response can be highly variable. Only 30-40% of all patients treated with anti-VEGF gain 15 or more ETDRS letters after one year.[93] In real world clinical practice, patients are often under-treated and under-monitored, which may result in even less favorable results.[93] In case of insufficient treatment response, switching to another anti-VEGF agent or to corticosteroids may result in better visual outcomes, although more research is need to quantify the beneficial effect.[94-96] At this moment, we are unable to know on beforehand who will respond well to treatment and who will not. Several attempts have been made to address this question, but with limited success. In post-hoc analyses of large randomized controlled trials, younger age, male sex, and higher baseline visual acuity were repeatedly, but not consistently associated with higher final visual outcomes.[97-101] In addition, imaging characteristics have been studied to identify prognostic biomarkers. The DRCRN reported that retinal thickness on OCT only accounts for 27% for the variability in visual outcomes, therefore, additional factors are hypothesized to play a role.[102] OCT biomarkers such as inner retinal layer integrity, photoreceptor layer integrity and hyperreflective foci have been investigated, but have so far not resulted in the development of a reliable prediction model.[103-105] Stratification of patients to find the best fitting treatment approach remains one of the biggest oncoming challenges in the management of DME.

Surgical management

In the early 1970s, revolutionary surgical approaches for vitrectomy through the pars plana were contemplated, independently and simultaneously by Machemer and Peyman. Before that time, ophthalmologists unanimously consented that 'instrumentation of the vitreous was tantamount to surgical malpractice'.[52] These experimental approaches opened doors for patients suffering from previously untreatable vitreoretinal disease, who now had a chance of vision recovery. The most important indications for pars plana vitrectomy as a consequence of DM are non-clearing vitreous hemorrhage, severe fibrovascular proliferation, and tractional retinal detachment threatening the macula. In these cases, vitrectomy allows for clearance of media opacities, disscection of fibrovascular tissue, and permanent reattachment of the retina using photocoagulation and intraocular tamponade

with gas or silicone oil. Furthermore, laser photocoagulation can be applied in order to decrease the neovascular drive or treat retinal breaks.[106] The Diabetic Retinopathy Vitrectomy Study (DRVS) has demonstrated the benefit of early vitrectomy in patients with severe vitreous hemorrhage and in the management of severe active fibrovascular proliferation.[107, 108] Since the DRVS reports, surgical techniques have been subject to advances, such as improved vitreoretinal visualization, higher vitrector cutting rates and smaller gauge vitrectomy systems.[109, 110] The rates of phthisis following diabetic vitrectomy were as high as 19% in the DRVS reports, but have reduced to 0% to 1% in more recent data.[111] Useful vision, defined as 6/60 or better, can now be retained or restored in >70% of the patients, although visual outcomes are difficult to predict as there are many factors influencing the outcome.[112]

When counseling patients for a vitrectomy as treatment option for diabetic retinopathy, their overall condition should be taken into account. The presence of severe complications of diabetic retinopathy is often associated with widespread systemic macrovascular and microvascular complications, that may have consequences for the type of anesthesia or hemorrhagic risk for patients on anticoagulant therapy. In addition, the visual status of the fellow eye may influences treatment strategies.[113] Furthermore, it is important to acknowledge that the fellow eye has a high risk of developing sight-threatening complications of diabetic retinopathy that may require vitrectomy.[114] However, how these complications develop in the long term remains largely unknown. Therefore, further studies are required that address the long-term outcome for patients undergoing a vitrectomy for advanced diabetic retinopathy.

5. CURRENT CHALLENGES IN DIABETIC EYE CARE

The global diabetes epidemic reflects the far-ranging and rapid socioeconomic changes over the past few decades, mainly obesity and its correlate, insulin resistance. The growing number of diabetes patients is met with a revolution in the field of diabetic care, including diabetic eye care. Over the past two centuries, this revolution has led to the availability of an extensive medical armamentarium. However, providing the best available healthcare to all people with diabetes has a skyrocketing price tag. In addition, the demand for diabetic eye care is exceeding the available resources. In The Netherlands for example, the average waiting time for the ophthalmologic outpatient clinic is 6 weeks, but may take up to 3 months, far surpassing the maximum allowable norm of 4 weeks. With that, ophthalmology is the department with the second longest waiting time.[115] To keep healthcare

cost-effective, governments and healthcare providers are forced to improve resource allocation. Our goal should be to minimize the burden of diabetes-related visual complaints in our society, whilst sustaining an affordable healthcare system.

An important strategy for improving cost-effectiveness is to optimize the efficiency of our current approaches of diabetic eye care. The development of new imaging techniques has granted access to a wealth of data, but distilling a set of information useful for clinical practice is posing a serious challenge. Furthermore, the question is raised what combination of imaging techniques should be utilized in which setting. In a screening setting different requirements may apply than in a treatment clinic or in a research facility. Efficiency of image analysis could be improved by the employment of computer-aided detection software. This should improve analysis by elimination observer bias, should significantly reduce analysis time and would free the clinician for other tasks.

To further improve cost-effectiveness of current and future diabetic eye care, research should be aimed at personalizing medicine. This entails the stratification of a group of patients according to their predicted risk of developing a disease, or their chance of response to a specific treatment. Ultimately, a personalized risk estimate could for example aid in the decision what treatment is expected to be most effective for an individual patient, or when the next screening visit should take place. The large numbers of patients, the high phenotypic heterogeneity and the variability in response to the various treatment options, make diabetic retinopathy an interesting candidate for the development of individualized approaches. Personalized medicine can only be achieved with comprehensive knowledge of associated risk factors. Future research should therefore be direct towards thorough investigation of established risk factors for development and progression of diabetic retinopathy, and the identification of new risk factors.

6. AIMS AND OUTLINE OF THIS THESIS

In order to provide sustainable diabetic eye care that is both affordable and accessible, a shift towards more efficient resource allocation is needed. This thesis focuses on different elements that should contribute to this process. We contribute to the foundations of personalized medicine by the identification of prognostic markers for development and progression of diabetic retinopathy and treatment outcomes. We explore new biomarkers that can aid in more careful phenotyping that could be used for risk and treatment prediction models. In a study that is almost directly of use in daily clinical practice, we validate an Icelandic model for

personalized screening intervals in a Dutch setting. Finally, we have developed automated algorithms for the computer aided detection of specific diabetic retinopathy characteristics on OCT.

Development and progression of disease

Chapter 2.1 investigates the incidence of development and progression of diabetic retinopathy in a tertiary care population of patients with type 1 DM, and further explores the associated risk factors. In addition, the long term visual outcomes in these patients are evaluated.

Chapter 2.2 is a validation study of a previously developed model for personalized diabetic retinopathy screening. The model was built and validated in populations with a vast majority of persons with type 2 DM. In this chapter we validate the model in Dutch persons with type 1 DM.

Imaging characteristics

Chapter 3.1 analyzes the distribution of hyperreflective foci on OCT in patients with different stages of diabetic retinopathy with or without DME, and investigates the association between hyperreflective foci and clinical and morphological characteristics in a population of patients with type 1 DM. In addition, the relationship between hyperreflective foci and visual acuity is further explored.

Chapter 3.2 describes the development and evaluation of a deep learning algorithm for the automated detection and quantification of hyperreflective foci on OCT. This chapter also investigates whether the proposed algorithm can effectively be applied to predict the response to treatment based on automatically detected hyperreflective foci.

Chapter 3.3 evaluates the topographical and morphological appearance of microaneurysms on OCTA in patients with DM. Furthermore, the clinical properties of microaneurysms are compared to leakage on FA and retinal thickening on OCT.

Treatment outcomes

Chapter 4.1 assesses the association between hyperreflective foci on OCT and treatment response to anti-VEGF in terms of visual acuity improvement and decrease in retinal thickness. In addition, the location of hyperreflective foci in the neuroretina and their reaction to anti-VEGF therapy is studied.

Chapter 4.2 studies the long-term outcome and associated risk factors regarding visual function in patients undergoing vitrectomy for consequences of proliferative diabetic retinopathy.

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2

Development and progression of disease

2.1

Risk factors for development and progression of diabetic retinopathy in Dutch patients with type 1 diabetes mellitus

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ABSTRACT

Purpose: To investigate risk factors for the development and progression of diabetic retinopathy (DR) and long-term visual outcomes in Dutch patients with type 1 diabetes mellitus (T1DM).

Methods: Cumulative incidences were calculated for DR, vision threatening DR, defined as (pre)proliferative DR and diabetic macular edema, and best corrected visual acuity <0.5 and <0.3 at the most recent eye examination. The following factors were assessed: duration of diabetes, age of onset of T1DM, gender, mean HbA1c, HbA1c variability (defined as coefficient of variation of five separate HbA1c measurements), mean arterial blood pressure, body mass index, albuminuria and lipid profile. We used multivariable Cox regression models to identify factors associated with DR development and progression to VTDR.

Results: We found 25-year cumulative incidences of 63% for DR, 21% for VTDR, 2% for BCVA <0.5, and 1% for BCVA <0.3. Mean HbA1c (HR 1.023, p<0.001), HbA1c variability (HR 1.054, p<0.001), age of onset of T1DM (HR 1.024, p<0.001), HDL cholesterol (HR 0.502, p=0.002) and total cholesterol (HR 1.210, p=0.029) showed an independent association with faster development of any form of DR. Mean HbA1c (HR 1.023, p<0.001) and the presence of albuminuria (HR 2.940, p=0.028) were associated with faster progression to VTDR.

Conclusion: These data show relatively low cumulative incidences of DR, vision threatening DR and visual impairment. Higher mean HbA1c, HbA1c variability, age of onset of T1DM and total cholesterol were independently associated with the risk of DR development, and a protective association was found for HDL cholesterol in subjects with T1DM. Mean HbA1c and presence of albuminuria were associated with progression of DR.

INTRODUCTION

Diabetic retinopathy (DR) is a potentially sight threatening microvascular complication of diabetes mellitus (DM), affecting a third of all DM patients worldwide.[1, 2] DR is characterized by retinal microvascular damage, based on ischemic changes and increased capillary permeability. Visual function can be affected by diabetic macular edema (DME), macular ischemia or as sequelae of proliferative diabetic retinopathy (PDR), notably vitreous haemorrhage and tractional retinal detachment. The substantial impact of DR on visual performance requires attention to prevention and early detection of this sight threatening condition.

DR is a complex multifactorial disease and many risk factors are involved. The most obvious and important predictive factor for the development and progression of DR is hyperglycaemia. The Diabetes Control and Complications Trial clearly showed that very intensive glycemic control can reduce the incidence of DR by 76% and the progression of DR by 54%.[3] Other major systemic risk factors include hypertension, dyslipidemia, and high body mass index, although there is substantial variation in the consistency and strength of the association with these risk factors.[1, 2, 4] Risk of development and progression of DR is furthermore influenced by a number of non-modifiable risk factors, such as duration of diabetes, pregnancy, puberty, and population-based diversity.[1, 5]

To the best of our knowledge, there are currently no cohort studies on development and progression of DR, and associate risk factors in Dutch patients with T1DM. The purpose of this study was to further explore the risk factors for the development and progression of DR in this patient population. In addition, we evaluated the long term visual outcomes in these patients.

METHODS

Subjects

This study was conducted in a cohort of patients with T1DM who were recruited at the outpatient diabetes clinic of the Internal Medicine Department at the Radboud university medical center between 2006 and 2008 for a study on hypoglycaemia awareness.[6] Patients were eligible for the current analysis when the DR screen had take place at the department of Ophthalmology of the Radboud university medical center. This study adhered to the tenets of the Declaration of Helsinki, and was approved by the local Institutional Review Board. Study participants provided written informed consent.

Eye determinants

Patients underwent complete ophthalmological examinations yearly or more often in those at high risk of visual decline. Examinations were standardized and included Snellen best-corrected visual acuity (BCVA) and retinal evaluation with biomicroscopy. In case of any signs of DR or DME, diagnosis was confirmed with colour fundus photography (Topcon TRC 50 IX, Topcon Corporation, Tokyo, Japan) and optical coherence tomography (Spectralis™ HRA+OCT, Heidelberg Engineering, Heidelberg, Germany). Stage of DR and DME was determined according to the International Clinical Diabetic Retinopathy Severity Scale.[7] The eye with the most severe retinopathy was included in further analyses. The two most severe stages of DR/DME of the International Clinical Diabetic Retinopathy/Macular Edema Severity Scale were defined as vision-threatening DR (VTDR), i.e., DR corresponding with the 4-2-1 rule or proliferative DR, retinal edema or hard exudates approaching or involving the fovea. Non vision-threatening DR (NVTDR) was defined as mild or moderate non-proliferative DR or DME distant from the fovea.

Visual impairment was defined as a BCVA <0.3 in the best seeing eye according WHO International Statistical Classification of Diseases and Health Problems.[8] We also used BCVA <0.5 as a cut-off, as this is below the visual requirement for a European driving license.[9, 10] In addition to presence of DR/DME and BCVA, all treatments which were administered during the study period were registered.

Data collection

We assessed the variables age at diagnosis of T1DM, gender, and T1DM duration from medical charts. Serum measurements were obtained at first diagnosis of DR, or within 6 months around the date of the most recent eye examination in those without DR. Mean glycated haemoglobin (HbA1c) level was calculated as the average of 5 measurements at 3 month intervals. The coefficient of variation (CV), a measure of HbA1c variability, was calculated from the mean and standard deviation of the HbA1c measurements.[11] Other laboratory measurements were total cholesterol (mmol/l), high-density lipoprotein (HDL) cholesterol (mmol/l), and albuminuria (mg/l). Albuminuria, the most important marker of diabetic nephropathy, was defined as a urinary albumin excretion of ≥30 mg/l in the absence of other renal pathology.[12] Mean arterial pressure was (MAP, mmHg), was assessed by the equation: MAP ≈ diastolic blood pressure + ⅓ (systolic blood pressure – diastolic blood pressure); body mass index (BMI, kg/m^2) was calculated as weight/height^2. Medical files were assessed between April and July 2015.

Statistical analysis

For For continuous variables, values were displayed as mean ± standard deviation (SD) for normal distributions, and as median with corresponding interquartile range (IQR) for skewed distributions. For categorical variables, values were presented as proportions in percentages. Binary logistic regression analysis was used to compare patient characteristics of the NVTDR group and the VTDR group with the no DR group. Time of follow up was defined as the time in years after first diagnosis of diabetes mellitus. Primary outcome variable was presence of DR, secondary outcomes were VTDR, BCVA <0.5 and BCVA <0.3. Cumulative incidences of outcome variables were estimated using Kaplan Meier survival analysis. Values at the extreme end were pooled due to limited numbers. Incidence rate was calculated by dividing the number of new cases by the total number of person years.

We used a multiple imputation approach for randomly missing data with twenty iterations, incorporating both determinants and outcome variables. Multivariable Cox proportional hazards regression was used to identify predictors for DR and VTDR, with backward stepwise selection eliminating variables with P≥0.1. Risks were displayed as hazard ratios (HR) with corresponding 95% confidence interval (CI). Survival curves were plotted as for low and high risk profiles. Profiles were based on significant variables from the Cox analyses using cut-off levels according to established clinical criteria. For variables for which standardized reference levels were not available, 25th and 75th percentiles were used to represent low and high risk. Using these criteria, low risk was defined as HbA1c=53 mmol/mol, total cholesterol=5.2 mmol/l, and HDL=1.6 mmol/l, HbA1c variability=4.9, and age of onset of T1DM=15 years. High risk was defined as HbA1c=74 mmol/mol, total cholesterol=6.2 mmol/l and HDL=1.0 mmol/mol, HbA1c variability=9.1, and age of onset of T1DM=30 years.[13-15]

To obtain insight in the discriminative ability of our models, a risk score was calculated for each individual by multiplying the estimated beta coefficients from multivariate Cox regression analysis with the registered data points. Subsequently, risk scores were categorized and plotted in a histogram for no DR vs. DR, and for NVTDR vs. VTDR. All statistical analyses were conducted using SPSS version 20 (SPSS, Chicago, IL, USA).

RESULTS

A total of 415 subjects were eligible for the current study. Follow up time varied from 7 to 65 years, with a median of 29 years. Total number of patients who developed DR was 284 (68%). Of these, 119 (42%) progressed to VTDR. Patient characteristics of the three groups are displayed in Table 1. As expected, duration of T1DM was longer in patients with NVTDR and VTDR than in patients without DR, and age of T1DM onset was lowest in the VTDR group. HbA1c determinants and blood pressure were less favourable in the NVTDR and VTDR groups, and HDL cholesterol was lowest in VTDR.

Table 1 Patient characteristics

Variables	No DR (n=131)	NVTDR (n=164)	VTDR (n=120)
Age, years	48 ± 37	52 ± 14	56 ± 13 *
Male gender, n (%),†	60 (46%)	80 (49%)	55 (46%)
Age of onset of T1DM, years	23 ± 11	21 ± 12	18 ± 13 **
Duration of T1DM, years	22 ± 10	32 ± 12 **	38 ± 11 **
Mean HbA1c, mmol/mol ‡	61 (56-67)	65 (57-72) *	78 (67-89) **
HbA1c variability, CV ‡	5.9 (4.4-8.1)	6.9 (5.0-9.1) *	7.8 (5.8-11.0) **
Mean arterial pressure, mmHg	91 ± 10	95 ± 10 *	96 ± 11 *
Body mass index, kg/m^2 ‡	25 (22-27)	26 (23-28)	24 (22-27)
HDL cholesterol, mmol/l	1.56 ± 0.37	1.46 ± 0.42	1.38 ± 0.35 *
Total cholesterol, mmol/l	4.9 ± 1.0	4.9 ± 0.8	5.1 ± 1.0
Albuminuria, n (%)†	10 (10%)	7 (7%)	6 (15%)

Data are means ± SD. *P<0.05; ** P<0.001; P-values addressed differences between no DR versus NVTDR/VTDR and were adjusted for age and gender. † Data are number of subjects with %. ‡ Data are median with interquartile range. DR = diabetic retinopathy; NVTDR = non-vision threatening diabetic retinopathy; VTDR vision threatening diabetic retinopathy; n = number; T1DM = type 1 diabetes mellitus; CV = coefficient of variation; HDL = high density lipoprotein.

The overall incidence rate of DR was 0.033 per person-years and 0.014 per person-years for VTDR. Ten year cumulative incidence of DR was 5%, 20-year cumulative incidence 43%, 25-year cumulative incidence 63%, and 30- and 40-year cumulative incidences were 75%, and 85%. For VTDR, 20-year cumulative incidence was 10%, 25-year cumulative incidence 21%, and 30- and 40-year cumulative incidences were respectively 34%, and 51%. Fifty percent of the total study population had developed DR after 22 years duration of DM, and VTDR after 40 years (Figure 1A). With respect to visual acuity, 2% developed BCVA <0.5 after 25 years of T1DM, and 28% after 58 years; 1% developed BCVA <0.3 after 25 years of T1DM, and 8% after 58 years (Figure 1B), during which period the treatments were applied that are shown in Supplemental Table 1.

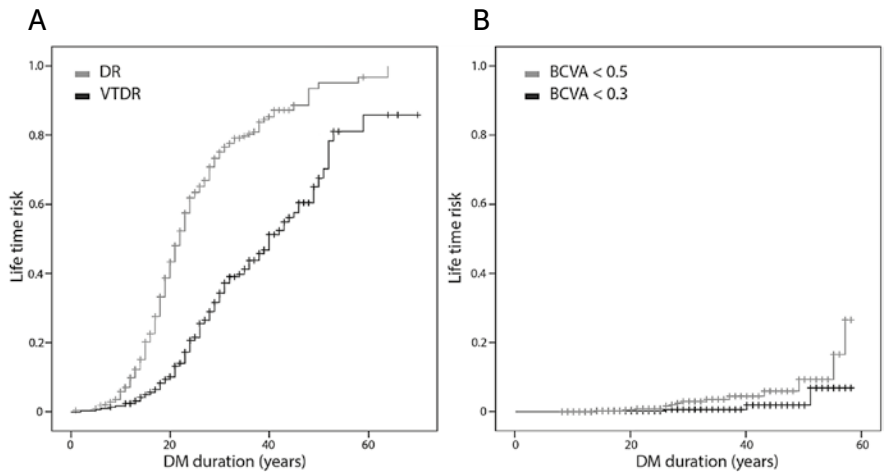

Figure 1 Lifetime risk as a function of DM duration for: (**A**) DR and VTDR; (**B**) visual impairment. DR = diabetic retinopathy; VTDR = vision threatening diabetic retinopathy; DM = diabetes mellitus; BCVA = best corrected visual acuity.

Lab assessments which coincided with eye examinations were available in 380 subjects (126 without DR, 158 NVTDR, and 96 VTDR). In the multivariate Cox regression analysis based on these subjects, higher age of onset of T1DM, mean HbA1c, HbA1c variability, total cholesterol and low HDL cholesterol were associated with a faster development of DR (Table 2). Mean HbA1c and presence of albuminuria were associated with a faster progression of DR to VTDR (Table 3). We compared the hazard of DR development for high and low risk profiles with Cox regression survival curves (Figure 2). Remarkably, high risk profiles showed a fast decline in DR free fraction around DM duration of 20 years, while low risk profiles showed a gradual decline. With respect to progression to VTDR, high risk profiles risks accumulated gradually after first diagnosis of DR to virtually all affected after >15 years; low risk profiles only progressed to VTDR in 50% of cases.

Risk scores for the study population were calculated and plotted in a histogram (Supplemental Figure 1). Although those developing DR/VTDR and those who did not showed somewhat overlapping risk scores, those with higher risk scores more often developed DR and VTDR.

Table 2 Hazard of developing DR for known risk factors from multivariate Cox regression analysis

	HR	95% CI	P-value
Age of onset T1DM (years)	1.024	1.013-1.035	<0.001
Mean HbA1c (mmol/mol)	1.023	1.014-1.033	<0.001
HbA1c variability (CV)	1.054	1.028-1.081	<0.001
HDL cholesterol (mmol/l)	0.502	0.325-0.775	0.002
Total cholesterol (mmol/l)	1.210	1.020-1.436	0.029

HR = hazard ratio; etc; 95% CI = 95% confidence interval; CV = coefficient of variation; HDL = high density lipoprotein. The hazard ratio for continuous variables should be interpreted as hazard per point of increase of the variable per time unit. For example: when the HbA1c level increases with 10 points, the instantaneous risk of DR development is 1.025^{10} = 1.280 or 28.0% higher.

Table 3 Hazard of developing VTDR for known risk factors from multivariate Cox regression analysis

	HR	95% CI	P-value
Mean HbA1c (mmol/mol)	1.023	1.011-1.036	<0.001
Albuminuria	2.940	1.130-7.648	0.028

HR = hazard ratio; etc; 95% CI = 95% confidence interval. The hazard ratio for continuous variables should be interpreted as hazard per point of increase of the variable per time unit. For example: when the HbA1c level increases with 10 points, the instantaneous risk of DR progression is 1.024^{10} = 1.268 or 26.8% higher.

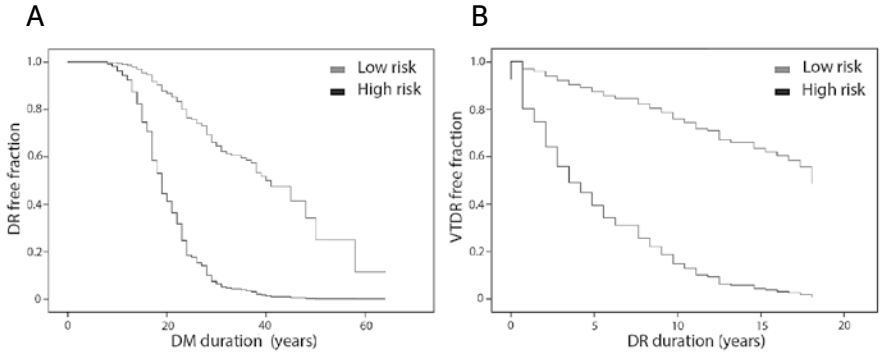

Figure 2 Disease free fraction for low versus high risk profiles in: (**A**) development of DR; (**B**) progression to VTDR. DR = diabetic retinopathy; DM = diabetes mellitus; VTDR = vision threatening diabetic retinopathy.

DISCUSSION

In this single centre cohort of subjects with T1DM, mean HbA1c, HbA1c variability, HDL cholesterol, total cholesterol and age of onset of T1DM were independently associated with the hazard of developing DR. The mean HbA1c and albuminuria were associated with the hazard of progression of DR to VTDR.

The effect of hyperglycaemia on development and progression of DR has been studied thoroughly in previous research, and our study confirms the important contribution of HbA1c.[1, 3, 16] HbA1c variability has been investigated to a much lesser extent. Hietala et al. and Herman et al. also reported HbA1c variability as a risk factor for development of DR independently of average glycemic control.[17, 18] Kilpatrick proposed several explanatory mechanisms for this correlation.[19] For example, it is possible that with an increase of HbA1c the risk of microvascular complications rises exponentially, therefore people that experience more HbA1c variation are exposed to a higher average risk. Another possible explanation is that improving glycemic control can lead to a short-term 'early worsening' in retinopathy, before a subsequent net improvement in the long term, which is a phenomenon that has been reported by the Diabetes Control and Complications Trial.[20] As a consequence of the fast alterations in glycemic control, the retina may have insufficient time to recover from the damaging effects of previously high HbA1c during periods where HbA1c is low.

In our study, lower HDL cholesterol land higher total cholesterol levels were associated with an increased hazard of DR development. This effect corroborates some, but not all studies, as clinical evidence for a relation between dyslipidemia and DR is controversial.[21] The potential mechanism of action of the association between lipids and DR is also unclear. It has been hypothesized that lipoproteins leak through the disrupted blood-retinal-barrier and have a cytotoxic effect on retinal cells.[22] Others hypothesized that lipoproteins interact with the activation of protein kinase C and advanced glycation end product formation – two pathways involved in DR pathophysiology.[21]

We found that a higher age of onset of T1DM was associated with a more rapid development of DR. This confirms earlier reports of associations between increasing age of onset of T1DM and development and progression of DR.[23-25] We hypothesize that subjects with a higher age of onset of T1DM develop DR faster, because the natural aging process contributes to retinal degeneration, independently of hyperglycaemia. Various microvascular alterations can be observed in the aging retina, such as increased vessel leakage and declining RPE cell integrity.[26, 27]

Additionally, age-related inflammatory changes are believed to contribute to the pathogenesis of DR.[28] This could make patients with an older age of onset of T1DM more vulnerable to development of complications.

The link between advanced DR stages and albuminuria has been well established.[16, 29] However, only few studies have investigated the relationship between albuminuria and progression to VTDR in T1DM patients. Lloyd et al. reported an association between increased albumin excretion rate and progression to proliferative DR over a 2-year interval.[30] In contrast, no relationship was found between albuminuria and DR progression in two other studies.[16, 31] This discrepancy is possibly the result of a different definition of progression, as in the latter two studies DR progression was referred to as an increase in DR severity level. Further research is warranted to investigate the discriminative ability of albuminuria as predictor for imminent VTDR in patients with T1DM.

The 25-year cumulative incidence of DR development (63%) and progression to VTDR (21%) was relatively low when compared the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) group who found rates for 25-year cumulative incidences of 97% for DR, and 42% for proliferative DR, while Broe et al. reported 16-year cumulative incidences of 95% for DR, and 31% for proliferative DR.[16, 32] In our cohort, cumulative incidences for BCVA <0.5 and <0.3 were 3% and 1% respectively. The WESDR group reported a 25-year incidence of 13% for BCVA ≤0.5. A possible explanation for these disparities may lie in the glycemic control of the patients in our cohort. The measured mean HbA1c level in our cohort was 68±15 mmol/mol, vs. 91±22 mmol/mol in the WESDR, and 81±17 mmol/mol in the study by Broe et al.[32, 33]

One of the major strengths of this study was the long duration of follow up. The average duration of follow up was 29 years, thereby offering good insight in the rates of development and progression of DR. Another strength was the use of five separate HbA1c measurements to define the mean HbA1c. This makes the HbA1c level a more reliable parameter and provides a good reflection of HbA1c variability in the one year period prior to diagnosis of DR or the most recent examination. The present study also has its limitations, besides the drawbacks that generally apply to a retrospective study design. In some instances, information on a single measurement was extrapolated in time, whereas patients might have changed their behaviour over time and the investigated variables may have been subject to fluctuations. Furthermore, the study population in this cohort represents a carefully selected phenotyped tertiary care population that may not fully reflect the general population.

In conclusion, the current study provides an overview of the risk factors that are responsible for development and progression of DR in patients with T1DM in a tertiary referral centre. This adds to the growing body of evidence that for proper glycemic control we should not only focus on absolute HbA1c levels, but also on the level of HbA1c variation. We therefore advise health care professionals involved in the prevention and early detection of retinopathy in patients with T1DM, to take HbA1c variability into account when optimizing glycemic control.

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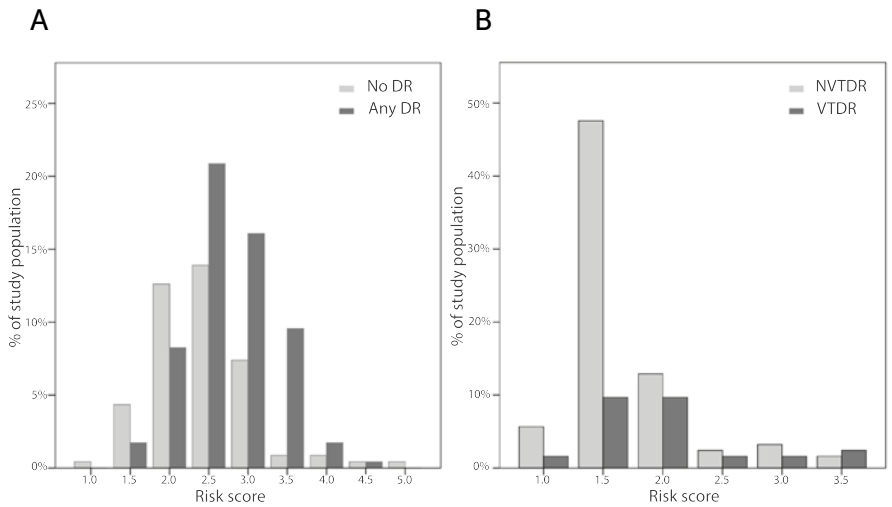
SUPPLEMENTARY MATERIAL

Supplemental Table 1 Treatments provided during the study period

	No DR Total n=131	NVTDR Total n=164		VTDR total n=120	
	%	%	Mean ± SD	%	Mean ± SD
Panretinal photocoagulation	0	6	4.1 ± 2.9	82	16.4 ± 10.1
Focal photocoagulation	0	8	2.4 ± 2.7	70	3.8 ± 3.5
Micropulse photocoagulation†	0	1	1.0	15	2.1 ± 1.1
Intravitreal injection	0	0	N/A	14	9.5 ± 8.2
Bevacizumab	0	0	N/A	14	6.9 ± 4.7
Ranibizumab	0	0	N/A	1	14.0 ± 5.7
Aflibercept	0	0	N/A	0	N/A
Triamcinolone	0	0	N/A	7	1.6 ± 1.0
Vitrectomy	0	0	N/A	21	1.7 ± 1.0
Cataract surgery	21	15	1.7 ± 0.5	42	1.8 ± 0.4

† No SD is shown, because only one subject in either group received micropulse photocoagulation.
DR = diabetic retinopathy; NVTDR = non vision threatening diabetic retinopathy; VTDR vision threatening diabetic retinopathy; n = number; N/A = not applicable



Supplemental Figure 1 Distribution of risk score for: (**A**) DR; (**B**) VTDR. Risk scores were calculated by multiplying the estimated beta coefficients from multivariate Cox regression analysis with the registered data points for each individual. The risk scores were subsequently grouped and plotted in a histogram. DR = diabetic retinopathy; NVTDR = non-vision threatening diabetic retinopathy; VTDR = vision threatening diabetic retinopathy.

2.2

Validation of a model for the prediction of retinopathy in persons with type 1 diabetes

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ABSTRACT

Background/aims: To validate a previously developed model for prediction of diabetic retinopathy (DR) for personalized retinopathy screening in persons with type 1 diabetes.

Methods: Retrospective medical data of persons with type 1 diabetes treated in an academic hospital setting were used for analysis. Sight threatening retinopathy (STR) was defined as the presence of severe non-proliferative DR, proliferative DR or macular edema. The presence and grade of retinopathy, onset of diabetes, systolic blood pressure, and levels of HbA_{1c} were used to calculate an individual risk estimate and personalized screening interval. In persons with STR, the occurrence was compared to the calculated date of screening. The model's predictive performance was measured using calibration and discrimination techniques.

Results: Of the 268 persons included in our study, 24 (9.0%) developed STR during a mean follow-up of 4.6 years. All incidences of STR occurred after the calculated screening date. By applying the model, the mean calculated screening interval was 30.5 months, which is a reduction in screening frequency of 61% compared to annual screening and 21% compared to biennial screening. The discriminatory ability was good (Harrell's C statistic = 0.82, 95% CI 0.74 – 0.90) and calibration showed an overestimation of risk in persons who were assigned to a higher risk for STR.

Conclusion: This validation study suggests that a screening programme based on the previously developed prediction model is safe and efficient. The use of a personalized screening frequency could improve cost-effectiveness of diabetic eye care.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that is growing universally.[1] Diabetic retinopathy (DR) and diabetic macular oedema (DME) are well-known complications of diabetes mellitus and are a main cause of blindness in working-age adults in developed countries.[2] Microvascular changes in the retina are the cause of DR which may be asymptomatic in the early stages but may progress to sight threatening retinopathy (STR) if left untreated.[2] Eye screening at regular intervals is a powerful tool to diagnose DR, which supports prompt treatment and prevention of blindness.[3] However, efficient and cost-effective screening strategies are required to keep up with the rising prevalence of diabetes in developed countries.

The cost-effectiveness of DR screening can be increased by personalizing the screening interval using a prediction model which estimates the risk of retinopathy based on an individual risk profile.[4, 5] One of these models, developed by Aspelund et al., is applicable for both type 1 and type 2 diabetes because it uses different algorithms for both types of diabetes. It estimates the risk of developing STR, and calculates a screening interval ranging from 6 to 60 months.[4] Four studies that validated Aspelund's model were all performed in a primary care setting, and people with type 1 diabetes were underrepresented.[4, 6-8] Therefore, the aim of this study is to validate Aspelund's model in persons with type 1 diabetes using an academic hospital setting.

METHODS

Study population and data sources

Persons with type 1 diabetes treated by the Department of Ophthalmology from the Radboud University Medical Center were eligible for this cohort study. Clinical data from a random sample of 405 persons examined between 1 January 2005 and 31 December 2009 were obtained from medical records. This cohort consisted of persons who received regular diabetes care in the same centre, but also persons referred for ophthalmological reasons while diabetes care was performed elsewhere. We excluded persons based on clinical features (diagnosis of STR at baseline, history of laser photocoagulation, pregnancy at baseline or during follow-up) or insufficient follow-up (incomplete data or loss to follow up). This study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Study participants provided written informed consent.

Baseline data

The baseline measurement used to calculate future retinopathy risk and screening interval was set at the first visit between 1 January 2005 and 31 December 2009. Demographics included sex and age. The following clinical hallmarks were obtained: diabetes duration and age at diagnosis, systolic and diastolic blood pressure in mmHg measured by a sphygmomanometer (Speidel & Keller, Juningen, Germany), levels of HbA_{1c} in mmol/mol as determined by HPLC (Menarini Diagnostics, Florence, Italy), body mass index (BMI) in kg/m^2, and lipid spectrum was determined by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany). The following lipid measurements were assessed: total cholesterol in mmol/l, HDL-cholesterol in mmol/l, LDL-cholesterol in mmol/l, and triglycerides in mmol/l. In case eye examination was not performed on the same date as the other clinical examinations, a range of 6 months was permitted between these measurements.

Clinical outcome measure

All persons underwent a standardized eye examination which included ophthalmoscopy after mydriasis by topical 0.5% tropicamide and 5.0% phenylephrine. Optic disc, macula and peripheral fundus were inspected during examination. Non-stereoscopic fundus photographs or optical coherence tomography were obtained when indicated. All people had an annual eye exam with more frequent follow-up when diabetic pathology (e.g. hard exudates) was found. DR and DME were graded according to the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scale.[9] DR was graded as no apparent DR, mild non-proliferative DR, moderate non-proliferative DR, severe non-proliferative DR or proliferative DR. DME was graded as present or absent. Background DR was defined as the presence of mild or moderate non-proliferative DR, whilst STR was defined as the presence of severe non-proliferative DR, proliferative DR or DME. The eye with the worst grade of DR was used to describe DR severity.

Statistical analysis

Baseline characteristics were described as proportions, means (SD) or medians (interquartile range (IQR)). To assess differences in baseline characteristics between patients with and without background DR, we used independent samples t-tests for parametric distributions, and Mann-Whitney U tests for non-parametric distributions. To compare proportions, a Chi-square test was used. The prediction model developed by Aspelund et al. was used to estimate the individual 5-year risk for STR and a tailored screening interval based on diabetes type, sex, presence of a less severe form of DR, duration of diabetes, HbA_{1c} and systolic blood pressure at baseline.[4] In persons who developed STR within 5 years after baseline, we investigated

whether the incidence occurred before or after the recommended time of screening. The model's predictive performance was measured using discrimination and calibration. Discriminatory ability was assessed by calculating Harrell's C-statistic, which is similar to the area under the receiver operating characteristic curve, taking into account the censored nature of the data.[10] Calibration is the agreement between the predicted STR risk according to the model and the observed STR incidence during follow-up, which was evaluated by plotting the predicted risk of STR in quintiles of the population against the observed incidence for each quintile. Missing clinical data were handled as follows: missing blood pressures were set at 130 mmHg, and missing HbA_{1c} values at 8% or 64 mmol/mol. Blood pressure measurements were missing in 3 persons (1.1%) and HbA_{1c} level was missing in 1 person (0.4%). IBM SPSS Statistics (version 20.0.0) and R (R Core Team 2016) was used to perform the analysis. A value of $P < 0.05$ was considered as statistically significant.

RESULTS

Of the random sample of 406 people, 114 were excluded due to a history of previous laser photocoagulation or diagnosis of STR at baseline. After further exclusion of persons with missing data or without follow-up (n=17) and pregnant women (n=8), 268 persons were available for analysis. No statistically significant differences in clinical data were found between persons with insufficient data or length of follow-up and those included for analysis.

Of the remaining 268 persons, 80 (29.9%) had a background DR at baseline. Baseline demographic and clinical characteristics of this cohort are shown in Table 1. In comparison to patients without DR at baseline, patients with background DR were significantly older (50 $\pm$ 13 years versus 40 $\pm$ 13 years) and had longer diabetes duration (26 $\pm$ 9 years versus 19 $\pm$ 11 years). Additionally, patients with background DR had higher HbA_{1c} (67 $\pm$ 13 mmol/mol versus 63 $\pm$ 13 mmol/mol, higher systolic blood pressure (138 $\pm$ 16 mmHg versus 129 $\pm$ 14 mmHg), and higher BMI (25 [IQR: 22-37] kg/m^2 versus 26 [IQR: 23-28] kg/m^2) levels than patients without DR at baseline.

During a mean follow-up of 4.6 years (SD: 1.1), STR occurred in 24 people (9.0%). All incidences of STR occurred after the calculated screening date, as is shown in Table 2. By applying Aspelund's model, the mean calculated screening interval was 30.5 months for the total study population, which is a reduction in screening frequency of 61% compared to annual screening, and 21% compared to biennial screening. In 196 persons (73%) the calculated screening interval was $\geq$ 1 year.

Table 1 Baseline characteristics by retinopathy grade

	No DR (n=188)	Background DR (n=80)
Male, n (%)	93 (49%)	40 (50%)
Age, years	40 (13)	50 (13)*
Age of diabetes diagnosis, years	22 (11)	24 (14)
Diabetes duration, years	19 (11)	26 (9)*
HbA_{1c}, mmol/mol	63 (13)	67 (13)*
Systolic blood pressure, mmHg	129 (14)	138 (16)*
Diastolic blood pressure, mmHg	75 (9)	76 (9)
Body mass index, kg/m^2	25 (22-37)	26 (23-28)*
Total cholesterol, mmol/l	4.9 (0.9)	4.9 (0.7)
HDL cholesterol, mmol/l	1.5 (0.4)	1.5 (0.3)
LDL cholesterol, mmol/l	2.9 (0.7)	2.9 (0.7)
Triglycerides, mmol/l	0.9 (0.7-1.3)	1.0 (0.8-1.4)

DR=diabetic retinopathy; n=number; HDL=high density lipoprotein; LDL=low density lipoprotein.
All data are expressed as proportions, means (SD), or median (interquartile range). *P<0.05.

Table 2 Details of screening after application of the model for the total population and stratified by level of retinopathy at baseline.

	Total population (n=268)	No DR (n=188)	Background DR (n=80)
Follow-up (years)	4.6 (1.1)	4.7 (0.9)	4.4 (1.3)
Incidence of STR, n (%)	24 (9.0%)	7 (3.7%)	17 (21.3%)
Screening interval (months)	30.5 (19.3)	39.5 (15.7)	9.1 (3.5)
Cases of STR before calculated screening date, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

STR=sight threatening retinopathy; DR= diabetic retinopathy; Background DR = mild or moderate DR.
All data are expressed as means (SD) or proportions.

Harrell's C-statistic was 0.82 (95% CI 0.74-0.90), which means the model is able to distinguish clinically between those who develop STR and those who do not. In Figure 1 the agreement between the predicted risk of STR and observed incidence is plotted. The risk of STR was overestimated in persons who were assigend a higher STR risk.

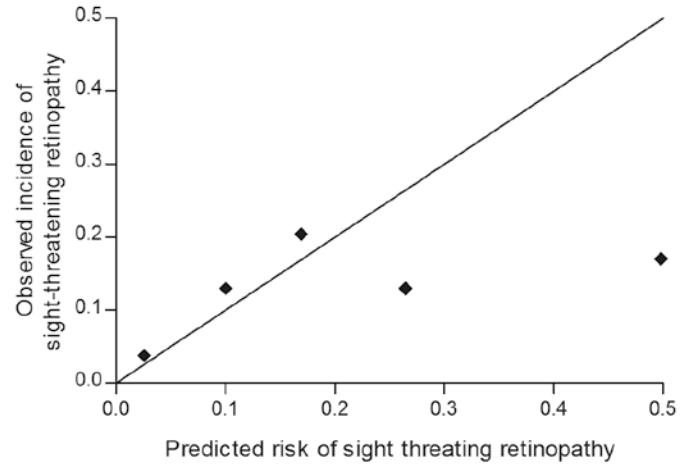

Figure 1 Calibration plot presenting quintiles of predicted sight threatening retinopathy risk versus observed incidence of sight threatening retinopathy.

DISCUSSION

This study externally validates Aspelund's model for people with type 1 diabetes treated in an academic hospital setting. The model showed a reliable performance and no events of STR occurred before the calculated time of retinopathy screening, meaning the model was safe for all individuals in this study. By applying Aspelund's model, screening frequency can be safely reduced by 61% compared to annual screening and 21% compared to biennial screening, resulting in a structural improved cost-effectiveness in the current diabetic eye-screening.

Annual and biennial eye screening are currently the most common methods for early detection of retinopathy in persons with no or minimal signs of DR in developed countries.[11-13] This is regardless of diabetes type, although it is known that people with type 1 diabetes do have a higher risk of developing DR than people with type 2 diabetes.[14] Nevertheless, earlier studies indicated that more extended screening intervals are permitted in persons with type 1 diabetes without retinopathy.[15] Using Aspelund's model, we confirm that the time between consecutive screenings can safely be extended in the majority of the population.

Previous validation studies have all been performed in a primary care context and focused on type 2 diabetes.[4, 6-8] Our findings are consistent with previous studies

in persons with type 2 diabetes validating Aspelund's model in which reductions in screening frequency ranged from 40% to 61% compared to annual screening. In the current study, we found a Harrell's C-statistic of 0.82, indicating that the discriminatory ability of the model is good. This corroborates the previous validation studies that reported AUC's between 0.74 and 0.83. Our calibration plot showed an overestimation of the risk of STR, especially in those at higher risk of STR. This has been a consistent finding in previous reports, and might suggest that the screening interval for those at high risk of STR could be even longer than is calculated by the current model.

Recently, a similar risk model was proposed by the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications study group based on persons with type 1 diabetes.[5] This model predicts the risk of STR, incorporating retinopathy status and HbA_{1c}, and results in a 58% reduction in eye examination compared to annual screening. However, this model was based on data collected during the 1990s, and diabetes care has since been subject to significant improvements. Therefore, these findings need to be validated in other cohorts.

The limitations of our study need to be acknowledged. Despite the relatively small sample size, the results of this validation study are highly comparable to the previous studies in type 2 diabetes that used larger sample sizes.[4, 6-8] Furthermore, fundus photography is generally preferred for the grading of DR in a research setting, in order to increase reproducibility, but was not available for all persons. In clinical practice however, fundus photography is not always available either, and we therefore provide evidence that Aspelund's model is effective in a real life clinical care setting. This study was conducted in an academic hospital, also providing healthcare to persons experiencing difficulties with glucose regulation, which may result in a higher incidence of STR. We therefore hypothesize that the performance of the model may be even better in a primary or secondary care setting. Furthermore, health care providers applying Aspelund's model should be aware that it does not account for changes in HbA_{1c} levels and blood pressure.

Our study indicates that Aspelund's model may facilitate a diabetic eye screening that is more cost-effective than standard annual or biennial screening. Additional studies are required for further validation of Aspelund's model to confirm its safety in large cohorts of persons with type 1 diabetes.

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3

Imaging characteristics

3.1

Retinal hyperreflective foci in patients with type 1 diabetes

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ABSTRACT

Purpose: To investigate hyperreflective foci (HF) on spectral domain optical coherence tomography (SD-OCT) in patients with type 1 diabetes mellitus across different stages of diabetic retinopathy (DR) and diabetic macular edema (DME), and to study clinical and morphological characteristics associated with HF.

Methods: SD-OCT scans and color fundus photographs were obtained of 260 patients. SD-OCT scans were graded for the number of HF and other morphological characteristics. The distribution of HF across different stages of DR and DME severity were studied. Linear mixed model analysis was used to study associations between the number of HF and clinical and morphological parameters.

Results: Higher numbers of HF were found in patients with either stage of DME versus patients without DME ($p<0.001$). A trend was observed between increasing numbers of HF and DR severity, although significance was only reached for moderate non-proliferative DR ($p=0.001$) and proliferative DR ($p=0.019$). Higher numbers of HF were associated with longer diabetes duration ($p=0.029$), lower HDL cholesterol ($p=0.005$), and the presence of microalbuminuria ($p=0.005$). In addition, HF were associated with morphological characteristics on SD-OCT, including central retinal thickness ($p=0.004$), cysts ($p<0.001$), subretinal fluid ($p=0.001$), and disruption of the external limiting membrane ($p=0.018$).

Conclusion: The number of HF was associated with different stages of DR and DME severity. The associations between HF and clinical and morphological characteristics can be of use in further studies evaluating the role of HF as a biomarker for disease progression and treatment response.

INTRODUCTION

Diabetic retinopathy (DR) occurs as a complication of chronic hyperglycemia, the hallmark of diabetes mellitus (DM), and may lead to vision loss, most frequently as a consequence of diabetic macular edema (DME).[1] Although therapeutic options for DR have improved with the advent of anti-vascular endothelial growth factor (VEGF), only three out of ten people experience a visual acuity gain of three or more lines after one year.[2, 3] Furthermore, the multifactorial origin of DR leads to a heterogeneous clinical manifestation, and disease progression can be highly variable.[4, 5] It is, therefore, imperative that we optimize current diagnostic procedures as well as the therapeutic arsenal. In recent years, precision medicine is emerging, using prognostic biomarkers to establish a tailored therapeutic approach for the individual patient. Prognostic biomarkers may be of clinical or genetic origin but may also be found in imaging characteristics.

Cornerstones of the diagnosis and management of diabetic retinal disease are the non-invasive imaging modalities color fundus photography (CFP) and spectral domain optical coherence tomography (SD-OCT). CFP is a well-established technology that enables visualization of various hallmarks of DR, including microaneurysms, hard exudates, hemorrhages, and neovascularization.[1] SD-OCT can be used to capture cross-sectional imaging of the retina, and is widely applied to detect DME, and monitor treatment response to intravitreal medication, such as anti-VEGF and short and long acting corticosteroids.[6-9] Furthermore, its high resolution enables evaluation of morphological characteristics of DR that cannot readily be observed by CFP, such as retinal layer integrity or the presence of hyperreflective foci (HF).

HF are small, well-circumscribed deposits that show high reflectance on SD-OCT, and were first described by Bolz et al.[10] The reflectivity of HF is similar to that of hard exudates, but due to their small size, they cannot be detected on CFP as such.[10] This resemblance in reflectivity has led to the hypothesis that HF represent precursors of hard exudates. The notion that HF reflect other DR features such as microaneurysms and hemorrhages are less likely based on reflectance characteristics.[10-12] Conversely, HF can also be detected in various other retinal diseases that are not associated with the presence of hard exudates, such as non-neovascular age-related macular degeneration.[13-15] A common factor between these retinal diseases is its inflammatory-driven nature, and it has therefore been suggested that HF are cells involved in the inflammatory response, such as aggregations of activated microglia.[15, 16] Others hypothesized that HF could represent migrating retinal pigment epithelium (RPE) cells or degenerated photoreceptor cells, because

of their association with the disruption of the photoreceptor layer.[13, 17] They have been observed in patients with DR, as well as other retinal disorders such as non-neovascular age-related macular degeneration. [13-15] However,,it is uncertain whether the underlying substrate of HF is identical in these various disorders.

To reliably use HF in the risk assessment of diabetic retinal disease, a thorough understanding of the distribution of HF, and their association with clinical and retinal morphological characteristics is needed. The aim of this study was therefore to analyze the distribution of HF in patients with different severity stages of DR and DME, and to investigate the association between HF and clinical and morphological characteristics in a population of patients with type 1 DM. In addition, we aimed to further explore the relationship between HF and visual acuity.

METHODS

Study Population

This study was conducted in a population of patients with type 1 DM visiting the outpatient clinic of the department of Internal Medicine at the Radboud University Medical Center for routine clinical care between September 2011 and August 2016. Patients were included if CFP and SD-OCT of the same date were available. Exclusion criteria were poor image quality and the presence of concurring retinal disease, such as age-related macular degeneration or retinal vein occlusion. This study was approved by the Medical Ethics Committee of the Radboud University Medical Center, Nijmegen, and all participants provided written informed consent. This research was conducted in accordance with the tenets of the Declaration of Helsinki.

Data collection

All patients underwent a full ophthalmologic examination, that included history taking, determination of best corrected visual acuity (BCVA), slit lamp biomicroscopy, CFP and SD-OCT. The BCVA was measured by a certified operator using the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart. Standard 7-field 35° CFP was obtained after mydriasis, according to the ETDRS protocol, using the Topcon TRC 50 IX camera (Topcon Corporation, Tokyo, Japan). We acquired high resolution 20° x 20° SD-OCT scans centered on the fovea with a volume of 25 B-scans using a Spectralis™ HRA-OCT device (Heidelberg Engineering, Heidelberg, Germany).

Medical charts were assessed to obtain clinical information, including age, gender, DM duration, level of mean glycated hemoglobin (HbA1c, mmol/mol), blood pressure (mmHg), body mass index (BMI, calculated as weight/height^2, kg/m^2) total cholesterol (mmol/l), high-density lipoprotein (HDL) cholesterol (mmol/l), and albuminuria (mg/l). The presence of microalbuminuria was defined as a urinary albumin excretion of ≥30 mg/l in the absence of other renal pathology.[18]

Image grading

The level of DR was graded on CFP according to the International Clinical Diabetic Retinopathy Severity Scale, distinguishing the levels 'no DR', 'mild non-proliferative DR (NPDR)', 'moderate NPDR', 'severe NPDR', and 'PDR' by one experienced grader (VS).[19] For DME, we distinguished 'mild DME', 'moderate DME', and 'severe DME' on CFP and SD-OCT, based on the International Clinical Diabetic Macular Edema Severity Scale.[19] The presence of hard exudates was determined on CFP. SD-OCT scans were imported in a custom-built annotation workstation developed using MeVisLab (MeVis Medical Solutions AG, Fraunhover MEVIS, Germany) The retinal layers in the central 3mm of the fovea-centered B-scan were assessed for the presence of HF. We defined HF as small, round or oval-shaped, well-circumscribed dense particles with higher reflectivity than the surrounding background, and a size of <100 µm.[20] The infrared images were checked to exclude HF that corresponded to retinal vessels. The total number of HF between the inner nuclear layer and the external limiting membrane (ELM) was evaluated by means of manual annotations made by two trained graders (VS, AB), masked to all clinical information. The average of both graders was used for analysis. Furthermore, the presence of cysts, subretinal fluid, disruption of the ELM, disruption of the ellipsoid zone (EZ), and disruption of the retinal inner layers (DRIL) was graded in the central 1mm of the fovea-centered B-scan. Disagreements between graders were solved by open adjudication. Central retinal thickness (CRT) was defined as the average thickness within a 1mm diameter around the fovea, as derived from Heidelberg retinal mapping software with manual correction of segmentation if the automatic measurement was found to be unreliable.

Statistical analysis

Values for continuous variables were displayed as mean ± standard deviation (SD) in case of a normal distribution, or as median with corresponding interquartile range (IQR) in case of a skewed distribution. Values for categorical variables were displayed as a proportion in percentage.

For statistical analyses, BCVA was converted to the logarithm of the minimum angle of resolution (logMAR). In addition, to study the distribution of HF according to BCVA, groups were created, comprising normal vision (BCVA ≥ 20/25), mild to

moderate visual impairment (BCVA ≥ 20/60 and < 20/25), and low vision (BCVA < 20/60).[21] Intergrader agreement for the grading of HF was determined using an intraclass correlation coefficient. The inter-eye symmetry for the number of HF was assessed with a Pearson's correlation coefficient (r_p). Patient characteristics were compared across the groups 'no DR', 'mild-moderate NPDR' and 'severe NPDR-PDR' using a mixed-effects multinomial logistic regression model. We applied univariable and multivariable linear mixed model analysis to study associations with the total number of HF and logMAR BCVA as outcome measures. For the multivariable analyses, we used backward selection, eliminating variables with a P-value of ≥ 0.1. Multicollinearity was checked using Pearson's correlation coefficient (r_p) for parametric distributions, and Spearman's rank correlation coefficient (r_s) for non-parametric distributions or categorical variables. P-values <0.05 were considered statistically significant. Analyses were conducted using SPSS version 25 (SPSS, Chicago, IL, USA).

RESULTS

A total of 260 patients were recruited for this study. In four patients, one eye had become blind as a consequence of diabetic retinal disease, and therefore only one eye could be evaluated. We excluded five eyes of three patients due to either age-related macular degeneration or retinal vein occlusion, and eight eyes of six patients due to poor image quality. Subsequently, a total of 503 eyes of 256 patients were eligible for analysis.

We detected no signs of DR in 156 eyes (31%), mild NPDR in 177 eyes (35%), moderate NPDR in 127 eyes (25%), severe NPDR in 26 eyes (5%), and PDR in 17 eyes (3%). Regarding DME, we found no DME in 417 eyes (83%) and classified 34 eyes (7%) as having mild DME, 25 eyes (5%) as moderate DME, and 26 eyes (5%) as severe DME. Patient characteristics of these patients are shown in Table 1. Patients with mild or moderate NPDR were significantly older, had a longer duration of diabetes, higher levels of HbA1c and HDL cholesterol, and more often a history of PRP, than patients with no DR. Patients with severe NPDR or PDR had significantly higher levels of HbA1c, lower levels of HDL cholesterol and BCVA, and a history of PRP was more frequent compared to patients without DR.

Table 1 Patient characteristics according to the groups 'no DR', 'mild and moderate NPDR' and 'severe NPDR and PDR'

	No DR n=156	Mild-moderate NPDR n=304	Severe NPDR-PDR n=43
Demographics			
Gender, male, n (%)	69 (44%)	147 (49%)	16 (37%)
Age, years (mean ± SD)	50 ± 15	53 ± 13*	47 ± 15
Diabetes duration, years (mean ± SD)	28 ± 15	32 ± 12*	30 ± 11
Clinical characteristics			
HbA1c, mmol/mol (mean ± SD)	61 ± 11	66 ± 13**	64 ± 14*
Systolic blood pressure, mmHg (mean ± SD)	130 ± 14	132 ± 15	133 ± 17
Diastolic blood pressure, mmHg (mean ± SD)	73 ± 10	72 ± 11	72 ± 9
Body mass index, kg/m^2 (mean ± SD)	26 ± 4	27 ± 5	27 ± 5
Total cholesterol, mmol/l (mean ± SD)	4.7 ± 0.8	4.7 ± 0.9	4.8 ± 0.8
HDL cholesterol, mmol/l (mean ± SD)	1.56 ± 0.40	1.44 ± 0.39*	1.35 ± 0.35*
Microalbuminuria, n (%)	27 (17%)	60 (20%)	8 (19%)
Ophthalmological characteristics			
BCVA, logMAR (median, IQR)	0.00 (-0.08 – 0.10)	0.00 (-0.08 – 0.10)	0.10 (0.00 – 0.40)*
BCVA, Snellen equivalent (median, IQR)	20/20 (20/25 – 20/17)	20/20 (20/25 – 20/17)	20/25 (20/50 – 20/20)
History of panretinal photocoagulation, n (%)	35 (22%)	108 (36%)*	22 (52%)**
Color fundus photography features			
Presence of hard exudates, n (%)	0 (0%)	48 (16%)**	15 (35%)**
Optical coherence tomography features			
Number of hyperreflective foci, (mean ± SD)	1.6 ± 1.8	2.4 ± 2.6*	3.5 ± 4.4**
Central retinal thickness, µm (mean ± SD)	280 (261 – 295)	280 (260 – 296)	284 (260 – 345)**
Cysts, n (%)	3 (2%)	26 (9%)*	11 (26%)**
Subretinal fluid, n (%)	0 (0%)	2 (1%)	5 (12%)**
Disorganization of retinal inner layers, n (%)	15 (10%)	27 (9%)	5 (12%)
External limiting membrane disruption, n (%)	5 (3%)	6 (2%)	1 (2%)
Ellipsoid zone disruption, n (%)	5 (3%)	6 (2%)	1 (2%)

*P<0.05; ** P<0.001. Abbreviations: n=number; SD=standard deviation; IQR=interquartile range; DR=diabetic retinopathy; NPDR=non-proliferative diabetic retinopathy; PDR= proliferative diabetic retinopathy; HDL= high-density lipoprotein; BCVA=best corrected visual acuity logMAR=logarithm of the minimum angle of resolution

There was good agreement for the number of HF between the two graders, with an interrater coefficient of 0.86, 95% CI [0.81-0.90]. There was a weak, but significant correlation for the number of HF between both eyes of one patients (r_p 0.310, p<0.001). HF were detected in 414 eyes (82% of total), throughout all stages of DR, and scattered through all studied retinal layers (Figure 1). HF were detected in 96 out of 156 eyes (62%) without DR, in 125 out of 177 eyes (71%) with mild NPDR, in 108 out of 127eyes (85%) with moderate NPDR, in 21 out of 26 eyes (81%) with severe NPDR, and in 14 out of 17 eyes (82%) with PDR. Compared to no DR (1.6 ± 1.8), the number of HF was significantly higher in moderate NPDR (3.0 ± 3.1, p=0.001) and PDR (3.0 ± 2.1, p=0.019), after correction of the p-value for the interaction with the presence of central DME. No significant difference was found between the number of HF in patients with mild NPDR (2.0 ± 2.1, p=0.058) or severe NPDR (3.9 ± 5.4, p=0.801) versus no DR (Figure 2A). Higher numbers of HF were found for either mild, moderate or severe DME when compared to no DME (3.9 ± 2.6, p<0.001; 4.0 ± 3.3, p<0.001 and 7.4 ± 5.7, p<0.001 versus 1.7 ± 1.7 respectively, Figure 2B). In addition, we found higher numbers of HF in patients with prior PRP than in those without prior PRP (3.3 ± 3.0 versus 1.7 ± 2.2, p<0.001). When patients presented with hard exudates, the number of detected HF was higher than when no hard exudates were present (4.6 ± 4.7 versus 1.9 ± 2.0, p<0.001, Figure 2C).

We then assessed the relationship between clinical characteristics and number of HF. In univariable analysis, we found a longer diabetes duration (p=0.002), a lower HDL cholesterol (p=0.005), and the presence of microalbuminuria (p=0.001) to be significantly associated with higher levels of HF (Table 2). In multivariable analysis, longer diabetes duration (p=0.029), lower HDL cholesterol (p=0.005), and the presence of microalbuminuria (p=0.005) remained significantly associated with a higher number of HF (Table 2).

To gain insight in the relationship between HF and other morphological characteristics that can be distinguished on SD-OCT, we used mixed model analysis with HF as dependent outcome measure. We found significant associations for central retinal thickness (p<0.001), the presence of cysts (p<0.001), subretinal fluid (p<0.001), DRIL (p<0.001), ELM disruption (p=0.003) and EZ disruption (p=0.004) in univariable analysis. In multivariable analysis, central retinal thickness (p=0.004), the presence of cysts (p<0.001), subretinal fluid (p=0.001), and ELM disruption (p=0.018) were significantly associated with HF (Table 3).

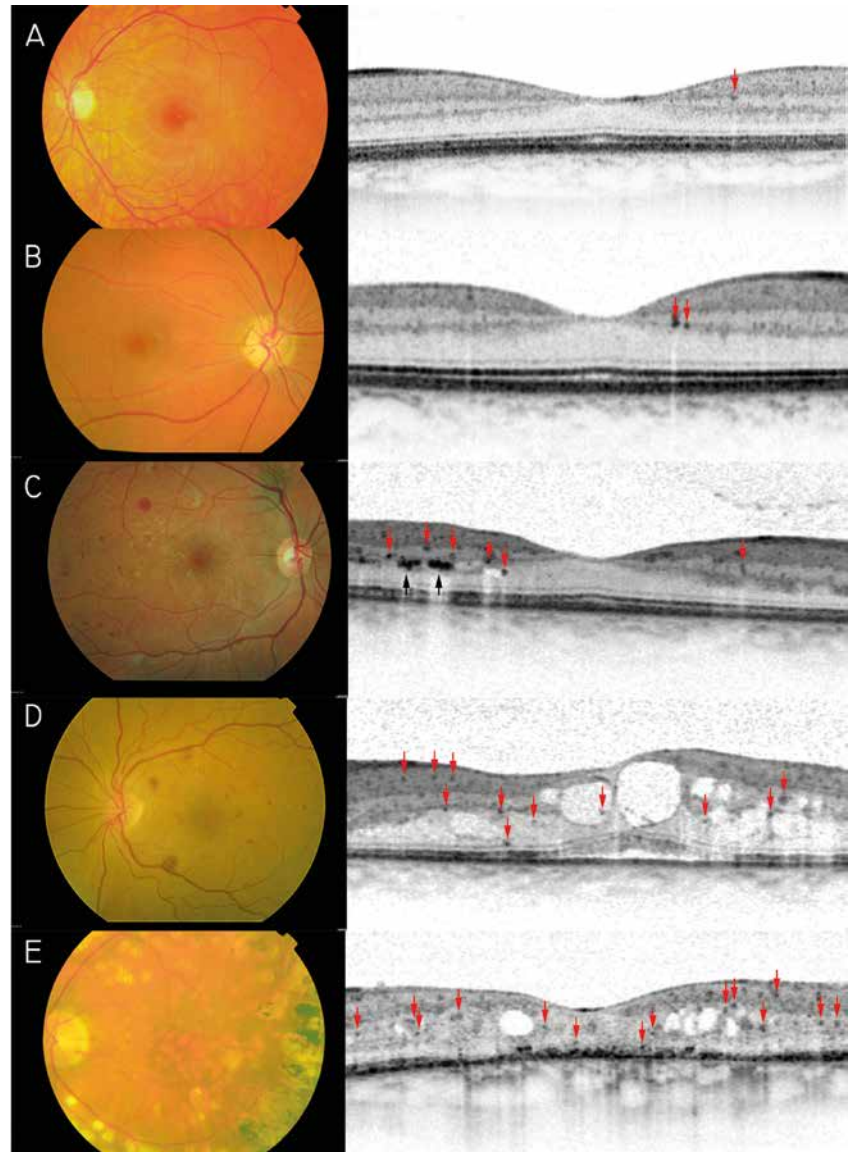

Figure 1 Color fundus photographs with corresponding 3mm fovea-centered optical coherence tomography-scan, showing representative examples of (**A**) no DR; (**B**) mild DR; (**C**) moderate DR, showing hard exudates on both SD-OCT and CFP, as indicated by the black arrows; (**D**) severe DR with severe macular edema; (**E**) longstanding macular edema with disruption of the external limiting membrane and inner segment/outer segment layer. Red arrows indicate hyperreflective foci.

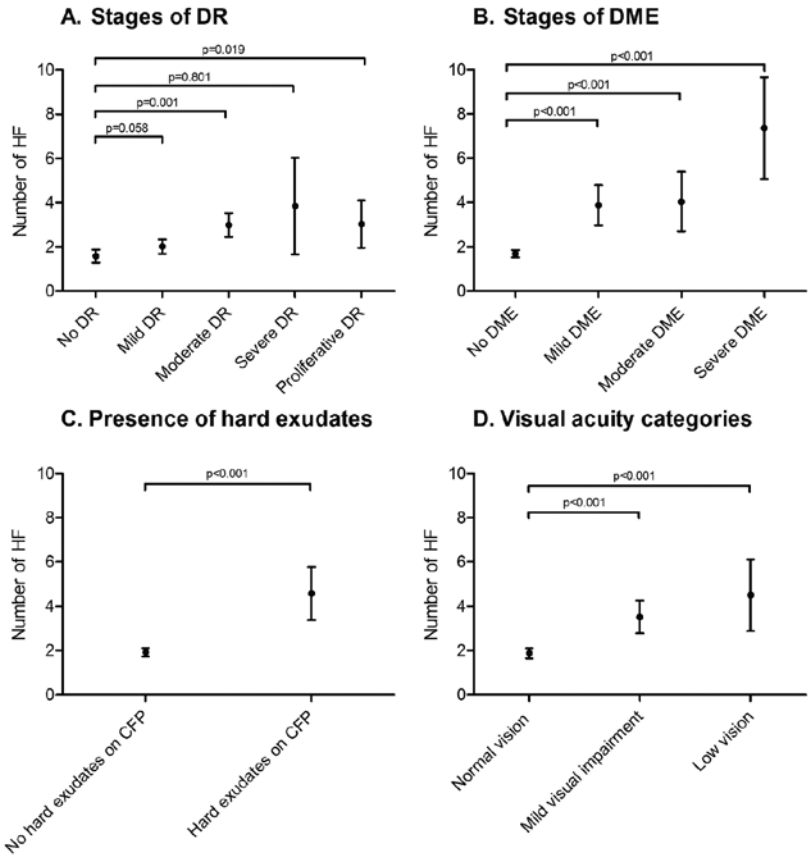

Figure 2 Distribution of hyperreflective foci, according to: (**A**) different stages of DR; (**B**) different stages of DME; (**C**) the presence of hard exudates; (**D**) visual acuity categories. Abbreviations: HF=hyperreflective foci; DR=diabetic retinopathy; DME=diabetic macular edema; CFP=color fundus photography.

Table 2 Univariable and multivariable linear mixed model analysis for the association of clinical variables with the number of HF.

	Estimate	95% CI	P-value
Univariable			
Gender	0.40	-0.13 – 0.94	0.139
Age	0.01	-0.01 – 0.03	0.210
Diabetes duration	0.03	0.01 – 0.05	0.002*
HbA1c	0.00	-0.03 – 0.02	0.738
Systolic blood pressure	0.02	0.00 – 0.04	0.062
Diastolic blood pressure	0.00	-0.03 – 0.02	0.968
Body mass index	0.01	-0.06 – 0.07	0.864
Total cholesterol	-0.09	-0.40 – 0.23	0.593
HDL cholesterol	-1.04	-1.72 – -0.37	0.003*
Microalbuminuria	1.00	0.42 – 1.59	0.001*
Multivariable			
Diabetes duration	0.021	0.002 – 0.040	0.029*
HDL cholesterol	-0.867	-1.468 – -0.267	0.005*
Microalbuminuria	0.839	0.257 – 1.422	0.005*

*P<0.05. Abbreviations: CI=confidence interval; HDL=high-density lipoprotein

The number of HF was significantly higher in patients with mild-moderate visual impairment (3.5 ± 1.9) or low vision (4.5 ± 2.9), when compared to patients with normal vision (1.9 ± 2.3, p<0.001 for both comparisons, Figure 2D). There was no significant difference in number of HF between patients with mild-moderate visual impairment and low vision patients (p=0.148). To further investigate the relationship between HF and phenotypic characteristics on SD-OCT with BCVA, we performed mixed model analysis. In univariable analysis, we found a higher number of HF, the presence of cysts, DRIL, ELM disruption and EZ disruption to be significantly associated with worse BCVA (Table 4). In multivariable analysis, we found independent associations between higher numbers of HF, and the presence of DRIL and ELM disruption with worse BCVA (Table 4).

Table 3 Correlation between the number of hyperreflective foci and other optical coherence tomography characteristics

	Estimate	95% CI	P-value
Univariable			
Central retinal thickness	0.02	0.01 – 0.02	<0.001*
Cysts	4.82	4.08 – 5.57	<0.001*
Subretinal fluid	6.84	4.95 – 8.73	<0.001*
DRIL	1.70	0.89 – 2.51	<0.001*
ELM disruption	2.31	0.81 – 3.80	0.003*
EZ disruption	2.22	0.73 – 3.72	0.004*
Multivariable			
Central retinal thickness	0.01	0.00 – 0.01	0.004*
Cysts	3.89	3.11 – 4.68	<0.001*
Subretinal fluid	3.20	1.33 – 5.07	0.001*
DRIL	0.72	-0.01 – 1.45	0.054
ELM disruption	1.76	0.31 – 3.22	0.018*

Central retinal thickness was evaluated as a continuous variable, explaining why the estimate is much lower in comparison to the other variables. In multivariable analysis, EZ disruption was excluded, because of the high correlation with ELM disruption and its relatively lower estimate in univariable analysis.
*P<0.05; DRIL=disruption of retinal inner layers; ELM=external limiting membrane; EZ=ellipsoid zone.

Table 4 Univariable and multivariable linear mixed model for the association of phenotypic characteristics on SD-OCT and logMAR BCVA

	Estimate	95% CI	P-value
Univariable			
Number of HF	0.018	0.010 – 0.025	<0.001*
Cysts	0.107	0.031 – 0.182	0.006*
Central retinal thickness	0.000	-0.001 – 0.001	0.916
Subretinal fluid	0.087	-0.089 – 0.263	0.331
DRIL	0.301	0.229 – 0.372	<0.001*
ELM disruption	0.762	0.645 – 0.878	<0.001*
EZ disruption	0.739	0.622 – 0.857	<0.001*
Multivariable			
Number of HF	0.010	0.003 – 0.016	0.005*
DRIL	0.152	0.844 – 0.220	<0.001*
ELM disruption	0.655	0.537 – 0.774	<0.001*

In multivariable analysis, EZ disruption was excluded, because of the high correlation with ELM disruption and its relatively lower estimate in univariable analysis.
*P<0.05; CI=confidence interval; HF=hyperreflective foci; DRIL=disorganization of retinal inner layers; ELM=external limiting membrane; EZ=ellipsoid zone.

DISCUSSION

With this study, we provide insight in the distribution of HF across different stages of DR and DME in patients with type 1 DM. We assessed the associations between HF and clinical characteristics, including BCVA, as well as other OCT parameters.

The potential clinical relevance of HF was demonstrated in previous studies, showing that HF may be a predictor of treatment response to intravitreal anti-VEGF or corticosteroid therapy in DME.[20, 22] In the current study, we observed significantly higher numbers of HF in all stages of DME versus patients without DME. The number of HF was especially high in severe DME, suggesting the presence of a strong link between HF and exudative retinal disease. We deem it unlikely that HF will replace other commonly used morphological biomarkers that predict or define treatment success, such as CRT, but they may be used in combination with other markers, thereby optimizing monitoring and potentially predicting treatment strategies. An advantage of HF with their high reflectance is that they may be more suitable for automated detection and inclusion in prediction models than other, less quantifiable, biomarkers that have shown associations with treatment response, such as DRIL.[23, 24] Moreover, the manual quantification of HF could be accelerated by the evaluation of en face OCT images, which is faster than grading single B-scan images.[25]

In this study, we furthermore aimed to relate the amount of HF to the severity of DR. We observed a trend for the association between higher numbers of HF and DR severity, however, these differences did not all reach statistical significance. For the early stages, our findings on the distribution of HF are in accordance with Vujosevic *et al.*, who reported a higher number of HF in patients with NPDR than in diabetic patients without DR.[12] In patients with severe DR, there was a large variation in the number of HF, potentially due to the high prevalence of DME in this group. After correction for the presence of DME, the size of the remaining subgroup may have been too small to detect significant differences. Another reason why the relationship between HF and DR severity in our study was not strong, may be that the observed area was limited to the central 3 mm surrounding the fovea. This area was selected because of its importance for visual function, and because the labor-intensive nature of HF grading impedes the evaluation of multiple B-scans per person. This is logical for diseases affecting the macula, such as DME, however, diabetic retinal disease activity is not restricted to the macula, and peripheral disease activity may not correspond to macular disease activity. As for now, we may conclude that macular HF are not a clinically relevant biomarker for DR severity.

We further substantiated the relationship between HF and disease activity by the correlation we found between HF and visual acuity, in consistence with previous reports.[17, 20, 26] The occurrence of HF in diabetic patients may act as an early warning sign for oncoming vision loss. The exact nature of the relation of HF and visual acuity is yet unclear. It is possible that DME is the reason for visual loss in patients with high numbers of HF, but this cannot fully account for the relationship we found: HF were independently associated with BCVA loss irrespective of the hallmarks of DME (CRT and the presence of cysts). In previous studies, a direct link between HF and visual impairment through degenerated photoreceptor cells was suggested.[17] However, HF were associated with visual acuity, independent of EZ disruption, and we therefore hypothesize that other processes pathognomonic for DR may affect retinal tissue integrity, such as activated microglial cell aggregations and precursors of hard exudates.[10, 12]

Regarding clinical patient characteristics, we found that a longer diabetes duration, lower levels of HDL cholesterol, and the presence of microalbuminuria were associated with higher numbers of HF. The HF in these type 1 DM patients appeared to be related to several other retinal morphological abnormalities on SD-OCT, including CRT, cysts, subretinal fluid, and ELM and EZ disruption, as is in line with previous reports.[17, 26-28] The variables that associated with HF are also known risk factors for DR or DME severity. To a certain extent, HF, DR severity and the investigated clinical variables may all be related to each other, and further research should be conducted to distinguish what is cause and what is consequence in this case.

A strength of this study is the large sample size with patients representing all stages of DR and DME. In addition, we used a homogeneous cohort with carefully phenotyped patients with type 1 diabetes. Our study also has several limitations. Because we only studied patients with type 1 diabetes, our findings should be replicated in other diabetic cohorts. However, due to the shared pathophysiological mechanisms, we hypothesize that it is likely that similar results could be found in type 2 diabetes. Another limitation is that the grading of HF is challenging and currently not standardized, which makes comparison to other studies difficult. Nevertheless, we found good interrater agreement, implicating that with this grading protocol replicable results can be obtained. In addition, the use of one fovea-centered B-scan for HF evaluation should be considered a limitation, as the number of HF may vary between B-scans. Automated detection of HF will make the detection of HF more objective, easier to apply in multiple B-scans or more peripheral areas, and more feasible for application in clinical practice.

In conclusion, our results confirm that HF are associated with diabetic retinal disease.. Future research should be directed towards the relationship between HF and progression of DR and histopathological studies should be conducted to unravel the origin of HF. Although hyperreflective foci were directly correlated to RPE cells and lipid filled non-RPE in age-related macular degeneration, their exact entity in diabetic retinal disease is still unknown.[29] Combining this knowledge could contribute to further development of personalized medicine through the implementation of HF as a prognostic biomarker for oncoming vision loss, and evaluation of treatment response to current and future treatments.

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3.2

Automatically detected hyperreflective foci in optical coherence tomography as a prognostic biomarker

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ABSTRACT

Importance: Hyperreflective foci (HRF), as seen in optical coherence tomography (OCT), are hypothesized to be predictive of treatment response in age related macular degeneration (AMD) and diabetic macular edema (DME). However, manually quantifying the number of HRF present in an OCT volume is extremely time-consuming.

Objective: To develop a deep learning algorithm for the automated detection and quantification of HRF in OCT volumes that allows for practical usage of HRF quantification as a biomarker.

Design, setting and, participants: The proposed algorithm is based on a so-called fully convolutional neural network (FCNN), where HRF are detected by performing a semantic segmentation of the OCT volume i.e., determining the probability for each pixel in an OCT scan as belong to a HRF or not. The proposed method was evaluated in a multicenter dataset consisting of AMD and DME OCT images, and was compared to manual annotations by two human observers. Finally, in a follow-up dataset of patients treated with anti-vascular endothelial growth factor (VEGF) for DME, we investigate if the proposed method can be effectively applied to predict treatment response based on the automatically detected HRF.

Main outcome measure: Performance of the developed FCNN is visualized using Free-response receiver operating characteristic (FROC) analysis and expressed by the intraclass correlation coefficient. Finally, the difference in number of HRF in patients with good response to anti-VEGF versus insufficient response is assessed.

Results: In the AMD dataset the proposed method achieved an ICC of 0.821, comparing favorably to the performance of the human observer with an ICC of 0.856. Similarly, for the DME dataset an ICC of 0.719 was obtained, approaching the performance of the human observer with an ICC of 0.792. Moreover, in patients with a good response to treatment, i.e., a decrease in central retinal thickness of 10% and an improvement in visual acuity of 0.1 $\geq$ logMAR, the proposed automated method detected more HRF at baseline compared to patients with insufficient treatment response (21.6$\pm$9.5 vs. 12.3$\pm$12.4, p=0.041).

Conclusion and relevance: The proposed automated method achieves HRF detection and quantification performance similar to human performance levels. This study demonstrates that HRF detected by the proposed method can be used as a prognostic biomarker, thereby potentially allowing faster and more accurate treatment assessment.

INTRODUCTION

In recent years, ophthalmological clinical practice has been revolutionized by the widespread adoption of spectral domain optical coherence tomography (SD-OCT) imaging. SD-OCT imaging provides a noninvasive, high resolution, three-dimensional visualization of the retina, allowing for the identification of different morphologic features. With the increase in resolution and imaging quality, improved differentiation of retinal structures became possible, allowing the identification of small structures such as hyperreflective foci (HRF). HRF are defined as discrete, well-circumscribed lesions with equal or greater reflectivity than the retinal pigment epithelium (RPE) band on SD-OCT[1]. Several studies suggest HRF to be correlated with disease severity, disease progression or could even be used as a prognostic biomarkers for treatment prediction[2, 3, 5, 8-17]. The presence and number of HRF was found to be related to the severity of age-related macular degeneration (AMD)[17,18]. Also, HRF are found to be predictive for the development of geographic atrophy (GA)[8,10,11] and choroidal neovascularization (CNV)[12] in AMD. Additionally, it has also been shown that HRF are predictive for treatment response in neovascular AMD indicated by a positive correlation between the number of foci and a decrease in central subfield thickness[13, 14], subretinal fluid (SRF) and intraretinal fluid (IRF)[15], or as a direct correlation to visual acuity[16]. Similarly in diabetic macular edema (DME), the number of HRF has shown to correlate with photoreceptor damage after anti–vascular endothelial growth factor (anti-VEGF) treatment[6, 9] and is positively correlated to complete resolution of edema[4]. Moreover, HRF are suggested to be an early sign for subclinical breakdown of the blood–retina barrier in DME[3]. Different hypothesis about the etiology of HRF exist[2]: while some authors suggest that they could be degenerated photoreceptor cells[6] or migrating RPE cells[7] others have argued that HRF are aggregates of activated microglia, cells involved in the retinal inflammatory response[8]. In DME, they have furthermore been hypothesized to be lipid extravasations acting as subclinical hard exudates[3-5]. A common denominator in studies addressing HRF is the dependency on manual detection and quantification of HRF. This can be a major limiting factor for the real-life implementation of HRF–based biomarkers as determining the presence and the number of HRF present in an SD-OCT volume is extremely time-consuming. Computer aided detection systems capable of automatically detecting and quantifying HRF might be the solution to this problem and open up the path for the usage of HRF as a biomarker in high throughput studies in clinical practice and in research settings.

In the last years, computer-based algorithms have shown their potential in the automatic analysis of retinal images and, particularly, in SD-OCT volumes. Algorithms for OCT analysis have been developed for the detection of anatomical

landmarks such as the fovea[19], and for the segmentation of the different intraretinal layers, either for healthy or mildly affected retinas[20], or for the total retina in the presence of severe disruptive pathology[21]. Automated methods for specific retinal lesions have also been proposed, e.g., segmentation of SRF[22], IRF[23], segmentation of GA[24] and detection of drusen[25]. In a recent study several biomarkers including HRF were automatically extracted to predict progression of AMD[26]. In this study, HRF were detected using a machine learning based approach trained on 150 annotated B-scans from 40 OCT volumes. While this study shows the possibility of using HRF as a biomarker, a thorough performance evaluation providing insight in the applicability and efficacy of algorithms for HRF detection is lacking. We therefore developed and evaluated a deep learning algorithm for the automated detection and quantification of HRF in SD-OCT volumes. The detection and quantification performance is evaluated in a large multicenter dataset consisting of AMD and DME cases annotated by two experienced human observers. Finally, we investigate if the proposed algorithm can be effectively applied to predict the response to treatment based on the automatically detected HRF.

METHODS

This study aimed to develop and validate a deep learning approach for the detection and quantification of HRF in SD-OCT volumes. The goal of this study was to compare the output of the proposed system to human graders, and ultimately to investigate if automated detection of HRF is reliable enough to use as a prognostic biomarker.

Data

For this study 100 OCT volumes from 79 patients with neovascular **AMD** were randomly selected from the European Genetic Database (EUGENDA, http://eugenda.org), a large multi-center database for clinical and molecular analysis of AMD[27, 28]. An additional multi-center dataset of 60 OCT volumes from 40 patients with center-involving **DME** were selected from the outpatient department of Ophthalmology of the Radboud University Medical Center and the Cologne University Eye Clinic. Additionally, a follow-up dataset comprised of 51 eyes from 38 patients with diabetes mellitus type 2 was included. For each eye OCT imaging was performed before and after treatment with intravitreal injections of bevacizumab, adding up to a total of 102 OCT volumes. This dataset was used in a previous study to investigate the possible correlations between the number of HF and treatment response. Further details regarding the inclusion/exclusion criteria and study design can be found in the associated publication[9]. Finally, an additional dataset of 157

OCT volumes from 74 patients with DME (61), AMD (72), or controls (24) were included for development and optimization of the deep learning algorithm, these OCT volumes were selected from the clinics at the department of Ophthalmology of the Radboud University Medical Center and the Cologne University Eye Clinic.

OCT volumes in all datasets were acquired using a Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) The study adhered to the tenets of the Declaration of Helsinki, and was approved by the institutional review boards and the ethics committees of all centers involved. Written informed consent was received from all participants.

Grading procedure

Annotation criteria

HRF were defined as small, well-circumscribed dense particles with higher reflectivity than the background. Annotation was performed in all layers ranging from the inner nuclear layer (INL) up to the external limiting membrane (ELM). The nerve fiber layer (NFL), the ganglion cell layer (GCL) and the inner plexiform layer (IPL) were excluded from the analysis as the intrinsic high reflectivity of these layers impedes the discrimination of HRF. For the same reason the layers below the ELM were excluded from analysis. Hyperreflective material with a diameter of $\geq$ 100 µm was also excluded, as in patients with DME these are hypothesized to be hard exudates.

Manual annotation of hyperreflective foci

Manual annotation of HRF was performed using a computer-assisted annotation platform which allows for zooming, panning, and modification of the contrast and brightness levels. Annotations were only performed within the central 3 mm of the B-scan containing the fovea. Manual annotations were performed by two trained and experienced independent graders (LA, VS) from two different institutes, i.e., the Cologne University Eye Clinic and the Radboud University Medical Center, respectively. The graders were masked to all clinical information. The AMD and DME dataset were annotated by each grader independently. Grader 1 was selected as the reference standard for evaluation of the proposed method while grader 2 was selected for comparative analysis of human performance and interrater agreement. The DME follow-up dataset was annotated by the two graders in consensus.

Deep learning based algorithm

The algorithm used for the automated detection of HRF is based on deep learning, specifically, a deep fully convolutional neural network[26] (FCNN), where HRF are detected by performing a semantic segmentation of the OCT volume, i.e., determining the probability for each pixel in an OCT scan as belong to a HRF or not.

The network automatically detects and quantifies HRF in OCT B-scans and can process an entire volume by iterative application.

In classical machine learning, determining this probability is typically achieved by considering many different distinguishing imaging characteristics (features) based on clinical knowledge to make a classification decision. The most difficult and essential step in developing such a classical machine learning system is translating and embedding this clinical knowledge into a robust algorithm that quantifies these imaging features. The large variation in HRF appearance (contrast, brightness, etc.) together with the noise introduced by the SD-OCT device makes development of robust handcrafted features a challenging task.

For the proposed algorithm, which is based on deep learning, this problem of developing handcrafted features based on clinical knowledge is completely circumvented. In deep learning, features are inferred from the data itself, i.e., the proposed network *learns* the distinguishing characteristics between HRF and other retinal tissue directly from the data, thereby avoiding the difficult step of manually translating and interpreting the prior clinical knowledge. The large modeling capacity of the proposed FCNN allows it to learn a wide range of different complex features, capable of capturing the wide variability in HRF appearance.

The proposed FCNN requires *training* to learn the features distinguishing HRF from other retinal tissue. This training is performed using the additional independent training dataset consisting of 157 OCT volumes from 74 patients. The parameters of the FCNN are optimized by performing backpropagation on the examples in the training data. The network learns directly from the provided examples how to characterize a HRF, and how to distinguish it from the background and other confounding structures.. Extensive details regarding the FCNN network architecture and the training procedure can be found in the appendix.

After applying the proposed deep learning algorithm we obtain a probability for every pixel in an OCT volume of belonging to a HRF. After discarding pixels with a probability lower than a certain threshold $th \in [0,1]$, we obtain a binary image containing clusters of detected pixels, i.e., candidate foci regions. For each of the candidate foci regions we determine the location of maximum probability, which is then selected as the final detected HRF location. The threshold *th* acts as a trade-off between the sensitivity and specificity of the proposed system, i.e., a lower threshold increases the sensitivity of the algorithm, but consequently also increases the number of false detections. Additional details regarding the detection of the local maxima can be also found in the appendix.

Algorithm evaluation

Detection of hyperreflective foci

To assess whether the proposed algorithm can accurately detect HRF, we compare the automatically obtained detections to the reference standard comprised of 60 OCT volumes from 40 patients with DME and 100 OCT volumes of 79 patients with AMD. Additionally, we compare the manual annotations by the second grader to the reference standard to assess the interrater agreement. Detection performance is assessed in the inner retina (INL), the outer retina (OPL, ONL), and both combined. To determine detection performance, we first match each *detected* HF to the closest *annotated* HF in the reference standard. A match can only be formed if the distance between the two is < 50 µm, i.e., less than the half the maximum diameter of a foci. If a detection and an annotation are a match, it is counted as a true positive. Otherwise, it is counted as a false positive. Finally if an annotation does not have a match, it is counted as a false negative. The detection performance is visualized using free-response receiver operating characteristic (FROC) analysis. An FROC curve provides a visual overview of the performance at different operating points, i.e., different values of the threshold *th*.

Quantification of hyperreflective foci

To investigate the ability of the proposed method to make an accurate quantification of the number of HRF in the retina, we compare the total number of detected HRF to the reference standard comprised of 60 OCT volumes from 40 patients with DME and 100 OCT volumes of 79 patients with AMD. Additionally, we compare the reference standard to the second grader. Detection performance is assessed in the inner retina (INL), the outer retina (OPL, ONL), and both combined. For this experiment only the total number of detected/annotated HRF is taken into account. HRF quantification performance is assessed by the intraclass correlation coefficient (ICC), a summarizing measure to indicate how well the number of HRF for the proposed method and the reference standard match. Additionally, the difference in total detected HRF is visualized using Bland-Altman plots comparing the proposed method and the second grader to the reference standard.

Automated detection of hyperreflective foci as a prognostic biomarker

In order to assess whether automated detection of HRF can be effectively used as a prognostic biomarker, we stratify the DME follow-up dataset into groups according to good and insufficient treatment response. Treatment response is assessed in two ways, by anatomical improvement, i.e., a decrease in central retinal thickness of ≥10%, and functional improvement, i.e., an improvement in visual acuity of 0.1 ≥ logMAR. 37 eyes (72%) showed a sufficient CRT decrease, while 16 eyes (31%) showed sufficient improvement in visual acuity. 13 eyes (25%) met both criteria. For

all eyes baseline and 3-month follow-up OCT data was available. After stratifying the dataset into good and insufficient treatment response groups, we assessed the association between baseline number of HRF and treatment response using an independent sample T-test. This analysis was performed using SPSS version 24 (SPSS, Chicago, IL, USA). P-values <0.05 were considered statistically significant.

RESULTS

Detection of hyperreflective foci

The FROC curves visualizing the HRF detection performance in the DME dataset for the inner retina, the outer retina and both combined are shown in Figure 1a, Figure 1c and Figure 1e, respectively. The performance of the second grader is indicated by the green cross. For the inner retina the second grader achieves a sensitivity of 0.69 at 2.03 false positives per B-scan. At this false positive rate the proposed automated method has a sensitivity of 0.763. For the outer retina the second grader achieves a sensitivity of 0.64 at 0.79 false positives per B-scan. At this false positive rate the proposed method has a sensitivity of 0.624. When taking the entire retina into account, the second grader achieves a sensitivity of 0.71 at 2.72 false positives per B-scan. At this false positive rate the proposed method has a sensitivity of 0.734.

Similarly, in Figure 1b, Figure 1d and Figure 1f, the FROC curves are visualized for the AMD dataset for the inner retina, the outer retina and both combined, respectively. The second grader achieves a sensitivity of 0.66 at 2.05 false positives per B-scan when analyzing the inner retina. The proposed method has a sensitivity of 0.570 at this false positive rate . For the outer retina the second grader achieves a sensitivity of 0.62 at 2.43 false positives per B-scan. At this false positive rate the proposed method has a sensitivity of 0.543. When taking the entire retina into account, the second grader achieves a sensitivity of 0.67 at 4.34 false positives per B-scan. At this false positive rate the proposed method has a sensitivity of 0.582.

Quantification of hyperreflective foci

The Bland-Altman plots indicating the difference in the number of detected HRF in the DME dataset for the proposed method compared to the reference standard for the inner retina, the outer retina and both combined, are shown in Figure 2a, Figure 2b and Figure 2c, respectively. The number of HRF detected by the second grader compared to the reference standard for the inner retina, the outer retina and both combined, are shown in Figure 2d, Figure 2e and Figure 2f, respectively. Similarly, in Figure 3a, Figure 3b and Figure 3c, the difference in the number of detected HRF in the AMD dataset for the inner retina, the outer retina and both

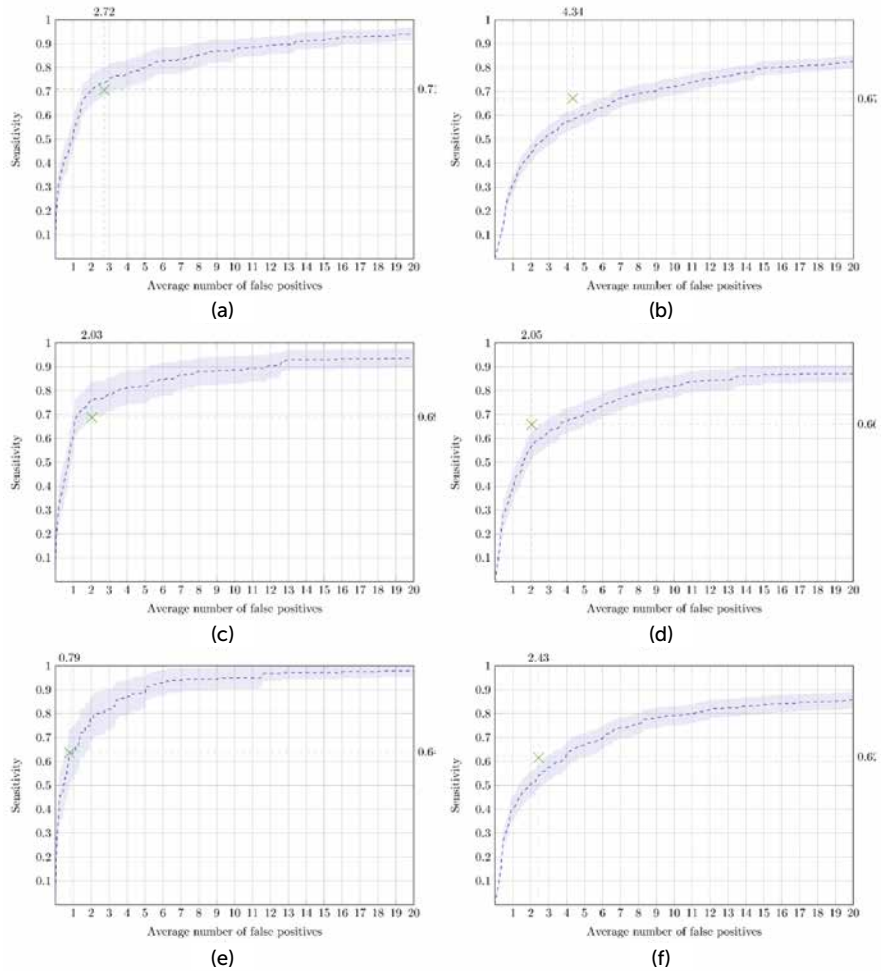

Figure 1 HRF detection performance: FROC curves of the proposed machine learning algorithm for detection of HRF in the DME dataset for (**a**) the total retina, (**c**) the inner retina, and (**e**) the outer retina. FROC curves for the AMD dataset in the (**b**) the total retina, (**d**) the inner retina, and (**f**) the outer retina. The performance of the human grader is indicated by the green cross.

combined are shown, respectively. The number of HRF detected by the second grader compared to the reference standard for the inner retina, the outer retina and both combined, are shown in Figure 3d-f, respectively. The obtained intraclass correlation coefficients for the proposed method and the second grader compared to the reference standard for the inner retina, outer retina and both combined in the DME and AMD dataset, are shown in Table 1.

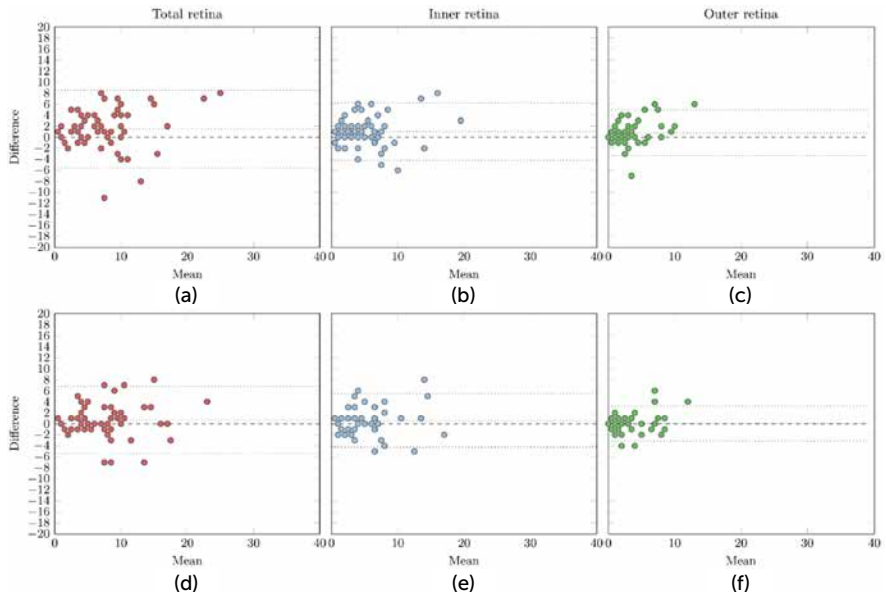

Figure 2 HRF quantification in DME: Bland-Altman plots visualizing the quantification performance of the proposed machine learning algorithm in the DME dataset compared to the reference standard for (**a**) the total retina, (**b**) the inner retina, and (**c**) the outer retina. The performance of the second grader compared to the reference standard is shown for (**d**) the total retina, (**e**) the inner retina, and (**f**) the outer retina. The dashed line indicates no systematic difference.

Automated detection of hyperreflective foci as a prognostic biomarker

Figure 4a-c show the number of HRF at baseline for the group of good responders and the group with insufficient response. When only considering anatomical improvement, a higher average number of HRF were found at baseline for eyes that had a good response to treatment as opposed to the group with an insufficient decrease in CRT, although statistically not significant (15.81 ± 10.28 vs. 11.36 ± 8.64, p=0.157). Similarly, a higher number of HRF were found at baseline for eyes with improved VA after treatment (18.69 ± 12.27 vs. 12.12 ± 7.60, p=0.024*). When considering both criteria for good treatment response, again a higher number of HRF were found at baseline for eyes that had a good response to treatment (20.31 ± 12.49 vs. 12.08 ± 7.65, p=0.014*).

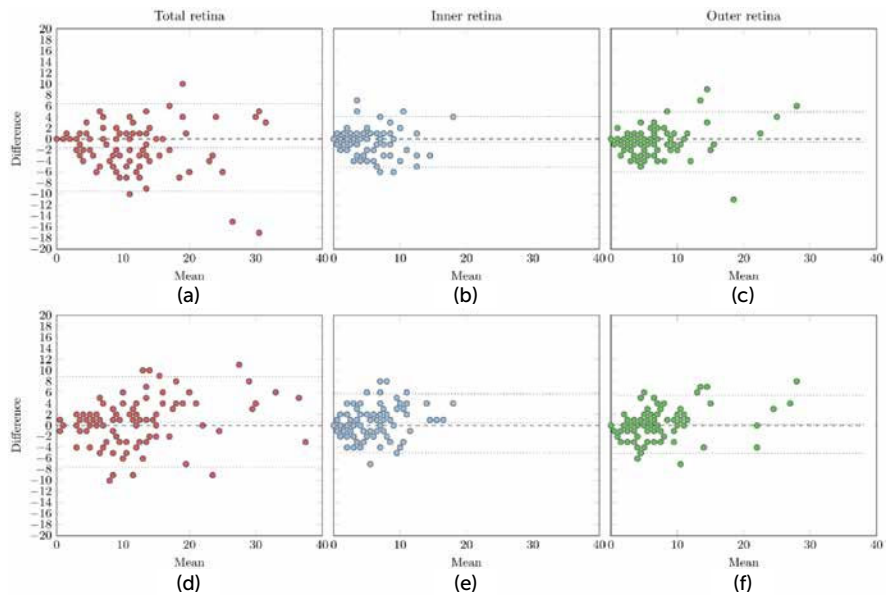

Figure 3 HRF quantification in AMD: Bland-Altman plots visualizing the quantification performance of the proposed machine learning algorithm in the AMD dataset compared to the reference standard for (**a**) the total retina, (**b**) the inner retina, and (**c**) the outer retina. The performance of the second grader compared to the reference standard is shown for (**d**) the total retina, (**e**) the inner retina, and (**f**) the outer retina. The dashed line indicates no systematic difference.

Table 1 HRF Quantification performance: Intraclass correlation coefficients for the proposed method and the second grader compared to the reference standard for the inner retina, outer retina and both combined in the DME and AMD dataset. 95% confidence intervals are indicated in brackets

	Proposed method		Second grader	
	AMD	*DME*	*AMD*	*DME*
Total retina	0.821 (0.718 – 0.884)	0.719 (0.538 – 0.831)	0.856 (0.793 – 0.900)	0.792 (0.672 – 0.871)
Inner retina	0.787 (0.698 – 0.852)	0.755 (0.596 – 0.853)	0.785 (0.696 – 0.850)	0.796 (0.676 – 0.874)
Outer retina	0.861 (0.799 – 0.905)	0.741 (0.579 – 0.843)	0.887 (0.836 – 0.922)	0.846 (0.754 – 0.906)

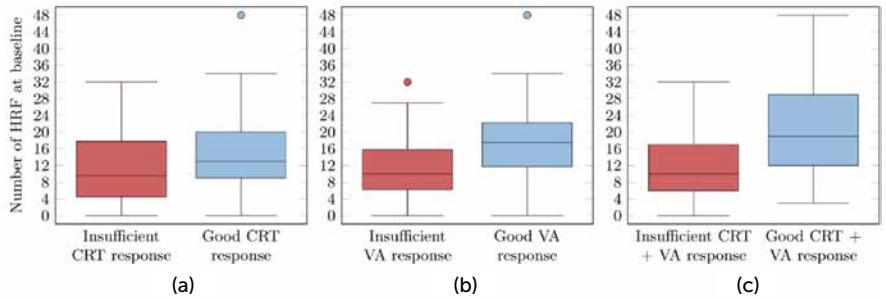

Figure 4 Difference in number of HRF at baseline for eyes with good response versus insufficient response: Box plots visualizing the difference in the number of HRF in groups based on (**a**) insufficient and good CRT response (p=0.157), (**b**) insufficient and good VA response (p=0.024*), and (**c**) insufficient and good combined CRT + VA response (p=0.041*).

DISCUSSION

In this study we assessed the performance of a deep learning algorithm for the automatic detection and quantification of HRF in SD-OCT volumes. Furthermore we investigated the possibility of using the automatically detected HRF as a prognostic biomarker for treatment response to anti-VEGF in DME. The experimental results show promising performance levels, for both detection and quantification of HRF, approaching those of trained physicians. Moreover, the number of HRF detected at baseline by the algorithm were associated with good response to treatment, in terms of CRT and VA, when compared to eyes with insufficient treatment response, indicating the possibility of using automatically detected HRF as a prognostic biomarker.

When comparing the obtained results in the DME dataset to the AMD dataset (Figure 1,2,3) differences can be observed in the performance of the algorithm, i.e., the algorithm performs substantially better in the DME dataset compared to the AMD dataset for the detection task, shown by the higher FROC curves in Figure 1. A similar decrease in performance can also be observed for the human graders. Considering the fact that DME and AMD are different diseases, presenting with different phenotypes, HRF may represent different entities in either disease and therefore also have different appearances. This could explain the difference in detection performance for both the algorithm and the human graders. Interestingly, for the quantification task a higher ICC is obtained in the AMD dataset, as shown in Table 1, and visualized in Figure 2 and 3. This can be explained by the fact that

detection and quantification are two different tasks, i.e., false positives and missed HRF do not matter in the quantification task as long as the total number is correct.

While scarce, other methods do exist for the identification of HRF. In recent years, a semi-automatic approach to quantify HRF in the vitreous was developed[29]. While using this method a difference between controls and eyes with DME was found, semi-automatic quantification makes the grading of large datasets infeasible. In another recent study several biomarkers including HRF were automatically extracted to predict progression of AMD[26]. In this study, HRF were detected using a machine learning based approach trained on 150 annotated B-scans from 40 OCT volumes. While this study shows the possibility of using HRF as a biomarker, a thorough performance evaluation providing insight in the applicability and efficacy of algorithms for HRF detection is lacking. Another method was developed based on a residual U-net deep learning architecture[30] showing good performance in segmenting HRF. The method does however not translate the segmented regions to individual HRF, i.e., a single number indicating the number of HRF is lacking. As the number of HRF is typically the variable of interest in studies finding a relation between HRF and disease progression, this is an important limitation of this algorithm. The algorithm proposed in this work does provide a fully automatic detection and quantification algorithm for HRF.

An interesting addition of the proposed algorithm arises from the use of deep neural networks. In deep neural networks the features are constructed based on what is most discriminative for HRF in the provided data. This means that the features that are learned do not necessarily have to be based on existing clinical definition and characteristics of HRF, i.e., it is possible that the algorithm has learned features to detect HRF that were previously unknown to or ignored by humans. Understanding what features the neural network has learned is currently an active area of research in the deep learning community.

A limitation in both the AMD and DME datasets used in this study is the relatively low interrater agreement between the two human observers in detecting HRF. This is visualized by the green crosses in Figure 1, indicating the sensitivity and average number of false positives per B-scan. This low interrater agreement is likely inherent to the difficulty of the task, i.e., variations in appearance and imaging characteristics increase the subjectivity in grading of HRF. Assuming the same level of disagreement is also present in the data used for training the algorithm, the interrater agreement serves as the maximum achievable performance for the proposed automated method. Given this upper limit, the proposed automated method reaches maximum performance in the DME dataset and approaches the performance of the second grader in the AMD dataset. However, even at this level of performance the proposed

method is capable of quantifying HRF to such a degree that the number of detected HRF can be effectively used as a prognostic biomarker.

In conclusion, we developed a fully automated system to detect and quantify HRF in SD-OCT volumes from patients with AMD and DME. The system proved to have performance compared to that of human observers and allows for fast quantitative HRF measurements that can possibly be used to predict treatment response.

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APPENDIX

Deep learning based algorithm

The proposed deep learning based algorithm automatically detects and quantifies HF in OCT B-scans and can process an entire volume by iterative application. First, an ensemble *E(B(x))* of fully convolutional neural networks (FCNN) is applied to obtain a semantic segmentation of a B-scan *B(x)*, i.e., it determines a probability *P(x)* for every pixel *x* in an OCT volume of belonging to a HF. Next, the obtained probability map *P(x)* is thresholded at threshold $th \in [0,1]$ to obtain a binary image *T(x)* containing clusters of detected pixels, i.e., candidate foci regions. Finally, we determine the local maxima in each cluster. The locations of the local maxima are then selected as the final detected HF. the individual steps will be discussed in more detail in the following subsections.

FCNN for the detection of candidate foci regions

For the initial detection of candidate foci regions we apply an ensemble of four individually trained FCNNs in parallel to increase performance and robustness. The ensemble *E(B(x))* is applied to a B-scan *B(x)* and produces a probability map *P(x)* indicating regions of high probability for containing HF, i.e.,

$$P(x) = E(B(x)) = \frac{1}{4}\sum_{i=1}^{i=4} P_i(x),$$

Where $P_i(x)$ are the outputs of the four individual FCNNs that constitute the ensemble, i.e.,

$$P_i(x) = C_i(B(x))$$

Each FCNN produces a probability map $P_i(x)$ that we average to merge the contribution of each FCNN. In contrast to traditional CNNs an FCNN does not use any fully connected layers, thereby preserving the spatial correlation between input and output[31]. This has the advantage that an FCNN can be applied efficiently to inputs of arbitrary size, and inference can be performed using whole images by dense feedforward computation, drastically increasing processing speed compared to patch-based networks. The architecture of each FCNN is identical, but each network is trained on a different training set. The proposed architecture is summarized in Table A1.

Table 1 Summary of the proposed network architecture used for the segmentation of candidate foci regions

Layer (i)	Filter size	type	channels	Receptive field
1	3x3	Convolution	32	3x3
2	3x3	Convolution	32	5x5
3	2x2	Max pooling	-	10x10
4	3x3	Convolution	64	12x12
5	3x3	Convolution	64	14x14
6	2x2	Max pooling	-	28x28
7	3x3	Convolution	128	31x31
8	-	Dropout	-	31x31
9	3x3	Convolution	128	33x33
10	-	Dropout	-	33x33
11	1x1	Convolution	64	33x33
12	1x1	Convolution	2	33x33

The network consists of the repeated application of 3x3 convolutions (C3), each followed by a batch normalization unit (BN) and a leaky rectified linear unit (ReLU) with leakiness 0.01, i.e.,

$$C3 \rightarrow BN \rightarrow ReLu$$

After every two convolutional layers a 2x2 max pooling operation with stride 2 is performed to increase the receptive field. After each max pooling step the number of feature channels is doubled as to not reduce the modeling capacity of the network too rapidly. After the last two 3x3 convolutional layers dropout with a probability 0.5 is applied to reduce overfitting of the network. Finally, the last two convolutional layers perform feature combination using 1x1 convolutions, similarly to a fully connected layer in a traditional CNN. At the output layer a softmax function is applied over the two classes, candidate foci and background.

As the network contains max pooling operations the resolution of the final output probability map is decreased. We apply the shift-and-stitch technique to recover the lost resolution[32].

The proposed network architecture has a final receptive field of 33x33 pixels, meaning that a neighborhood of 33x33 pixels (397.5 µm x 128.7 µm) surrounding a pixel is taken into account when determining the foci probability for a pixel. Considering HF are defined to have a diameter ≤ 100 µm in this study, this receptive field is chosen sufficiently large.

FCNN training procedure

The network parameters of the four FCNNs were optimized using four fold cross validation in the ***training data***, i.e., each FCNN is trained with 3 folds and validated on the remaining fold using a different combination of the four folds for each FCNN.

At every iteration, a batch of 200 image patches of size 33x33 are selected from the ***training data*** for optimization of the system parameters. A batch consists of 100 randomly selected patches centered on an annotated foci location, and 100 randomly extracted patches from the remaining background. A *dead zone* of 3 by 3 pixels around an annotated foci location is used where no background pixels can be selected. This avoids ambiguous background pixels to be extracted and prevents confusion of the network.

To artificially increase the variation in the training data and to increase robustness of the system, we applying a data augmentation strategy. The following data augmentations are randomly applied to every 33x33 image patch selected for training:

- Random rotation between -15 and +15 degrees
- Random mirroring
- Random multiplicative speckle noise with a magnitude between 0 and 0.4

The limits have been selected in such a way that after augmentation the resulting image patch is a plausible example observable in clinical practice.

Local maxima detection

After thresholding the obtained probability map *P(x)* at threshold *th* we obtain a binary image *T(x)* indicating candidate HF regions. We divide *T(x)* in separate isolated clusters using connected-component analysis[33]. Connected clusters smaller than 3 pixels are ignored to remove spurious detections. Next, we apply a local maximum filter to the probability map *P(x),* i.e., a point is a local maximum if it has a higher probability *P(x)* compared to all the pixels in a 3x3 neighborhood surrounding the pixel. Only local maxima that are inside a valid cluster in *T(x)* are included. Note that a single cluster can contain multiple individual local maxima. Finally, the locations of the remaining local maxima are selected as the final detected HF, i.e., the number of HF is equal to the number of local maxima.

3.3

Morphological and topographical appearance of microaneurysms on optical coherence tomography angiography

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ABSTRACT

Aims: To investigate retinal microaneurysms in patients with diabetic macular oedema (DME) by optical coherence tomography angiography (OCTA) according to their location and morphology in relationship to their clinical properties, leakage on fundus fluorescein angiography (FFA) and retinal thickening on structural optical coherence tomography (OCT).

Methods: OCTA and FFA images of 31 eyes of 24 subjects were graded for the presence of microaneurysms. The topographical and morphological appearance of microaneurysms on OCTA was evaluated and classified. For each microaneurysm, the presence of focal leakage on FFA, and associated retinal thickening on OCT was determined.

Results: Of all microaneurysms flagged on FFA, 219 out of 513 (57%) were also visible on OCTA. Microaneurysms with focal leakage and located in a thickened retinal area were more likely to be detected on OCTA than not leaking microaneurysms in non-thickened retinal areas (P=0.001). Most microaneurysms on OCTA were seen in the intermediate (23%) and deep capillary plexus (22%). Of all microaneurysms visualized on OCTA, saccular microaneurysms were detected most often (31%), as opposed to pedunculated microaneurysms (9%). Irregular, fusiform, and mixed fusiform/saccular shaped microaneurysms had the highest likeliness to leak and to be located in thickened retinal areas (P<0.001, P<0.001, and P=0.001).

Conclusions: Retinal microaneurysms in DME could be classified topographically and morphologically by OCTA. OCTA detected less microaneurysms than FFA, and this appeared to be dependent on leakage activity and retinal thickening. Morphological appearance of microaneurysms (irregular, fusiform and mixed saccular/fusiform) was associated with increased leakage activity and retinal thickening.

INTRODUCTION

Diabetic macular oedema (DME) is the major cause of vision loss in patients with diabetes mellitus.[1] It is characterized by accumulation of fluid in the macula due to breakdown of the blood-retinal barrier by leakage from capillaries and micro-aneurysms, protrusions of the retinal capillary wall. Microaneurysms are the earliest clinically visible signs and a hallmark of hyperglycaemic retinal damage.[2] Identification of microaneurysms is important, as their location or turnover rate yields prognostic information on the development of vision-threatening diabetic retinopathy.[3, 4] In addition, microaneurysms presenting with focal leakage and increased retinal thickness might be treated effectively by focal photocoagulation.[5]

Fundus fluorescein angiography (FFA) is currently the gold standard to examine the retinal vasculature. In DME, FFA can be performed to identify dye leakage, microaneurysms, as well as ischemia. However, because FFA provides summative information on the entire retinal vascular network, not all abnormalities may be detected by FFA, and especially visualization of the deeper capillary network is incomplete.[6-8] Moreover, the use of intravenous dye may result in undesirable adverse events, varying from nausea and dizziness to rare but serious events like anaphylaxis and hypovolemic shock or even death.[9, 10]

Optical coherence tomography angiography (OCTA) is a non-invasive imaging technique that is based on the decorrelation of OCT signal amplitude due to blood flow, and does not require dye administration.[11] It enables acquisition of high-resolution images of the retinal vasculature in three dimensions. A major drawback of OCTA, however, is the inability to visualize vascular leakage and low flow rates. In the macula, three distinct retinal capillary plexi and the choroid can be evaluated separately, in contrast to conventional FFA that does not provide depth resolution.[12]

While microaneurysms on conventional FFA usually appear as dot-like hyperfluorescent spots, a range of morphologic appearances can be visualized on OCTA. Saccular and fusiform appearances, and coiled capillary ends have already been identified.[13-16] Dubow et al. proposed a classification incorporating focal bulging, saccular, fusiform, mixed saccular/fusiform, pedunculated, and irregular micro-aneurysms, based on adaptive optics FFA.[17] To the best of our knowledge, this full range of morphological appearances has not been described for OCTA.

In this study we evaluated the topographical and morphological appearance of microaneurysms on OCTA and compared this to their clinical properties, leakage on FFA, and retinal thickening on structural OCT. By this, we aimed to gain more

knowledge about microaneurysm characteristics, which may provide a better understanding of the pathophysiology of microaneurysms, and their role in the development of DME.

MATERIAL & METHODS

Patients

We conducted an observational case series study on 52 eyes of 33 patients with clinically significant DME that visited the outpatient clinic of the Ophthalmology department of the Radboud university medical center Nijmegen, The Netherlands, between November 2015 and June 2016. This study adhered to the tenets of Helsinki, and ethical approval was waived by the Research Ethics Committee of Radboud university medical center Nijmegen. Patients were included if FFA and OCTA were performed on the same day in the context of regular clinical practice. Exclusion criteria were presence of retinal vascular pathology other than DME, or poor quality images due to severe motion or blinking artefacts. Demographic and clinical characteristics were collected by reviewing patients' medical files. Diabetic retinopathy was staged according to the International Clinical Diabetic Retinopathy Severity Scale.[18]

Image acquisition and processing

3x3 mm OCTA images were obtained using a swept source OCTA device (DRI Triton™ OCT, Topcon Corporation, Tokyo, Japan). The images were manually segmented into three retinal capillary plexus using IMAGEnet™ (Topcon Corporation, Tokyo, Japan) processing software.[12] For the superficial capillary plexus (SCP), the boundaries were set at the inner border of the inner limiting membrane to the superficial portion of the inner plexiform layer (IPL). The borders of the intermediate capillary plexus (ICP) were set between the deep portion of the IPL and the superficial portion of the inner nuclear layer (INL). The boundaries of the deep capillary plexus (DCP) were set between the deep portion of the INL and the outer boundary of the outer plexiform layer (Figure 1).

Spectral domain OCT and FFA were obtained using the Spectralis™ HRA+OCT device (Heidelberg Engineering, Heidelberg, Germany). FFA was acquired after intravenous administration of 2.5 ml of 2.5% fluorescein solution, and images for analysis were selected from the early (30 seconds-2 minutes) and late phase (5-15 minutes) of the dye transit.

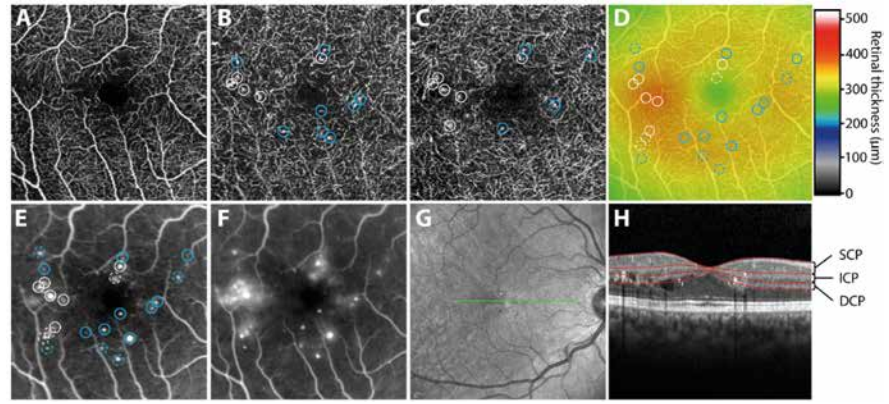

Figure 1 Illustration of the investigated imaging characteristics of microaneurysms in a patient with diabetic maculopathy. Optical coherence tomography angiography of the (**A**) superficial capillary plexus (SCP); (**B**) intermediate capillary plexus (ICP); (**C**) deep capillary plexus (DCP); (**D**) SCP with superimposed the retinal thickness map. Fluorescein angiography of the (**E**) early phase; (**F**) late phase. (**G**) infrared image with the green arrow indicating the cross section of the optical coherence tomography B-scan. (**H**) Optical coherence tomography of the foveal centred B-scan, showing the segmentation boundaries of the SCP, ICP and the DCP. White circles indicate leaking microaneurysms; blue circles indicate non-leaking microaneurysms (**A–C**), as determined by late phase fluorescein angiography; solid circles indicate high-flow microaneurysms; dashed circles indicate low-flow microaneurysms that were non-detectable on optical coherence tomography angiography (**E, F**).

Image analysis

All OCTA and FFA images were exported to custom image analysis software created with Mevislab (MeVis™ Medical Solutions AG, Fraunhofer MEVIS, Germany). FFA images were resized and matched to the 3x3 mm OCTA image slabs for pixel-wise analysis. Two independent experienced graders (AD, VS) evaluated all images. Microaneurysms were defined as hyperfluorescent dots on FFA. A synchronized cursor assisted in detecting the according locations on OCTA to analyze flow signals on the same location in all retinal layers. All locations were checked throughout the total thickness of the retina to avoid false negative results by segmentation errors. Microaneurysms non-visible on OCTA due to blood flow below the detection threshold were classified as low flow microaneurysms, in contrast to their high flow counterparts visible on OCTA. In case microaneurysms were detected in more than one retinal capillary plexus, we used the according high flow signal in the uppermost layer where the signal was detected for analysis to account for projection artefacts.

According to Dubow et al., we classified the shape of the microaneurysms on OCTA into either saccular, fusiform, mixed saccular/fusiform, pedunculated, irregular or focal bulging.[17] We furthermore graded the microaneurysms for the presence of focal leakage on the late phase FFA. Focal leakage was defined as the presence of one or several microaneurysms within a surrounding area of fluorescein leak with fluorescein signal intensity decreasing to the periphery of the microaneurysm in the center.[19] Discrepancies between graders were solved by open adjudication.

A retinal thickness map derived from the Spectralis HRA+OCT was superimposed and aligned onto the FFA and OCTA images according to retinal landmarks using Adobe Photoshop (cs5 Adobe Systems Incorporated, San Jose, CA). The distribution of low flow and high flow microaneurysms was studied within the different categories mentioned above, based on local leakage and retinal thickening. The threshold for increased retinal thickening was ≥325 µm in the central subfield of the ETDRS grid centred to the fovea, and ≥375 µm in the para- and perifoveal areas.

Statistical analysis

Intergrader agreement was assessed using the intraclass correlation coefficient (ICC) for the number of microaneurysms per eye. We used generalized estimating equations binary logistic regression analysis to evaluate the influence of leakage and thickening status on the visibility of microaneurysms on OCTA, using 'no leakage and no thickening' as reference category. The same analysis was used to study the influence of different microaneurysm appearances on the presence of focal leakage or increased thickening,,indicating the category with the lowest predictive probability as reference category. Results are presented as odds ratio (OR) with corresponding 95% confidence interval (CI). P-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 22 (SPSS, Chicago, IL, USA).

RESULTS

Patients

Fifty-two eyes of 33 patients were diagnosed with clinically significant DME and underwent simultaneous FFA and OCTA. Of those, we excluded 18 eyes of 12 patients due to poor OCTA quality, and 3 eyes of 2 patients due to poor FFA quality. Eventually, 31 eyes of 24 patients were eligible for evaluation in our study. Demographic and clinical features of study patients are displayed in Table 1.

Table 1 Demographic and clinical characteristics of study participants

Variable (n = 31 eyes of 24 subjects)	
Gender, n (%)	
Male	14 (58%)
Female	10 (42%)
Age in years, mean (SD)	57 (13)
Diabetes type, n (%)	
Type 1	8 (33%)
Type 2	16 (67%)
Diabetic retinopathy stage, n (%)	
Mild NPDR	5 (16%)
Moderate NPDR	16 (52%)
Severe NPDR	5 (16%)
Proliferative DR	5 (16%)
Duration DM in years, mean (SD)	24 (12)
Central retinal thickness in µm, median (IQR)	335 (304-351)
Edema type, n (%)	
Cystoid	25 (81%)
Diffuse	6 (19%)
Prior treatment, n (%)*	
Intravitreal anti-VEGF	19 (61%)
Intravitreal corticosteroids	10 (32%)
Focal photocoagulation	20 (65%)
Micropulse photocoagulation	14 (45%)
Panretinal photocoagulation	20 (65%)

Abbreviations: n=number; SD=standard deviation; NPDR=non-proliferative diabetic retinopathy; DR=diabetic retinopathy; DM=diabetes mellitus; IQR=interquartile range; VEGF=vascular endothelial growth factor.
* Because some patients received multiple treatments, the percentages do not add up to 100%.

Microaneurysms

The ICCs for the number of microaneurysms on FFA and the SCP, ICP and DCP of OCTA were 0.911, 0.974, 0.774, and 0.765 respectively. Of all 513 microaneurysms flagged on FFA, 219 (57%) also showed an increased flow signal on OCTA. Most increased flow signals associated with microaneurysms were located in the ICP (117 out of 513, 23%), closely followed by the DCP (111 out of 513, 22%). The SCP contained the least amount of increased flow signals (66 out of 513, 13%).

All microaneurysms were characterized according to leakage and flow signal properties and projected on a topographical retinal thickness map (Figure 1). Focal leakage was present in 154 out of 513 (30%) microaneurysms visible on FFA and 200

out of 513 (39%) microaneurysms were located within a thickened area. High flow microaneurysms did not necessarily leak, nor were they all located in a thickened area. Microaneurysms that showed focal leakage and were located in a thickened area, were more often detected on OCTA than not leaking microaneurysms in non-thickened retinal areas (OR 2.6, 95%CI [1.4-4.6], P=0.002, Figure 2 A). The percentage of microaneurysms showing focal leakage ranged from 33% in the SCP to 36% in the ICP, and 41% in the DCP (Figure 2 B).

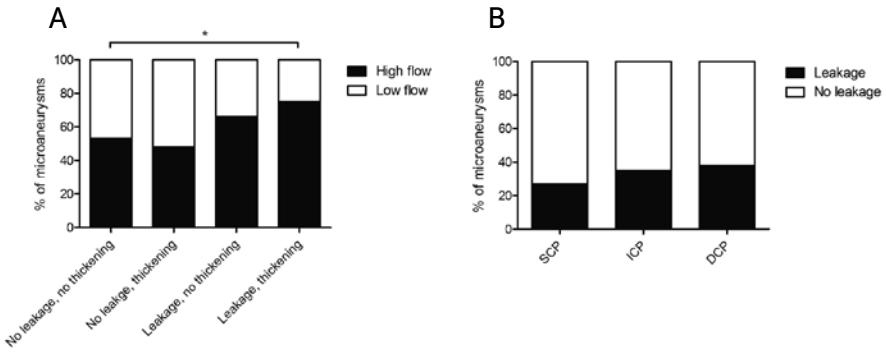

Figure 2 Distribution of (**A**) high-flow and low-flow microaneurysms across different categories according to leakage and thickness information in patients with diabetic maculopathy; (**B**) leaking and not-leaking microaneurysms according to their location within the plexi. * p<0.05. Abbreviations: DCP=deep capillary plexus; ICP=intermediate capillary plexus; SCP=superficial capillary plexus.

Focal bulging, saccular, fusiform, mixed saccular/fusiform, pedunculated, and irregular microaneurysms were all observed on OCTA (Figure 3). Saccular microaneurysms were detected most often (89 out of 219, 31%), while pedunculated microaneurysms were detected least often (26 out of 219, 9%, Figure 4 A). The morphological appearances of microaneurysms were distributed across all three capillary plexi (Figure 4 B). All types of microaneurysms were present throughout all stages of DR (Supplementary Table 1). Irregular, fusiform, and mixed fusiform/saccular shaped microaneurysms were more often showing focal leakage than focal bulging ones (OR 7.6, 95%CI [2.4-23.8], P<0.001; OR 5.3, 95%CI [1.6-17.6], P=0.006; and OR 4.1, 95%CI [1.9-9.1], P<0.001 respectively, Figure 4 CD). There was no statistical significant difference in the presence of retinal thickening across the six morphological groups. The position of microaneurysms within the different capillary plexi did not differ among the various morphological features (data not shown).

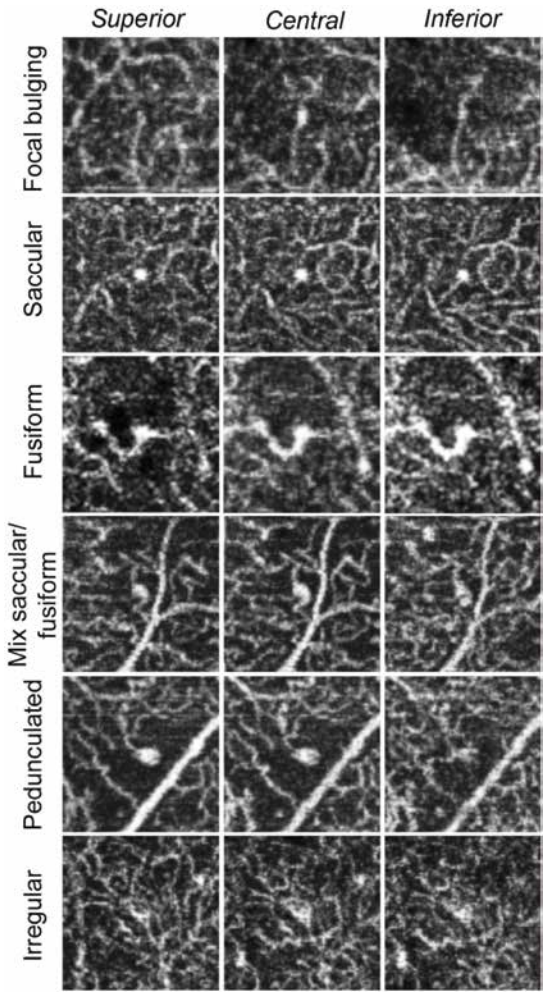

Figure 3 Morphology of microaneurysms detected by optical coherence tomography angiography in patients with diabetic maculopathy. The detected appearances include (first row) focal bulging; (second row) saccular; (third row) fusiform; (fourth row) mixed saccular/fusiform; (fifth row) pedunculated; (sixth row) irregular.Orientation of segmentation was determined by the location of the microaneurysm. Optical cuts were performed (left column) superior, (middle column) central and (right column) inferior tangentially.

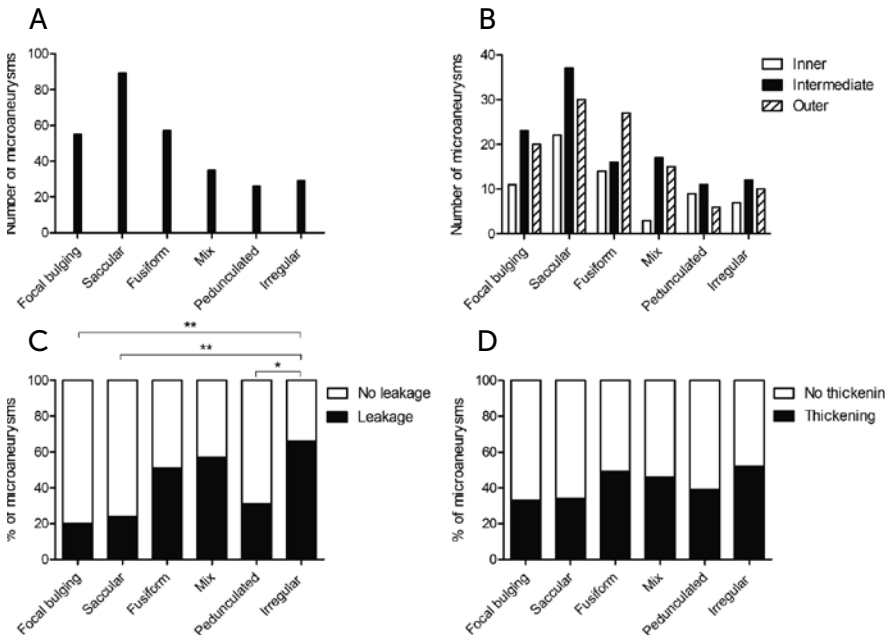

Figure 4 Distribution of microaneurysm types on optical coherence tomography angiography (OCTA) in patients with diabetic maculopathy. (**A**) Total number of microaneurysms throughout the retina; (**B**) number of microaneurysms according to their intraretin allocation on OCTA. Properties of microaneurysms in terms of leakage and retinal thickening in patients with diabetic maculopathy. (**C**) Percentage of microaneurysm types according to leakage; (**D**) percentage of microaneurysm types according to retinal thickening.*p<0.05; **p<0.001.

DISCUSSION

We investigated retinal microaneurysms in DME according to their location and appearance on OCTA in correlation to their clinical properties, leakage on FFA and retinal thickening on structural OCT. OCTA detected less microaneurysms than conventional FFA, but did provide information on the location of the microaneurysms in the retinal layers as well as their morphological appearances that formerly could only be appreciated using adaptive optics FFA.[17] In addition, we could correlate the morphology of the microaneurysms observed on OCTA to their clinical properties, like leakage and retinal thickening.

Of all microaneurysms detected on FFA, increased flow signals associated with microaneurysms were found in 57%. This is in the same range as the previously reported frequency of 41% by Miwa et al., the 62% reported by Couturier et al., and

the 64% reported by Salz et al.[14-16] The variance in detected microaneurysms by OCTA is, at least in part, based on divergent grading definitions for microaneurysms in both FFA and OCTA. Moreover, these studies might not be directly comparable, as both swept-source and spectral-domain OCT systems were employed.

We hypothesized that microaneurysms showing no signal on OCTA did not meet the minimal blood flow velocity in order to gain a positive flow signal. Besides slow flow, some microaneurysms may not contain (moving) erythrocytes at all, as demonstrated in a histopathologic study reporting occlusion of the vessel lumen.[20] Furthermore, not all hyperfluorescent dots on FFA may represent true microaneurysms. Fluorescein may cause extensive staining of capillary walls or dye leakage from an impaired vessel, mimicking a microaneurysm, but not causing an increased flow signal on OCTA.[13, 16]

The majority of previous OCTA studies only assessed the SCP and DCP, derived from preset commercial image analysis software, dividing the intermediate capillary plexus across those two plexi. We argue that one should take the structural and the developmental differences of three distinct retinal capillary plexi into account in the analysis of macular diabetic vascular changes, as this best represents the anatomical organization of the retinal vascular network. However, distinction of three capillary plexi is challenged by the presence of projection artefacts, more so than when only identifying two plexi.[12] To account for projection artefacts, we classified microaneurysms into the most superficial layer in which they were detected, although this method might result in an underestimation of microaneurysms in the deeper plexi. Therefore, to avoid over- or underestimation of the presence of microaneurysms, one should carefully slide through the complete OCTA volume in advance of preset OCTA slabs. By this, confounders like local capillary tortuosity (pseudo-microaneurysm if observed tangentially in a single slab) and microaneurysms displaced to an underlying layer (false negative if single slabs above are studied) can be detected, resulting in more reliable study outcomes. Future improvements in anatomic segmentation software may reduce the number of false positives and negatives.

We found microaneurysms to be distributed throughout all retinal capillary plexi, although most microaneurysms were located in the ICP and DCP. This corroborates findings from histopathologic studies localizing microaneurysms mainly in the INL, that contains the lower portion of the ICP and the upper portion of the DCP.[21] Therefore, we hypothesize that when using the preset segmentation method, most microaneurysms will be detected in the DCP, as it contains the entire INL.

Recent retinal blood flow studies suggest a serial perfusion of retinal capillaries, with direct flow from the large retinal vessels to ICP and DCP, and SCP.[22] Hence,

blood flow velocity in general may be higher in the ICP and DCP, and consequently, in the microaneurysms located there, facilitating detection by the OCTA.

Microaneurysms showing focal leakage on FFA and located in a thickened area on OCT were more often high flow than not leaking microaneurysms that were not located in a thickened area. However, high flow in a microaneurysm did not necessarily mean leakage activity, neither did low flow mean the opposite. Leakage of microaneurysms on FFA is an important observation in the diagnostic workup of DME, as these microaneurysms may effectively be treated by focal laser treatment. Although the intravitreal administration of steroids or anti-VEGF agents has replaced primary laser treatment in patients with central macular oedema, this information is still useful in cases with persisting oedema, if despite anti-VEGF additional laser treatment is indicated.[23] Currently, laser treatment is often guided by FFA, but may be replaced by a non-invasive imaging technique such as OCTA if it enables identification of microaneurysms that are considered treatment targets. Our present study shows that for this purpose, FFA cannot be replaced by OCTA, as not all leaking microaneurysms were detected by OCTA. Therefore, future research should be directed towards improving OCTA techniques and processing software.

As with adaptive optics FFA, we observed different shapes of microaneurysms by OCTA and our results corroborated the frequency of the single subtypes, despite using a different imaging technique.[17] In addition to adaptive optics FFA, we were able to localize the microaneurysms three-dimensional in the retina and correlate these morphological subtypes with focal leakage and retinal thickening. Irregular, fusiform and mixed shaped microaneurysms tended to leak more often than other types of microaneurysms. Irregular microaneurysms may have a greater luminal surface area that may increase the risk of damage of the basement membrane and thus breakdown of the inner blood-retina barrier.[3] Wang et al., on the other hand, demonstrated that size of the aneurysms on FFA does not necessarily correlate with FFA leakage status.[17, 24] Therefore, we argue that the morphology of retinal microaneurysms is a clinically more important biomarker than size and should be considered in upcoming studies.

The small study population is a limitation of our current study. Furthermore, we included patients with various stages of DME, regardless of whether or not treatment was administered, resulting in a heterogeneous study population. Further research is warranted in a large, prospective, and preferably treatment-naïve cohort, to replicate our findings, and to evaluate the clinical relevance. In addition, a consensus is needed for the definition of microaneurysms in FFA as well as OCTA to increase the reliability of the ground truth in future studies.

In conclusion, we have described the morphology and three-dimensional distribution of diabetic retinal microaneurysms in relation to their clinical properties in terms of leakage and retinal thickening. Defining the morphology of microaneurysms on OCTA increases our understanding of pathophysiological mechanisms of DME and may aid in finding the optimal therapeutic approach. Future research should be directed towards the clinical relevance of the various morphological manifestations as biomarkers for disease progression or treatment response.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 Morphological appearance of microaneurysms according to the diabetic retinopathy stage

Shape	DR stage							
	Mild DR		*Moderate DR*		*Severe DR*		*Proliferative DR*	
Focal bulging	11	(21%)	34	(23%)	5	(9%)	5	(14%)
Saccular	16	(30%)	49	(33%)	17	(31%)	7	(20%)
Fusiform	8	(15%)	29	(20%)	10	(18%)	10	(29%)
Mix	9	(17%)	13	(9%)	9	(16%)	4	(11%)
Pedunculated	3	(6%)	11	(7%)	8	(15%)	4	(11%)
Irregular	6	(11%)	12	(8%)	6	(11%)	5	(14%)

Abbreviations: DR=diabetic retinopathy

4

Treatment outcomes

4.1

Long term outcomes of vitrectomy for complications of proliferative diabetic retinopathy

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ABSTRACT

Purpose: To investigate the long term outcomes of a vitrectomy for the consequences of proliferative diabetic retinopathy.

Methods: Cumulative incidences were calculated for low vision (<0.3), re-vitrectomy in the study eye, and fellow eye vitrectomy. To identify potential prognostic factors that associate with these outcomes, we used multivariable Cox regression models.

Results: In a total of 217 patients, we found 1-, 5-, and 10-year cumulative incidences of low vision in the study eye of 24%, 31%, and 39%, respectively. For both eyes, these rates were respectively 10%, 14%, and 14%. Low vision in both eyes was associated with higher age and worse contralateral visual acuity. The 1-, 5-, and 10-year cumulative incidence for re-vitrectomy in the study eye were 16%, 27%, and 27%, respectively, and for a vitrectomy in the fellow eye 24%, 40%, and 54%, respectively. Re-vitrectomy of the study eye was associated with worse contralateral visual acuity, while vitrectomy of the fellow eye was associated with shorter diabetes duration, worse contralateral visual acuity, higher HbA1c level, and the diabetic retinopathy severity stage of the fellow eye.

Conclusion: Functional visual acuity in at least one eye was achieved or preserved in most patients. After 10 years, about a quarter of all patients underwent a re-vitrectomy, while more than half of the patients needed a vitrectomy of the fellow eye. Knowledge of these long-term outcomes is essential when counseling patients for a vitrectomy.

INTRODUCTION

Proliferative diabetic retinopathy (PDR) is a sight-threatening complication of diabetes mellitus (DM), affecting 7% of all 425 million people living with DM.[1] The relative incidence of PDR and associated blindness have declined over the past decades due to improvements in diabetes care. These include a tighter grip on blood glucose levels through self-monitoring devices and various modern types of insulin, implants with real-time analysis and metered insulin delivery, as well expanded screening programs for the ophthalmic complications of diabetes. Moreover, the introduction of anti-vascular endothelial growth factors (VEGFs) and the refinement of surgical techniques have greatly improved diabetic eye care, including PDR.[2]

Unfortunately, the current diabetic epidemic with its steep incline of diabetic patients still leads to a rising prevalence of PDR. PDR therefore continues to be a major burden on public health.[2] If severe consequences of PDR develop, a vitrectomy can be required to maintain and/restore visual function. The indications according to the American Academy of Ophthalmology (AAO) for a vitrectomy include: non-clearing vitreous hemorrhage; significant recurring vitreous hemorrhage, despite use of maximal panretinal photocoagulation; dense premacular subhyaloid hemorrhage; tractional retinal detachment involving or threatening the macula; combined tractional and rhegmatogenous retinal detachment; red blood cell-induced glaucoma and "ghost cell" glaucoma; and anterior segment neovascularization with media opacities preventing panretinal photocoagulation.[3] The yearly incidence of a vitrectomy in patients with PDR is estimated to be 6%.[4]

The majority of the patients that require a vitrectomy for PDR are relatively young and have jobs and families to maintain. Vision loss for these individuals has a large impact on their everyday life and society as a whole. Since PDR is a bilateral disorder, the long-term visual prognosis in these patients is a major concern. Most studies investigating the outcomes of vitrectomy for PDR report however short-term results and largely focus on the operated eye. Arguably, for diabetic patients, the long-term prognosis for bilateral visual function is important. A recent study by Ostri et al. reported that two thirds of the patients undergoing vitrectomy for PDR obtained a visual acuity of $\geq$ 0.3 in the operated eye after 10 years.[5] Studies on bilateral visual outcomes after vitrectomy date back at least fifteen years ago, and report that 80% of the patients maintained or obtained a visual acuity of $\geq$ 0.1 in at least one eye, while 43% underwent fellow eye vitrectomy.[6, 7] Recent studies on bilateral long-term outcomes of vitrectomy are lacking, and little studies cover a study period of 5 years or longer.[5, 8] The aim of this study was therefore to study the long-term outcome of eyes following vitrectomy for PDR as well as the visual prognosis in broader sense. In addition, we studied candidate prognostic factors that may be used in future risk stratification.

METHODS

Study population

This study included all patients who underwent primary pars plana vitrectomy for complications of PDR at the Radboud University Medical Centre Nijmegen, The Netherlands, between September 2006 and June 2012. We employed the following exclusion criteria: a follow-up duration of less than 6 months, prior vitrectomy in either eye, missing intraoperative data of the primary vitrectomy, and pre-existing low vision in the operated eye due to non-diabetic comorbidity. Low vision was defined as a best corrected visual acuity (BCVA) of <0.3, and blindness as a BCVA of <0.05, according to the standards of the World Health Organization.[9] Follow-up data were collected both from the tertiary health care center where the vitrectomy was performed as well as from the referring ophthalmologist. This study was approved by the medical ethical committee of the Radboud University Medical Center Nijmegen and adhered to the tenets of Helsinki.

Study outcomes

The primary outcomes of this study were the occurrence of low vision and blindness in either eye and for both eyes, the need for a re-vitrectomy in the study eye, and the need for a vitrectomy in the fellow eye. Removal of silicon oil tamponade was not considered as a re-vitrectomy. Secondary outcome measures were survival rate, survival of the crystalline lens, the occurrence of post-operative complications, and the associations of baseline variables with low vision in both eyes.

Data collection

Preoperative and annual postoperative decimal BCVA in both eyes was collected, and a patient was classified as having low vision if two consecutive BCVA measurements were <0.3. General patient characteristics and preoperative variables that were recorded included sex, age, age of onset of diabetes, diabetes type, duration of diabetes, the presence of nephropathy, amputation or ischemic heart disease, glycated hemoglobin (HbA1c, mmol/mol), body mass index (BMI, kg/m^2), and mean arterial pressure (MAP, mmHg), calculated by the equation: MAP ≈ diastolic blood pressure + ⅓ (systolic blood pressure – diastolic blood pressure). In addition, the following ophthalmological parameters were collected: lens status, indication for vitrectomy, diabetic retinopathy (DR) stage in the fellow eye during the time of vitrectomy, and prior treatment with panretinal photocoagulation or anti- VEGF. Mortality data for all patients were retrieved from the Dutch population register.

Statistical analysis

Decimal BCVA was converted to the logarithm of the maximum angle of resolution (logMAR) for statistical analysis. Patients with vision loss resulting in the non-numerical vision of perception of light or no perception of light were assigned a logMAR value of 2.78, which is the approximate equivalent of a Snellen VA of 1/300, the lowest VA observed in the cohort.[10, 11] Continuous variables were displayed as mean ± standard deviation (SD) when normally distributed, and as median with interquartile range (IQR) when the distribution was skewed. Categorical variables were displayed as a proportion with corresponding percentage. A paired t-test was performed to investigate the difference in visual acuity before and after vitrectomy. Differences in complications rate for 20- or 23-gauge instruments were tested using a Pearson Chi-square test. Kaplan-Meier survival analysis was used to generate survival curves and cumulative incidences for the occurrence of low vision, re-vitrectomy of the study eye, vitrectomy of the fellow eye, survival, and cataract surgery. To compare the mortality in our population to the general population, we simulated age- and sex-matched survival rates retrieved from the public registry Statistics Netherlands with 100 iterations.

Randomly missing data was imputed using multiple imputation with twenty iterations. We used multivariable Cox regression analysis with backwards stepwise elimination of variables with p<0.05 to identify variables that were associated with low vision in the best eye, re-vitrectomy of the study eye, and vitrectomy of the fellow eye. Variables were checked for multicollinearity using Pearson´s coefficient for parametric distributions and Spearman´s rank for non-parametric distributions. Hazard ratios with corresponding 95% confidence intervals are displayed for significantly associated variables. Analyses were limited to those patients who had a follow-up duration of at least one year. A p-value <0.05 was considered statistically significant. All analyses were conducted using SPSS version 20 (SPSS, Chicago, IL, USA).

RESULTS

Patient demographics and follow-up

A total of 273 patients underwent primary vitrectomy for PDR during the study period, of which 40 were excluded due to a follow-up duration of <6 months, nine due to missing intraoperative data, and seven due to comorbidity causing pre-existing low vision. Subsequently, a total of 217 patients were included in the current study. In 59 patients (27%) 23-gauge vitrectomy was performed, and the remaining 158 patients (73%) underwent 20-gauge vitrectomy. Silicon oil tamponade was

used in 36 patients (13%). The course of follow-up during the study period is displayed in Fig. 1. The mean follow-up duration for all patients was 5.4 years (SD 2.9 years). Patient characteristics at baseline are shown in Table 1. In 2/3 of the patients, vitrectomy was performed because of a non-clearing or recurrent vitreous hemorrhage. In 7% of the patients, vitreous hemorrhage was associated with a tractional retinal detachment involving or threatening the macula. Sight threatening tractional retinal detachment was present in 13% of patients in the absence of a vitreous hemorrhage. Neovascular glaucoma was a rare (1%) indication for surgery. A relatively common indication that did not meet with the pre-defined indications for diabetic vitrectomy was severe fibrovascular proliferation, unresponsive to laser therapy (12%). In these patients, surgery is considered a last resort to restrain neovascular activity. Almost half of all patients (46%) had a severe non-proliferative or proliferative stage of DR in the fellow eye, mild stage or absent DR was present in the fellow eyes of 62 (29%) patients.

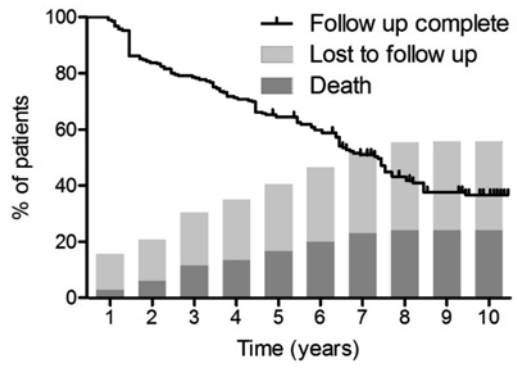

Figure 1 Course of follow up during the study period. Follow up completion was estimated by Kaplan-Meier survival analysis, censoring patients with complete follow up. Yearly cumulative incidence of deceased patients and patients lost to follow up were simultaneously plotted on the x-axis.

Vision outcomes

The cumulative incidence of low vision in the study eye was 31% at 5 years, and 39% at 10 years, as is shown in Fig. 2A. For low vision in the fellow eye, cumulative incidences at 5 and 10 years were 25% and 29% respectively, while preoperative low vision in the fellow eye was present in 55 eyes (20%). The cumulative incidence of low vision in both eyes was 14% at 5 years. In the follow-up > 5 years, we observed no new cases of bilateral low vision.

Table 1 Patient characteristics at baseline

Demographics	N=217
Age, years, mean ± SD	55 ± 16
Sex, n (%)	
Male	122 (56%)
Female	95 (44%)
DM Type, n (%)	
Type 1	80 (38%)
Type 2	133 (62%)
Duration of DM, years, mean ± SD	21 ± 12
Age of onset DM, years, mean ± SD	
Type 1 DM	14 ± 12
Type 2 DM	46 ± 12
Systemic characteristics	
HbA1c, mmol/mol, mean ± SD	63 ± 15
Mean arterial pressure, mmHg, mean ± SD	101 ± 14
Body mass index, kg/m^2, mean ± SD	28.3 ± 6.6
Nephropathy, n (%)	56 (27%)
Amputation, n (%)	17 (9%)
Ischemic heart disease, n (%)	52 (24%)
Ophthalmological characteristics	
Indication for vitrectomy	
Proliferative DR with vitreous hemorrhage	178 (82%)
Proliferative DR without vitreous hemorrhage	30 (14%)
Tractional retinal detachment with vitreous hemorrhage	2 (1%)
Tractional retinal detachment without vitreous hemorrhage	7 (3%)
Preoperative BCVA, decimals, median (IQR)	
Study eye	0.02 (0.003-0.20)
Fellow eye	0.50 (0.25-0.79)
Duration of DR, years, median (IQR)	6 (2-12)
Prior treatment for DR, n (%)	
None	25 (12%)
Photocoagulation	174 (83%)
Intravitreal anti-VEGF	35 (17%)
Lens status study eye, n (%)	
Phakic	166 (77%)
Pseudophakic	50 (23%)
DR stage fellow eye, n (%)	
None	34 (16%)
Mild	28 (13%)
Moderate	51 (24%)
Severe	13 (6%)
Proliferative	85 (40%)

Abbreviations: n=number; SD=standard deviation; DM=diabetes mellitus; BCVA=best corrected visual acuity; IQR=interquartile range; DR=diabetic retinopathy; anti-VEGF=anti-vascular endothelial growth factor

For blindness, the 5- and 10-year cumulative incidences in the study eye were 12% and 16%. In the fellow eye, preoperative blindness was present in 18 eyes (9%), and after 5 and 10 years, we found cumulative incidences of blindness of 10% and 11%, respectively. A total of 6 persons (3%) suffered from blindness in both eyes after a median of 2 years [IQR 1-3] and were considered legally blind.

Further surgical interventions

In total, 42 patients (21%) required a re-vitrectomy in their study eye. If patients needed a re-vitrectomy, the median interval was 6 months [IQR 2-15] and in 98% of the cases the procedure was performed within three years after the first vitrectomy in the study eye, resulting in a 5-year cumulative incidence of 27% (Fig. 2B). In one patient an evisceration of the study eye was performed after 10 months of follow-up, because of unbearable and uncontrollable ocular pain due to glaucoma as result of chamber angle neovascularization. In the fellow eye, a total of 81 patients (40%) required a vitrectomy, most often for the indication of proliferative DR with a non-clearing vitreous hemorrhage (55% of all vitrectomies in the fellow eye). Of the 85 patients with proliferative DR in their fellow eye at baseline, 50 (59%) required a fellow eye vitrectomy at some time point during follow-up, versus 10 out of 62 (16%) in the patients with no or mild DR in their fellow eye at baseline. The 1-, 5- and 10-year cumulative incidences for a vitrectomy in the fellow eye were 24%, 40%, and 54% respectively (Fig. 2B). Another eight people needed a re-vitrectomy in their fellow eye, and one person needed a second re-vitrectomy.

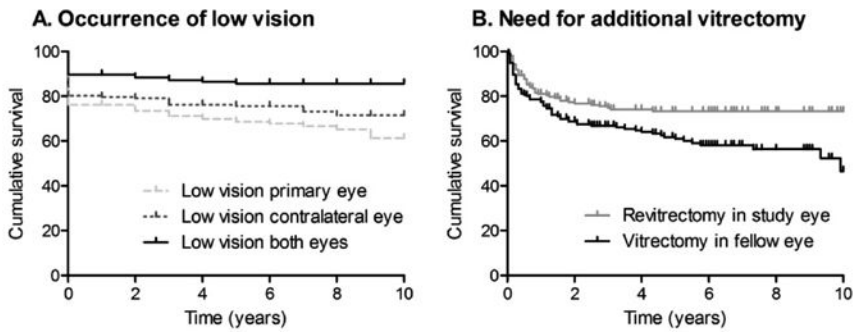

Figure 2 Kaplan-Meier survival plots for (**A**) low vision, and (**B**) the need for additional vitrectomy.

Prognostic factors

We subsequently studied factors predictive for low vision in both eyes, re-vitrectomy of the study eye, and vitrectomy of the fellow eye using multivariable Cox regression analysis. Low vision in both eyes in the follow-up period was significantly associated with higher age and a lower preoperative BCVA in the fellow eye (Table S1). Lower BCVA in the fellow eye was the only significant predictor for re-vitrectomy in the study eye (Table S1). Shorter DM duration, higher HbA1c, lower BCVA in the fellow eye, and more severe stages of DR in the fellow eye were associated with the need for vitrectomy in the fellow eye (Table S1).

Survival

We then looked at the secondary outcome measures of this study. A total of 80 patients (31%) died during the period of follow-up. The 5- and 10-year survival rates were 81%, and 67% respectively, as shown in Fig. 3A. In the simulated normal population, the 5- and 10-year survival rates were 94% and 88%.

Cataract surgery

Prior to surgery, 50 people (23%) were pseudophakic and 167 (77%) were phakic. A total of 81 (49%) of all phakic patients at baseline underwent cataract surgery, after a median interval of 16 months [IQR 5-31]. Besides vitrectomy, age is an important factor in the development of cataract. We therefore stratified the cohort according to age and analyzed patients older and those younger than 50 years separately. In patients below the age of 50, the cumulative incidence of cataract surgery was 40% after 5 years, and 52% after 10 years (Fig. 3B). In people of 50 years and older, the 5-year cumulative incidence of cataract surgery was 83%. The maximum follow-up duration in this group was 8 years, resulting in an 8-year cumulative incidence of 91% (Fig. 3B).

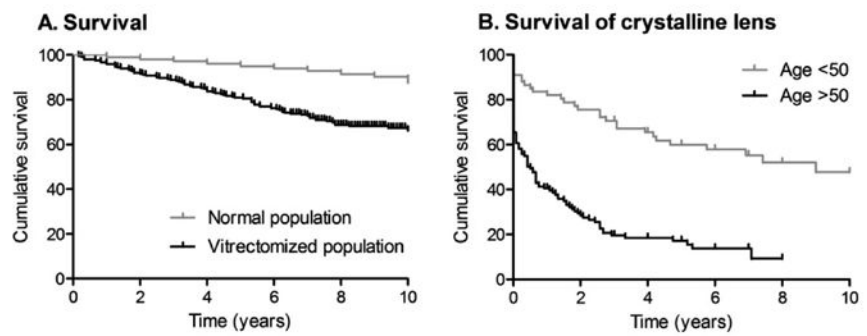

Figure 3 Kaplan-Meier survival plots for (**A**) life, and (**B**) survival of the crystalline lens.

Long-term complications

We then investigated the complications that occurred in the study eye (Table 2). Most complications occurred within 1 year after the primary vitrectomy. Elevated IOP was the most frequent complication and occurred in 81 out of 217 (37%) patients. A recurrent vitreous hemorrhage was observed in a total of 31 patients (of which 20 (65%) required a re-vitrectomy. Tractional retinal detachment after the primary vitrectomy occurred in 16 patients (7%), and a combined tractional and rhegmatogenous retinal detachment in 9 patients (4%). There was no statistical difference in complication rate between 20- and 23-gauge vitrectomy (data not shown).

Table 2 Incidence of complications in the study eye after the initial vitrectomy

	<1 year	1-5 years	>5 years	Total
Recurrent vitreous hemorrhage*	24 (11%)	5 (2%)	2 (1%)	31 (14%)
Retinal detachment	22 (10%)	3 (1%)	0 (0%)	25
Tractional	13 (6%)	3 (1%)	0 (0%)	16
Rhegmatogenous + tractional	9 (4%)	0	0 (0%)	9
Macular edema	39 (18%)	19 (9%)	5 (4%)	63
Elevated intraocular pressure	71 (33%)	6 (3%)	4 (3%)	81
Neovascular glaucoma	7 (3%)	1 (0.5%)	0 (0%)	8
Valid number	217	207	140	217

*Only comprises delayed recurrent vitreous hemorrhage (≥1 month after primary vitrectomy)

DISCUSSION

In the current study, we investigated the long-term outcome in patients undergoing vitrectomy for PDR. In this relatively young patient population, besides the outcome of the operated eye, important outcome measures are the risk of visual loss of the fellow eye, the prognosis for the bilateral visual function and the need for additional surgery, because they have a large impact on everyday functioning. In the consultation room, knowledge of the long-term prognosis in these patients is essential for management of expectations and long-term planning.

In the majority of patients (86% after 5 years), relative good (>0.3) visual function was maintained in at least one eye, and blindness in both eyes was observed in only 6 persons (3%). Moreover, no new cases of low vision in both eyes occurred after 5

years, suggesting that visual function in the long term can be stabilized with proper medical care. Higher age, lower BCVA in the fellow eye and the presence of tractional retinal detachment at baseline were significantly associated with an increased risk of developing low vision (0.05-0.3) in both eyes. These findings corroborate the results in previous reports, although these studies focused solely on visual outcome in the study eye.[12] Systemic measurements and the presence of other diabetic complications were also entered in the multivariate regression model, but none of these variables were associated with low vision in both eyes.

Re-vitrectomy was indicated in a total of 42 patients (21%) and was performed within the first 3 years in the vast majority (98%) of the patients. The re-vitrectomy rate in our study is slightly higher than the rates reported earlier, ranging between 14-19%. This might be explained by the relative short follow-up in these studies (mean or median <18 months).[13-15] In the fellow eye, a total of 81 patients (40%) required a vitrectomy. This shows that patients undergoing a primary vitrectomy for PDR are highly likely to have significant pathology in their fellow eye, potentially requiring vitreoretinal surgery, especially when PDR in the fellow eye is already present. Patients with no or mild DR in their fellow eye at the time of the primary vitrectomy are at a much lower risk of a fellow eye vitrectomy, although this risk is not negligible. Our findings are in correspondence with Vote et al., who described a 38% intervention rate for the fellow eye.[6] In our study, high HbA1c level, low preoperative BCVA in the fellow eye, severity of DR in the fellow eye, and short DM duration at the time of the primary vitrectomy were associated with an increased risk of fellow eye vitrectomy.

The presence and severity of DR have repeatedly been associated with increased mortality, irrespective of diabetes type.[16-18] The 5-year survival rate we found in our study was 81%. This is comparable to previous studies where 5-year survival rates ranging between 68%-86% were reported.[19-23] The 10-year survival rate in our study was 67%. Blankenship et al. provided a 10-year survival rate of 50% in patients vitrectomized between 1970-73, however, diabetes care has improves significantly in the last fifty years.[8] Our data indicate that patients undergoing a diabetic vitrectomy have a higher mortality risk than patients in the general population. Mortality in patients undergoing a diabetic vitrectomy is often due to other micro- and macrovascular complications, such as renal insufficiency and ischemic heart disease.[19-22]

Vitrectomy may accelerate the development of cataract. The underlying mechanism of this complication is unclear, but may relate to an increased oxygen tension after vitrectomy, iatrogenic damage and the type of tamponade.[24, 25] In addition, age is

a significant risk factor.[7, 26] In patients below the age of 50, we observed that 40% needed cataract surgery in the first 5 years of follow-up. This figure more than doubled to 83% in individuals of 50 years and older. Prior to vitrectomy, patients should be informed about this differentiated risk of cataract extraction. Given the high rate of post-vitrectomy cataract extraction in patients over 50, a combined vitrectomy and cataract extraction with intraocular lens implantation can have significant benefits, including faster recovery of visual function. However, a more conservative strategy is warranted in younger persons, as the implant of a non-multifocal intraocular lens will not compensate the loss of accommodation.

Strengths of this study include the long duration of follow-up, the relatively large sample size and the collection of initial vitrectomy data at a single tertiary center, reducing external causes of variability. In addition, we studied multiple endpoints, providing a comprehensive evaluation of the long-term outcomes of a vitrectomy for PDR. We furthermore employed broad inclusion criteria, studying all patients that underwent vitrectomy for the complications of PDR. Although not all patients met with any of the pre-defined indications for diabetic vitrectomy according to the AAO, we argue that exclusion of these patients would have lead to an incorrect reflection of real-life clinical practice. We should also acknowledge some limitations to the current study. A major limitation is the loss to follow-up, which may result in under- or overestimation of the reported outcomes. Additionally, the retrospective nature of the study did not allow standardization of the measurements. A limitation that inherently applies to the study of long-term outcomes, is that vitrectomies were performed 6-13 years ago. Retinal surgery as it is performed today has inevitably advanced in relation to the studied period, for example regarding preoperative administration of anti-VEGF of the use of smaller instruments. Although smaller instruments certainly have advantages over larger 20-gauge instruments in terms of duration of surgery, patient comfort and recovery, we did not find a difference in any of the long-term outcomes between 20- and 23-gauge vitrectomy.[27]

In conclusion, this study provides insight in the long-term outcomes after vitrectomy for PDR and its consequences. In the majority of patients, visual function of >0.3 in at least one eye is maintained over the course of 5 to 10 years, allowing patients to perform daily activities without far-reaching visual restrictions. We furthermore identified vision loss in the fellow eye to be predictive for a poor prognosis, with respect to both low vision and re-vitrectomy. These results can be used for counseling patients with PDR by providing insight in the prognosis following vitrectomy including visual function of the fellow eye.

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SUPPLEMENTARY MATERIAL

Table S1 Predictive variables for low vision in both eyes, re-PPV in the study eye, and PPV in the fellow eye, using Cox regression survival analysis.

Low vision in both eyes

	HR	95% CI	p-value
Age, years	1.030	1.000-1.061	0.048
Contralateral preoperative BCVA, logMAR	2.768	1.836-4.173	<0.001

Re-vitrectomy in the study eye

	HR	95% CI	p-value
Contralateral preoperative BCVA, logMAR	1.678	1.177-2.391	0.004

Vitrectomy in the fellow eye

	HR	95% CI	p-value
DM duration, years	0.976	0.954-0.998	0.036
HbA1c, mmol/mol	1.023	1.006-1.041	0.008
Contralateral preoperative BCVA, logMAR	1.57	1.107-2.189	0.011
Preoperative DR stage fellow eye			
Moderate NPDR	3.320	1.056-10.439	0.040
Severe NPDR	4.578	1.201-17.453	0.026
Proliferative DR	6.474	2.302-18.202	<0.001

Variables that entered the model: Age, sex, surgical indication, diabetes duration, age of diabetes onset, diabetes type, HbA1c, mean arterial blood pressure, body mass index, presence of nephropathy, history of ischemic heart disease, previous amputation, preoperative BCVA, contralateral preoperative BCVA, contralateral diabetic retinopathy stage, prior laser therapy, prior anti-VEGF therapy, sclerotomy size (20 vs 23 gauge).
Abbreviations: HR=hazard ratio; CI=confidence interval; BCVA=best corrected visual acuity; DM=diabetes mellitus; DR=diabetic retinopathy; NPDR=non-proliferative diabetic retinopathy.

4.2

Hyperreflective foci on optical coherence tomography associate with treatment outcome for anti-VEGF in patients with diabetic macular edema

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ABSTRACT

Purpose: To investigate the relationship between baseline number of hyperreflective foci (HF) on spectral domain optical coherence tomography (SD-OCT) in patients with diabetic macular edema (DME), as well as the dynamics of HF during treatment with anti-vascular endothelial growth factor (VEGF, and treatment response.

Methods: We evaluated patients diagnosed with DME scheduled for treatment with intravitreal bevacizumab. Eyes were classified as adequate or insufficient treatment responders based on logMAR visual acuity improvement and central retinal thickness (CRT) decrease after three consecutive injections. Associations between number of HF at baseline and treatment response, the change in HF over the course of treatment, and the distribution of HF within the retinal layers were evaluated.

Results: In 54 eyes of 41 patients, mean number of HF and CRT decreased after intravitreal treatment with bevacizumab ($p=0.002$ and $p<0.001$ respectively). Decrease in CRT after 3 months was independently associated with a higher number of HF at baseline (estimated effect -2.61, 95% CI [-4.42–-0.31], $p=0.006$). Eyes with adequate treatment response presented with more HF at baseline (OR 1.106, 95% CI [1.012-1.210], $p=0.030$) than eyes with insufficient treatment response. Most HF were located within the inner retinal layers, and decrease of HF was mostly due to a decrease of inner retinal HF.

Conclusions: In patients with DME treated with anti-VEGF, higher baseline numbers of HF have predictive value for treatment response in terms of visual acuity improvement and CRT decrease after 3 months. In addition, HF were responsive to anti-VEGF therapy.

INTRODUCTION

Diabetic macular edema (DME) is a sight threatening complication of diabetes mellitus (DM) and one of the most frequent causes of vision loss.[1] Because vascular endothelial growth factor (VEGF) plays a central role in the development of centre-involved DME, anti-VEGF agents have been implemented as the treatment of choice for this condition. However, not all patients respond equally well to the initiated treatment, in which case patients are redirected to treatment with an alternative anti-VEGF agent or long acting corticosteroids.[2] Currently, we are not able to choose the best treatment option for an individual patient a priori, because information on baseline characteristics that associate with treatment outcomes is lacking. Any delay in finding the most effective personalized treatment strategy may result in irreversible visual impairment and also increases the costs of health care.[3]

Although visual function is the most relevant outcome measure, it is a subjective measure of treatment response, and can be influenced by for example fluctuations in glucose levels or the presence of other ocular disorders. Conversely, anatomical measurements such as central retinal thickness (CRT) on spectral domain optical coherence tomography (SD-OCT) are a more objective and reliable outcome measure for treatment response. The diabetic retinopathy clinical research network (DRCR.net) employs a combination of both outcome measures and defined insufficient treatment response as a CRT decrease of ≤10%, or a gain of ≤5 letters on the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart, the equivalent of 0.1 logMAR.[4, 5]

Hyperreflective foci (HF) are well-circumscribed dots that can be visualized on SD-OCT in all retinal layers. They were first described in patients with DM by Bolz et al. and have since been associated with the presence of DME, as well as with non-proliferative stages of diabetic retinopathy.[6, 7] Hypotheses about their etiology diverge: some authors suggested they could be lipid extravasations acting as subclinical hard exudates.[6, 8, 9] Others have argued that HF are migrating RPE cells since the reflectivity of HF corresponds with that of the RPE,[10] or that they might be degenerated photoreceptor cells.[11] Another theory is that HF are aggregates of cells involved in retinal inflammatory response, such as activated microglia.[12]

The purpose of this study was to investigate the association between baseline number of HF and treatment response to anti-VEGF in terms of visual acuity (VA) improvement and CRT decrease. We also studied the location of HF in the neuroretina and the behavior of HF during anti-VEGF treatment.

MATERIAL & METHODS

Population

We reviewed the medical files of DM type 2 patients with DME who were treated with intravitreal injections of bevacizumab (1.25 mg) at the department of Ophthalmology of the Radboud University Medical Center between November 2010 and May 2013. We restricted inclusion to treatment naive patients who received a complete loading dose of three consecutive injections with a 4-6 weeks interval, and of whom baseline and 3 month follow up data were available. Other exclusion criteria were: laser treatment or intraocular surgery within 12 weeks prior to the first injection, active proliferative diabetic retinopathy, vitreous hemorrhage or tractional retinal detachment at baseline visit, and presence of other retinal vascular diseases. This study adhered to the tenets of Helsinki. The Research Ethics Committee of the Radboud university medical center Nijmegen approved this study and waived the requirement for informed consent (2018-4424). All data was fully anonymized before it was accessed by the investigators.

Data collection

The following clinical characteristics were assessed: gender, age, duration of DME, and DR staging according to International Clinical Diabetic Retinopathy Severity Scale.[13] At baseline and 3 month visits, Snellen VA was measured and converted to the logarithm of the maximum angle of resolution (logMAR). We used SD-OCT-scans (Spectralis™ HRA+OCT, Heidelberg Engineering, Heidelberg, Germany) to automatically measure CRT as the average thickness in an area of 1000 µm diameter surrounding the foveal centre. Adequate response was defined as a CRT decrease of >10% and a VA improvement of >0.1 logMAR.[4, 5]

Image grading

We selected baseline and 3 months B-scans centered on the fovea and evaluated these scans for the presence of HF within the central 3000 µm around the fovea (Fig 1). HF were defined as small, round or oval-shaped, well-circumscribed dense particles with higher reflectivity than the background. The size could not exceed 100 µm, as we hypothesized this to be clumps that can be visualized as hard exudates on fundus photographs. The total number of HF in each scan was counted, as well as the number of foci within the inner (inner nuclear layer) and outer (outer plexiform layer to outer border of the external limiting membrane) retinal layers. The nerve fiber layer through the inner plexiform layer, and the photoreceptor layer and the retinal pigment epithelium (RPE) were excluded from analysis, because the naturally high reflectivity of these layers impedes the evaluation of HF. Additionally, the number of HF with a higher reflectivity than the

RPE were counted. Grading was performed by two experienced independent graders (LA, VS), masked to all clinical information. In case of disagreement in counted number of HF between the two graders exceeded 20%, differences were resolved through discussion. For all other observations, the average of both graders was used for analysis. In addition, the observers graded the central B-scans for the presence of cysts, subretinal fluid, foveal disruption of the external limiting membrane and the photoreceptor layer, and the presence of disorganization of the retinal inner layers (DRIL).

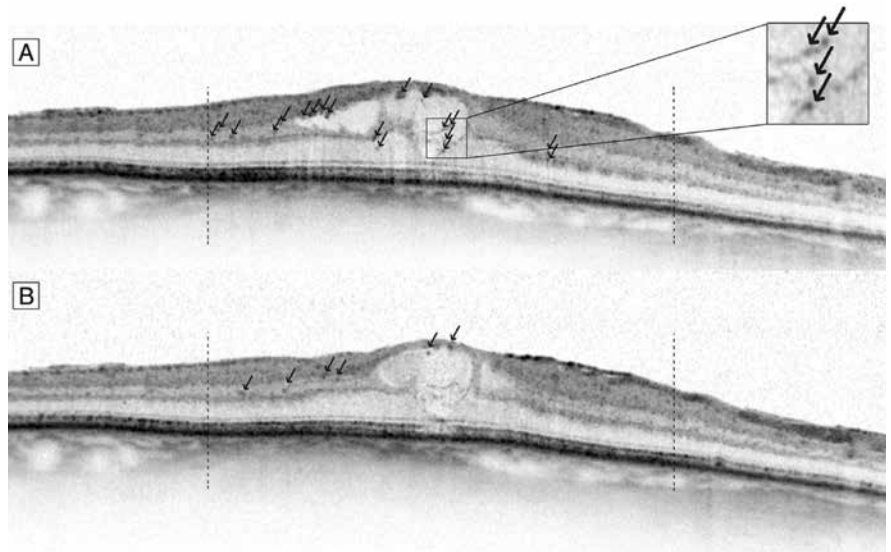

Figure 1 Hyperreflective foci on SD-OCT before and after treatment with anti-VEGF. Foveal centered spectral domain optical coherence tomography (SD-OCT) B-scan image of a patient with DME before (**A**) and after (**B**) 3 injections with anti-VEGF. Black arrows indicate hyperreflective foci, within 3000 µm of the fovea (dashed bars).

Statistical analysis

An intraclass correlation coefficient for the number of HF was calculated to assess interrater agreement. To account for the relationship between both eyes, univariable linear mixed model analyses were used to assess the difference in CRT, VA and number of HF before and after treatment, and to evaluate the association between baseline number of HF and (change in) CRT and VA. To correct for potential confounders, multivariable linear mixed model analyses were used, applying

stepwise backward deletion of non-significant confounders with an influence of <10% on the estimate of HF at baseline. These analyses were conducted using SPSS version 22 (SPSS, Chicago, IL, USA). Mixed effect logistic regression analysis was used to assess associations between baseline number and change of HF with adequate or insufficient treatment response, expressed as an odds ratio (OR) with 95% confidence interval (CI). These analyses were performed in SAS Statistical Analysis Software 9.2 (SAS Institute, Cary, NC, USA). P-values <0.05 were considered statistically significant.

RESULTS

Fifty-four eyes of 41 subjects were evaluated over the course of 3 months of treatment with bevacizumab. Patient characteristics at baseline are described in Table 1. Mean CRT at baseline was 482 ± 128 µm, and decreased significantly after treatment with bevacizumab to 419 ± 127 µm (p<0.001, Fig 2A). LogMAR VA was 0.54 ± 0.36 (Snellen 20/69) at baseline and improved to 0.48 ± 0.35 (Snellen 20/60) at the 3 month visit, although this difference did not reach statistical significance

Table 1 Baseline characteristics.

Variable (n = 54 eyes of 41 subjects)	
Gender, n (%)	
Male	22 (54%)
Female	19 (46%)
Age, mean (SD), years	67 (12)
Duration DME per eye, median (IQR), months	8 (2-21)
DR stage per eye, n (%)	
Mild	9 (17%)
Moderate	41 (76%)
Severe	4 (7%)
Presence of cysts per eye, n. (%)	51 (94%)
Presence of subretinal fluid per eye, n (%)	13 (24%)
Foveal disruption of ELM per eye, n (%)	20 (37%)
Foveal disruption of PR layer per eye, n (%)	21 (39%)

Abbreviations: n=number; SD= standard deviation, DM=diabetes mellitus; DME=diabetic macular edema; IQR=interquartile range; DR=diabetic retinopathy; ELM=external limiting membrane; PR=photoreceptor

(p=0.168, Fig 2B). For HF measurements, the intraclass correlation coefficient was 0.85 [0.79-0.89]. Mean number of HF at baseline was 14.8 ± 9.7, and decreased to 10.7 ± 6.5 (p=0.002, Fig 2C).

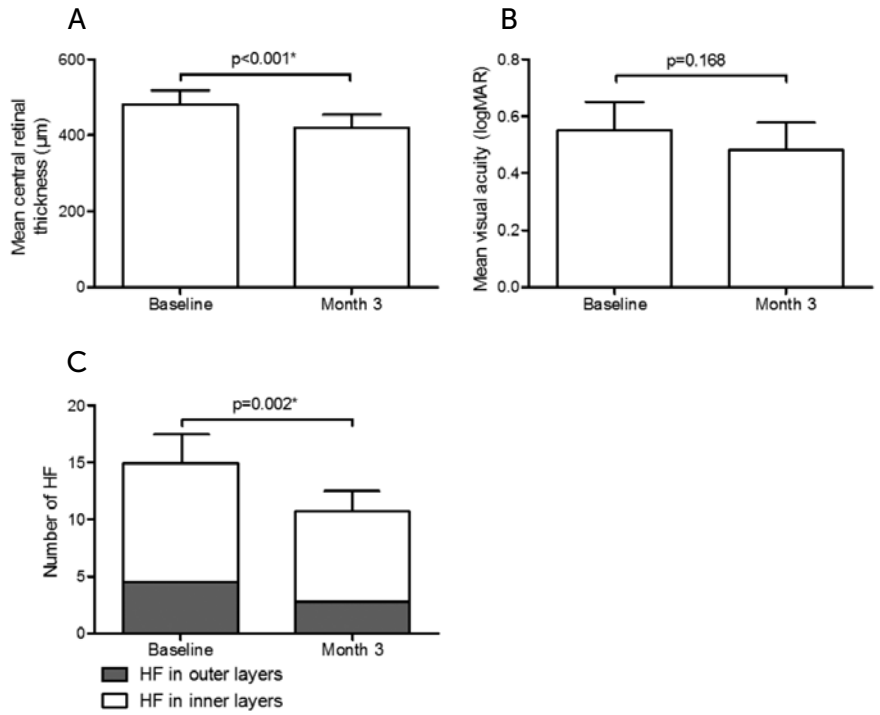

Figure 2 Differences between before and after treatment with anti-VEGF. Mean changes in central retinal thickness (**A**), visual acuity (**B**) and number of hyperreflective foci (**C**) at baseline and after 3 injections with anti-VEGF. The bars represent mean ± 95% confidence interval. *P<0.05.

HF were scattered throughout all retinal layers. The mean number of HF in the inner retinal layers at baseline was 10.4 ± 7.3 versus 7.9 ± 5.0 after treatment (p=0.010, Fig 2C). In the outer layers the mean number of HF at baseline was 4.5 ± 4.9 compared to 2.8 ± 3.0 after treatment (p=0.013, Fig 2C). The percentage of HF with a reflectivity higher than the RPE was higher in the outer retinal layers (64% at baseline and 68% at 3 months) than in the inner retinal layers (36% at baseline and 25% at 3 months).

The number of HF at baseline was independently associated with a decrease in CRT after 3 months (p=0.006, Table 2). An estimate of -2.61 was found for this association, implicating that for every HF counted at baseline, CRT declines with 2.61 µm after treatment. There was no relation between the number of HF at baseline and baseline CRT (Table 2). When we investigated the relationship between HF number at baseline and VA outcome measures, we found that baseline VA was 0.008 points worse for every counted HF (p=0.047). No effect of baseline number of HF and change in VA after 3 months was found (Table 2).

Table 2 Linear mixed model analysis of the effect of baseline number of HF on baseline values of VA and CRT, and VA and CRT changes

	Univariable			Multivariable		
	Estimate	95% CI	P	Estimate	95% CI	P
CRT baseline, µm	-1.89	-5.71 – 1.94	0.325	-3.54	[-7.44-0.366]	0.074
CRT change, µm	-2.47	-4.64 – -0.31	0.026*	-2.61	[-4.42--0.79]	0.006*
VA baseline, logMAR	0.013	0.004 – 0.023	0.008*	0.008	[0.001-0.016]	0.047*
VA change, logMAR	-0.002	-0.009 – 0.004	0.473	-0.002	[-0.006-0.009]	0.661

Multivariable analyses were corrected for gender, age, duration of DME, DR stage, presence of cysts, presence of subretinal fluid, disruption of the external limiting membrane, disruption of the photoreceptor layer and the presence of disorganization of the retinal inner layers. Analyses of CRT and VA changes were also corrected for both CRT baseline and VA baseline. Analysis of CRT baseline was also corrected for VA baseline, and analysis of VA baseline was also corrected for CRT baseline.
Abbreviations: CI=confidence interval; HF=hyperreflective foci; CRT=central retinal thickness
*P<0.05.

Next, we stratified the cohort into two groups with either adequate or insufficient treatment response. A total of 30 eyes (56%) showed a CRT decrease of ≥10%, while 16 eyes (30%) showed an improvement in VA of ≥0.1 logMAR. Subsequently, 13 eyes (24%) met both CRT and VA criteria for adequate treatment response, classifying 41 eyes (76%) as insufficient responders. Eyes that were classified as adequate responders presented with higher numbers of HF at baseline than eyes with insufficient CRT and VA response (21.6±9.5 vs. 12.7±8.8, OR 1.106, 95% CI [1.012-1.210], p=0.030, Fig 3A-C). In eyes showing adequate CRT and VA response, 76% of HF were observed in the inner retinal layers, while this amounted to 66% in eyes with insufficient response (Fig 3C).

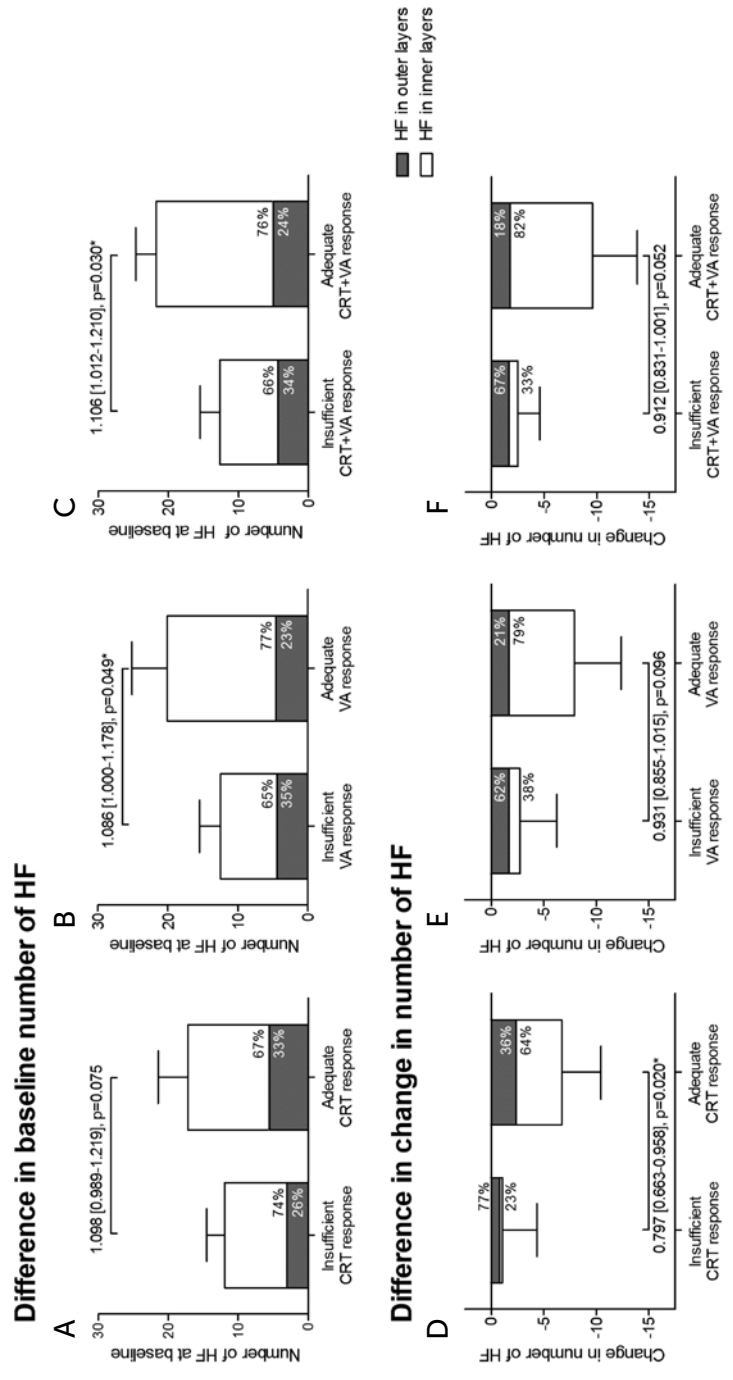

Figure 3 Difference in number of HF at baseline, and change in HF between eyes with adequate response versus insufficient response. Mean number of HF at baseline in groups based on insufficient and adequate CRT response (**A**); insufficient and adequate VA response (**B**); and insufficient and adequate combined CRT + VA response (**C**). Mean change in number of HF in groups based on insufficient and adequate CRT response (**D**); insufficient and adequate VA response (E); and insufficient and adequate combined CRT + VA response (F). The distribution of HF in the inner and outer retinal layers is displayed as a percentage. The values represent the odds ratio with corresponding 95% confidence interval and p-value. The odds ratio of adequate response is 1.106, which can be interpreted as an increase, or chance, for adequate response by 10.6 % for every HF at baseline; when the number of HF at baseline increases by 10, the chance for adequate response increases exponentially by 1.106^{10}=2.74 or 274%. The bars represent mean ± 95% confidence interval and are based on descriptive statistics. *P<0.05.

We did not observe a significant difference in HF change between eyes with an adequate CRT and VA response and eyes with insufficient CRT and VA response (-9.5±7.4 vs. -2.6±8.8, OR 0.912, 95% CI [0.831-1.001], p=0.052, Fig 3D-F). In eyes showing an adequate response, the decrease in HF was mostly seen in the inner retinal layers, whereas in insufficient responders this decrease was more prominent in the outer retinal layers (Fig 3F).

DISCUSSION

Higher numbers of HF at baseline were associated with adequate treatment response to anti-VEGF, in terms of CRT decrease and VA improvement. The relationship between baseline number of HF and CRT decrease was also apparent in linear mixed model analysis, showing that the effect was independent from potential confounders. In addition, HF were responsive to treatment with anti-VEGF, and were more often detected in the inner retinal layers than in the outer retinal layers. We also observed that a high number of HF at baseline was associated with poorer VA prior to treatment with anti-VEGF.

The observation that higher numbers of HF at baseline are associated with adequate treatment response could be of importance for clinical practice. For example, when poor response to anti-VEGF can be predicted based on baseline biomarkers, the decision to start with an alternative treatment can be made beforehand, thereby preserving visual performance. Ideally, these findings should be integrated in a prediction model that can reliably predict treatment response, including all other potential predictive factors, such as patient characteristics, environmental factors, genotype variables and other imaging biomarkers to be further investigated.

To the best of our knowledge, a relationship between high baseline number of HF and both adequate morphological and functional treatment outcomes in DME has not been established before, although it has been studied in other cohorts. An association between higher numbers of HF and final visual acuity at a mean of 7 months after treatment with bevacizumab in 33 eyes with DME accompanied by serous detachment was reported by Kang et al.[14] Contrarily, Hwang et al. recently reported fewer numbers of HF to be associated with good CRT response after 3 months of bevacizumab treatment. The reason for this disparity is unclear, although there are substantial differences in study design.[15] Hwang et al. studied particles with equal or higher reflectivity than the RPE, while we used a reflectivity higher than the surrounding tissue for a definition, which in our experience results in a more reliable detection of HF. Furthermore, proliferative diabetic retinopathy is

associated with an increased inflammatory response and exuberant activation of microglia, and we therefore hypothesize that neovascularization could influence the number of HF.[16] For this reason, we excluded patients with active neovascularization, while DR staging was not reported by Hwang et al. Vujosevic et al., who also studied the relationship between baseline HF and treatment response, did not find a association between baseline number of HF and change in CRT or VA after treatment with ranibizumab in respectively 20 and 26 eyes with DME, which might be due to small sample size.[17, 18] Moreover, different criteria for treatment response were used by all study groups: Hwang et al. chose for a treatment response definition of CRT <300 µm or a reduction by more than 50 µm, while Kang et al. and Vujosevic et al. defined treatment outcome by the continuous variables VA improvement and CRT reduction, respectively.[14, 15, 17, 18] We chose a definition of CRT decrease of >10% and a VA improvement of >0.1 logMAR, as proposed by the Diabetic Retinopathy Clinical Research Network, as a CRT reduction expressed as a percentage accounts for differences in baseline CRT, and the combination with VA improvement provides valuable information on visual function.[4, 5]

The association between higher baseline number of HF and poorer baseline VA is in line with previous studies,[11, 19] and could be a relevant observation for clinical practice. Often, vision loss is not the first manifestation of DME. Therefore, large-scaled screening programs are set up for patients with DM to detect early changes related to leakage of fluid into the retina, such as microaneurysms and hard exudates.[20] It has been suggested that HF are the missing link between the breakdown of the inner blood– retina barrier and the osmotic swelling of retinal layers.[8] HF, as an earlier representation of microvascular damage, may be important in the risk assessment for progression of DME and vision loss.

Various different hypotheses have emerged about the origin of HF that might in fact coexist. HF could be precursors of hard exudates,[6, 8, 9] migrating RPE cells,[10] degenerated photoreceptor cells,[11, 19] or aggregations of activated immune cells, such as microglia.[12] Microglia are highly ramified phagocytic cells in the retina and are required for neuronal homeostasis and immune defense. VEGF has been demonstrated to induce microglial activation,[21] engaging a feedback loop in which immune cells in the retina simultaneously express and release VEGF.[22] Our work shows that HF are responsive to anti-VEGF and reside predominantly in the inner retinal layers, which is in accordance with microglial cell behavior.[16, 23] Moreover, the decrease in HF after 3 months of bevacizumab was most prominent in the inner retinal layers, which supports the hypothesis that HF in these layers correspond to activated microglia, and that HF in the outer retinal layers may represent a different entity. It has also been hypothesized that HF are precursors of hard exudates, given

the equal reflectivity on SD-OCT. Hard exudates are commonly located in the outer plexiform layer, and unlike microglial cells, they are not subject to rapid regression after only 3 months of treatment, although these dynamics are unknown for precursors of hard exudates.[12, 24, 25] In the outer retinal layers, we found that most HF had a reflectivity higher than the RPE, in contrast to the HF in the inner retinal layers. The unresponsiveness to therapy and the hyperreflective appearance suggests that at least some of the HF in the outer layers are precursors of hard exudates or migrating RPE cells, as was proposed earlier.[10] Further research is warranted to study the etiology and clinical value of HF, by means of histological and epidemiological studies.

Overall, there was a significant decrease in CRT, but no significant improvement in VA after 3 injections of bevacizumab in this cohort. Earlier studies demonstrated that treatment outcomes in real life are often inferior to the results of controlled clinical trials.[26, 27] Moreover, the follow up duration in this study was relatively short, and it is known from large clinical trials that VA can improve even after the loading dose of 3 injections before stabilizing.[5] Most large clinical trials include both patients with type 1 or type 2 diabetes. In our study, we only included patients with type 2 diabetes, which may explain why these patients are slightly older than in most clinical trials. However, to the best of our knowledge, there is no evidence from previous studies that age or type of diabetes are associated with worse treatment outcomes.[28]

The use of a well-designed, detailed grading protocol was one of the major strengths of this study, evidenced by the good interrater agreement. This protocol enables replication of our findings and validation in other cohorts. Another strength was the well-defined cohort: we used strict inclusion criteria to limit the analysis to eyes with a completed loading phase and follow-up. Sample size and the short duration of follow-up can be considered limitations of our study, although it has been shown that VA response at 3 months is highly predictive for long term outcomes of anti-VEGF therapy.[29] Another limitation of this study is the grading of the central horizontal SD-OCT B-scan only, thus we may not be able to generalize our findings to peripheral parts of the macula. The process of HF grading is labor-intensive, and a computer assisted approach could facilitate fast and replicable grading. Furthermore, the development of software-based grading is inevitable when translating HF grading from research to clinical practice, in order to implement feasible evaluation of HF for the clinician.

In conclusion, our results suggest that in patients with DME, baseline number of HF are correlated with visual acuity as well as anti-VEGF treatment response in terms of CRT reduction and VA improvement. Replication of our findings in larger cohorts is needed to assess the validity of our study. Additionally, the relation between HF and other anti-VEGF agents and corticosteroids needs to be further explored. This knowledge may contribute to the development of proper risk assessment, and therefore enable personalized decision making and prevention of overtreatment.

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5

General discussion

GENERAL DISCUSSION

Diabetes mellitus is posing a major public health problem and is arguably the biggest epidemic of the twenty-first century. The increase in incidence largely reflects the trends of rapid urbanization, sedentary lifestyles and poor dietary habits. Until recently, diabetes was a disease typically associated with high-income countries. In the immediate future, however, the largest increases will take place in regions where economies are rapidly moving from low income to middle income levels, such as in parts of Africa and Asia, especially China.[1] Due to the increase of diagnostic and therapeutic options, diabetes is a disease that is now more and more characterized by chronicity. Subsequently, the financial burden of diabetes is expanding, and it is now estimated that already an average of 12% of all global healthcare expenditures are dedicated to the treatment of diabetes and related complications.[1] In ophthalmic practice, the proportion of costs attributable to patients with diabetes is even 21%, with the largest expenses attributable to severe stages of retinopathy.[2, 3] In order to pursue affordable and optimal diabetic eye care in the future, an increased focus on prevention and risk stratification strategies is essential.

The aim of risk stratification is to separate patients according to their risk of specific health outcomes. Assumed that this risk varies considerably within a population, patients at high-risk may require a different approach than those at low-risk. This way, healthcare can be more efficiently delivered to those who need it most, thereby improving cost-effectiveness. For diabetic eye care for example, this entails the predicted risk of developing diabetic retinopathy, the risk of progression to vision-threatening diabetic retinopathy, and the predicted response to treatment. In diabetic eye screening, longer screening intervals will reduce unnecessary visits in low-risk patients, while shorter screening intervals in high-risk patients will prevent unnecessary vision loss and associated costs. In patients with diabetic macular edema, risk stratification enables the development of individually tailored treatment strategies. For example, patients with indicators of poor treatment response to anti-VEGF at first presentation may be directed to therapy with corticosteroids. Irreversible vision loss as a result of delays in effective treatment can thereby be prevented.

In order to develop personalized strategies, extensive knowledge of biomarkers that associate with the risk of development and progression of diabetic retinopathy and treatment outcomes is needed. Identifying these biomarkers is a focus of this thesis, thereby providing building blocks needed to establish personalized medicine. This thesis furthermore aims to contribute to cost-effective diabetic eye care through recommendations for more efficient use of already available techniques.

In this chapter, we will discuss the implications for clinical practice and scientific research of our most important findings and outline future perspectives of diabetic eye care.

1. DEVELOPMENT AND PROGRESSION OF DIABETIC RETINOPATHY

1.1. Epidemiology

In Chapter 2.1, we analyzed the risk factors involved in development and progression of diabetic retinopathy. In our cohort of Dutch patients with type 1 diabetes, we found an estimated 25-year cumulative incidence of 63% for diabetic retinopathy and 21% for vision-threatening diabetic retinopathy, defined as the presence of (pre) proliferative diabetic retinopathy or diabetic macular edema. Three comparable studies in the United States and Denmark, dating roughly 10 years prior to our study, analyzed cohorts of type 1 diabetes patients and found a 25-year cumulative incidences of 83-97% for diabetic retinopathy and 42-53% for proliferative diabetic retinopathy.[4-6] Although direct comparison is speculative due to differences in study design and, to lesser degrees, differences in diabetic eye care, these numbers suggest a decline in the incidence and severity of diabetic eye disease. This trend is further confirmed in a meta-analysis by Wong et al. They calculated a pooled 4-year incidence of proliferative diabetic retinopathy of 20% for studies conducted between 1975-1985, versus 3% for studies conducted between 1986-2008.[7] A comparable declining trend is observed for visual impairment and blindness as a consequence of diabetic retinopathy. In our 2018 study, we found a 25-year cumulative incidence of 3% for visual acuity loss below 0.5. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) dating from 2008 reported a 25-year cumulative incidence of 13% for visual loss of 0.5 or lower.[4]

Despite continually improving diabetic eye care, an increasing number of individuals experience loss of vision due to diabetes-related eye disease worldwide. In 1990, an estimated 1.7 million people suffered from moderate-severe visual impairment or blindness as a consequence of diabetic retinopathy worldwide. In 2015, 25 years later, this number had nearly doubled to 2.9 million.[8] This increase, in spite of the advancements in diabetic eye care, are simply the result of the rapidly increasing number of individuals living with diabetes. This number has grown in a much higher pace, from 158 million in 1990 to 415 million in 2015 (Figure 1).[9, 10] From these data, we can calculate that the proportion of people with diabetes that suffer from visual impairment has declined from 1.1% in 1990 to 0.7% in 2015.

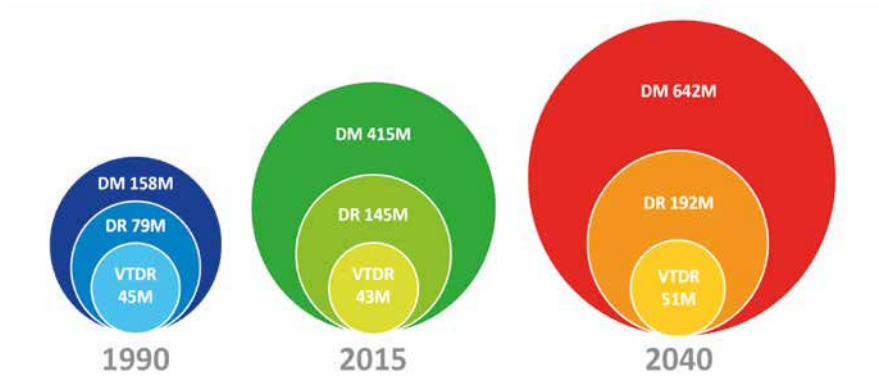

Figure 1 Estimated prevalence of diabetes mellitus, diabetic retinopathy and vision-threatening retinopathy in 1990, 2015 and 2040 respectively.[8-11] Abbreviations: M=million.

The improvements in diabetic eye care over the past 25 years that are responsible for the declining trend, include better screening programs, much tighter blood glucose control with new devices for self-monitoring and the administration of insulin through pumps, as well as the introduction of new and improved treatment options, both surgically and pharmaceutically, notably anti-VEGFs. However, little is known about the risk for people in developing countries, where the largest increase in diabetics is expected. In under-resourced countries, the accessibility of healthcare services is insufficient, posing a major challenge to public health. Efforts should be made to prevent diabetes and its complications in these countries, for example by promoting lifestyle changes, such as improving diet and physical exercise. In addition, more cost-effective approaches of diabetes care are required in order to provide these people with adequate healthcare.

1.2. Clinical biomarkers

From literature it is known that not all patients with diabetes are at equal risk of developing ophthalmic complications.[12, 13] To identify high-risk patients at an early stage, recognizing specific risk factors for development and progression of diabetic retinopathy is required. It is well established that HbA1c and diabetes duration are the most important risk factor for development and progression of diabetic eye disease. However, this combination only accounts for 11% of the total risk of retinopathy.[14, 15] It is of the essence that the risk factors that account for the remaining 89% of the total risk should be explored to improve our risk stratification.

Glycemic control is typically expressed as elevated levels of blood glucose or HbA1c. This is, however, a rather one-dimensional indicator of glycemic control. For instance, patients with identical mean HbA1c levels may vary considerably in their long-term glycemic variability. We identified HbA1c variability as an independent risk factor for the development of diabetic retinopathy in a population of patients with type 1 diabetes (**Chapter 2.1**). How HbA1c variability contributes to the development of retinopathy is currently unknown, but there are several hypotheses that are not mutually exclusive. It could be that the risk of retinopathy increases exponentially with higher HbA1c levels, putting patients with larger variation in HbA1c at a higher average risk. Another hypothesis is that HbA1c variability leads to microvascular damage through the paradoxical phenomenon of short-term worsening. This entails a worsening of retinopathy after a period of rapid improvement of glycemic control. In patients with large variability in HbA1c, this process is continuously triggered, resulting in sustained disease progression. A third hypothesis relates to the observation that periods of sustained hyperglycemia can be 'remembered' by the body, also known as 'metabolic memory'. This is evidenced by the observation that patients who underwent aggressive early glycemic control demonstrated a lower risk of microvascular complications than the conventionally treated patients, despite similar HbA1c values in the long-term follow-up that ensued.[16, 17]

Where HbA1c variability relates to changes in blood glucose levels over longer periods of time, it does not provide information on the intensity of glycemic excursions during the day, resulting from postprandial spikes and fasting hypoglycemia. Short-term glycemic variability has been related to oxidative stress, endothelial dysfunction and inflammation, and may therefore play an independent role in the development of microvascular complications.[18, 19] A relation between higher short-term glycemic variability and the presence of retinopathy was reported in patients with type 2 diabetes, but not in patients with type 1 diabetes.[20, 21] Explanations for this disparity may be found in differences in definitions of glycemic variability and interval length: the method of measuring variability may differ between studies (standard deviation, coefficient of variation or mean amplitude). We argue that the coefficient of variation, defined by the ratio of the standard deviation to the mean, is the preferred method to measure variability, because it corrects for an increase of variability that may accompany an increase of the mean. In long-term glucose variability, an interval of 3 months is most optimal, because this equals the average turnover rate of red blood cells. For short-term glucose variability, many studies used 7-point self-monitoring blood glucose profiles that offer a relatively crude measure of variability.[20] Modern devices, such as continuous glucose monitoring systems could facilitate standardized measurements of glycemic variability. The additive prognostic value of short- and long-term glycemic variability as a biomarker for retinopathy development and progression should be addressed in well-designed, prospective studies. In addition, future research should assess whether glycemic variability would make existing prediction models more effective.

In clinical practice, glycemic control is generally monitored by the levels of fasting blood glucose and HbA1c. The current guidelines use glycemic targets that only focus on the height of blood glucose or HbA1c. Because HbA1c variability is an independent risk factor for both micro- and macrovascular complications of diabetes, we argue that internists and general practitioners should take this parameter into account when assessing the effectiveness and safety of a management plan on glycemic control. In addition, to suppress large fluctuations in HbA1c in patients in need of insulin, continuous subcutaneous insulin infusion (pump therapy) could be considered, rather than multi-daily injections.[22, 23]

1.3. Screening

For diabetes patients, annual eye screening has long been - and in most clinical practices still is - the standard of care. However, as outlined previously, the risk of developing retinopathy and sight-threatening retinal complications differs for individual patients. To better address these differences in risk profiles, more tailor-made screening programs are needed. Personalized screening intervals based on individual risk profiles would significantly improve cost-effectiveness of diabetic eye screening. Recently, Aspelund and co-workers developed such an individualized screening model and tested the validity on a cohort of type 2 diabetes patients in Iceland. This model employs variables such as diabetes type and duration, the presence of diabetic retinopathy, and levels of HbA1c and systolic blood pressure. We validated Aspelund's model to calculate a personalized screening interval in a Dutch population with type 1 diabetes.[24] We showed that by using the model, eye screening frequency can safely be reduced by 61% compared to annual screening, and by 21% compared to biennial screening, with a corresponding reduction in costs (**Chapter 2.2**). Our findings in patients with type 1 diabetes are comparable to those reported by validation studies in patients with type 2 diabetes.[25-27]

Our calibration of the model showed that the risk of sight-threatening retinopathy was overestimated, especially in those at higher risk. This implicates that the observed risk of sight-threatening retinopathy is lower than the predicted risk, assigning these patients a shorter screening interval than needed. This is a consistent finding in previous validation studies,[25-27] and may be based on the assumption of the model that risk factors are independent, while in fact interaction is possible.

Another explanation may be that the model assumes that all risk factors have a linear effect, while this may not apply for high values. In addition, patients with high HbA1c and blood pressure levels may have received treatment to improve control, resulting in a lower observed risk. Despite the improvement in the cost-effectiveness of diabetic eye screening compared to current screening methods, the performance of the model to predict the risk of sight-threatening retinopathy is not perfect. Therefore, we should continue the search for biomarkers explanatory of the risk of retinopathy progression.

In Aspelund's model, the longest possible interval was set at five years. It was previously pointed out by McGhee et al. that this might be dangerously long, because patient's clinical risk factors could change during this period, which would affect the predicted risk.[28] However, in our study, no cases of sight-threatening diabetic retinopathy occurred within the screening interval, suggesting that the use of a five-year ceiling is a safe approach. Safety can only be guaranteed with good patient compliance.

With current screening protocols however, the screening uptake greatly varies, with recent studies reporting a range of 30-80%.[29-34] When adopting Aspelund's model, patients who are assigned a longer screening interval should be well-educated on the importance of regular screening uptake. Compliance can furthermore be improved by technological advances that enable the organization of screening facilities in the direct environment of the patient. For example tele-ophthalmology, smart-phone based imaging and automated image analysis have shown to improve access to healthcare, while reducing the pressure on specialist eye care and associated costs.[35-37] It is entirely feasible that, in the future, patients no longer need to cross their doorstep for diabetic eye screening.

Translating research into clinical practice is challenging and the implementation of an individualized screening system is no different. To facilitate the application of personalized screening intervals in clinical practice, the algorithm should be easy accessible for health care providers. The website www.risk.is provides a decision support system, applying Aspelund's algorithms. After the health care provider enters the risk factors for a specific person, the system calculates the recommended screening interval. However, during busy consultation hours, browsing to a website and manually filling out the electronic forms is time-consuming. A first necessary step would be to translate this website into a Dutch version and to inform health care professionals of the existence of this system of personalized screening intervals. However, a major leap in accessibility would be the integration of this algorithm in the electronic patient dossier (EPD). This would provide the additional advantage that risk factors, such as diabetes duration, HbA1c and blood pressure

levels, are already available in the system and could be pre-filled by the software, so there is no need for the healthcare provider to spend time on retrieving information. Integration with automated detection software for diabetic retinopathy on fundus photographs may further reduce the time needed for a health care provider to determine a person's risk profile.

2. IMAGING CHARACTERISTICS

2.1 Integration of new imaging modalities

Color fundus photography and ophthalmoscopy have long been, and currently still are, a cornerstone in the evaluation of diabetic retinal disease. The introduction of a standardized classification scale for diabetic retinopathy at the Airlie House conference in 1968 has been of crucial importance for diabetes care and this classification that is still in use today.[38] This was followed by the standardized classification of fluorescein angiography by the ETDRS in 1991, although this classification mainly served research purposes, as it is considered too complex for use in daily clinical practice.[39] However, over the last decades new imaging modalities, such as optical coherence tomography (OCT), OCT angiography (OCTA) and ultra widefield (UWF) photography have emerged that provide new information vital for optimal diabetic eye care. The spectral domain (SD) OCT, in particular, has become an invaluable diagnostic tool for central retinal pathology and has partially replaced fluorescein angiography in the evaluation of DME. These imaging techniques allow more detailed phenotyping than can be obtained by color fundus photography or fluorescein angiography alone, especially for diabetic macular edema. Improving our knowledge of the specific characteristics that can be observed in patients with diabetic retinopathy, enables better stratification of patients at risk of progression or treatment failure.

2.2. Exploring new biomarkers

In order to optimize risk stratification based on phenotypic characteristics, efforts should be made to appropriately quantify imaging characteristics. To establish phenotype-based stratification strategies for diabetic retinal disease, fundamentals have to be laid through exploration of potentially relevant biomarkers. In addition, the study of imaging characteristics can help us to unravel pathophysiological mechanisms. An imaging-derived biomarker should preferably be easy to measure, have low inter- and intra-observer variability, and should have a relationship with a specific endpoint, such as visual acuity, disease progression or treatment response.

One of the difficulties of exploring new biomarkers is that definitions have not previously been standardized. Sometimes researchers even have to establish a definition of their own. A clear definition of the biomarker to be evaluated is of crucial importance to obtain meaningful and replicable results. However, even established reference standards can be subject to substantial inter- and intra-observer variation.[40-45] We have experienced this difficulty as well in our own research projects where new biomarkers were tested (hyperreflective foci on OCT) or existing biomarkers were evaluated on new imaging modalities (microaneurysms on OCTA). From this experience we have learned that reaching consensus on a clear definition prior to start with grading is key. In addition, training of graders can substantially improve interobserver reliability.[46] Despite these improvements, manual annotations will to some extent always be a source of bias, because they rely human observation and interpretation.

2.2.1. OCT

OCT enables detection of disease characteristics that could previously not be appreciated by fundoscopy or fundus photography. The high-resolution, three dimensional imaging of the macular area allows for the detailed evaluation of morphological characteristics, providing more insight into structural-functional relationships in diabetic retinopathy.[47] For example, disorganization of the retinal inner layers (DRIL), disruption of the ellipsoid zone, and hyperreflective foci were found to associate with visual function in diabetic macular edema.[48-50] In this thesis we chose to focus primarily on hyperreflective foci, because of their potential inflammation-related origin, and our subsequent hypothesis that they could aid in predicting disease progression and treatment response to anti-VEGFs.

Various hypotheses circulate concerning the origin of hyperreflective foci. In diabetic retinal disease, these small dots could represent precursors of hard exudates, as they have similar reflective characteristics.[51] This is corroborated by our own work, where we show that hyperreflective foci are associated with the presence of hard exudates on color fundus photography (**Chapter 3.1**). However, hyperreflective foci also occur in other retinal diseases that do not typically present with hard exudates, such as age-related macular degeneration.[52] A common pathway in diabetic macular edema and age-related macular degeneration is the presence of inflammation. This knowledge has led to the hypothesis that hyperreflective foci could represent cells involved in the inflammatory response, such as aggregations of microglia or macrophages.[53, 54] Most hyperreflective foci are found in the inner retinal layers (**Chapter 4.2**). As the disease worsens, these foci migrate to the outer layers, which corresponds with the behavior of microglia cells.[55, 56] Lastly, it has been postulated that hyperreflective foci are migrating retinal pigment epithelial

cells or degenerated photoreceptor cells.[57] This may be true in some cases, but it cannot explain the presence of hyperreflective foci in early stages of diabetic retinopathy, where the retinal pigment epithelium and photoreceptor layers are generally intact. We therefore hypothesize that hyperreflective foci are representations of multiple entities, depending on their location within the retina and the type and stage of the disease. To obtain a better understanding of the origin of hyperreflective foci in diabetic macular edema, studies combining histological and OCT-derived information should be conducted.

In order to use hyperreflective foci as a prognostic marker for treatment response or disease progression, a thorough understanding of their distribution in the population of patients with diabetes is needed, as well as their relationship with other morphological and clinical variables. We therefore studied hyperreflective foci in different stages of diabetic retinopathy as well as diabetic macular edema in a population of patients with type 1 diabetes mellitus (**Chapter 3.1**). The number of hyperreflective foci increased as the macular edema severity increased. Meanwhile, the relation with diabetic retinopathy severity was less pronounced. This may point towards a more prominent role of hyperreflective foci in exudative rather than in ischemic diabetic retinal disease. However, due to the labor-intensive nature of hyperreflective foci grading, only one B-scan of the total OCT volume was assessed. We selected the central 3 mm of the fovea-centered B-scan, because of the importance of this area for visual function. This part of the macula is generally affected in case of diabetic macular edema, but diabetic retinal disease may not be restricted to this area. The availability of automated detection of hyperreflective foci (**Chapter 3.2**) enables the evaluation of total OCT volumes. The role of extramacular hyperreflective foci as a biomarker for retinopathy progression should be subject of future research.

2.2.2. OCTA

OCTA has emerged as a novel imaging modality that enables visualization of microvascular blood flow. OCTA has advantages over fluorescein angiography in the sense that it is non-invasive, has faster acquisition time, and provides greater detail. It provides, however, a static image of the blood flow and cannot depict vascular leakage. In diabetic retinopathy, multiple vascular abnormalities can be identified on OCTA, such as microaneurysms, intraretinal microvascular abnormality and neovascular complexes. In addition, OCTA allows for quantification of various metrics, such as size of foveal avascular zone, capillary perfusion density and fractal dimension to evaluate vascular branching.[58]

The high resolution of OCTA enables the study of microaneurysms in more detail. (**Chapter 3.3**). We identified a range of morphological appearances using a swept source OCTA device that were previously only described on adaptive optics fluorescein angiography, thereby demonstrating the capability of swept source OCTA in terms of resolution. However, only 57% of all microaneurysms identified on fluorescein angiography were also showed an increased flow signal on OCTA. This is most likely due to the inability of OCTA to detect slow blood flow.[59] that generally applies to studies investigating new techniques or new features is the lack of standardized definitions. We therefore used established grading protocols for microaneurysms on fluorescein angiography as a gold standard by default. It is known however, that the visualization of the deeper vascular networks with fluorescein angiography is incomplete, therefore potentially resulting in underdetection of microaneurysms in the intermediate and deep capillary plexi.[60, 61] Efforts should be made to develop standardized definitions of microaneurysms for OCTA, preferably by a group of experts in the field. Our work concerning the description of morphological appearances of microaneurysms could assist in developing such a classification scheme.

In this study, we furthermore used a multimodal approach to compare the morphological appearance and location of microaneurysms on OCTA to leakage on fluorescein angiography and retinal thickening on OCT. We found that microaneurysms within a focal area of leakage or retinal thickening were detected on OCTA more often than those outside an area of focal leakage or retinal thickening. Microaneurysms with an irregular, fusiform or mixed fusiform-saccular appearance on OCTA were more likely to leak and to be located in a thickened retinal area, although not all leaking microaneurysms were detected on OCTA. We can conclude that at this stage, OCTA cannot differentiate between leaking and non-leaking microaneurysms, and can therefore not replace fluorescein angiography for the guidance of focal laser treatment.

The clinical application of OCTA more likely lies in the quantification of biomarkers associated with disease progression and treatment response. Capillary nonperfusion area and enlargement of the foveal avascular zone were observed in diabetic individuals without clinical retinopathy.[62] In addition, capillary density was found to decrease with progressing retinopathy.[63] Hence, OCTA could play a role in the identification of patients at risk of retinopathy development and progression. In patients treated with anti-VEGF, decreased capillary density and a bigger foveal avascular zone in the deep capillary plexus at baseline were associated with poor response.[64] The role of OCTA parameters in the prediction of disease progression and treatment response should be addressed in future studies, applying multivariable models.

2.3 Automated detection

The evaluation of morphological characteristics is labor-intensive, and therefore unfeasible to perform in all consulting patients. Computer aided detection systems that can automatically identify these characteristics could increase repeatability as well as reduce image processing time. We developed a deep learning algorithm for the detection and quantification of hyperreflective foci that operated at a similar level as human graders (**Chapter 3.2**). This approach uses deep neural networks capable of self determining what features are most discriminative for hyperreflective foci within a given dataset. These features are independent of the existing definition of the foci, and may include properties that were not previously recognized by human observers. The self learning ability of the system may result in a more objective definition of hyperreflective foci, which is another advantage of this automated detection system. Moreover, where manual grading of hyperreflective foci was limited to the central B-scan due to its labor-intensive nature, automated grading allows grading of a total OCT volume. This way, the number of hyperreflective foci can be determined within a smaller margin of error.

This algorithm can readily be applied for research purposes, but translation to clinical practice requires additional efforts. First, the output of the algorithm needs to be visualized for the clinician, for example within the available image viewing software. Second, an integral approach needs to be developed, as it is unlikely that hyperreflective foci are the only biomarker of interest for the evaluation of diabetic retinopathy. As examples of this, recent work has addressed the automated quantification of cysts on OCT and microaneurysms and hemorrhages on color fundus photography.[65, 66] The one by one interpretation of all available automatically detected biomarkers would still be time-consuming. Ideally, the clinician should be provided with an automatically generated risk score or therapeutic advice based on the available relevant algorithms. Where for diabetic eye screening results of artificial intelligence-based systems are promising and are now even approved by the Food and Drug Administration,[67] an effective approach of automated treatment response prediction has not yet been developed. Future research should therefore be focused on pattern recognition and automated detection of phenotypes that associate with therapeutic outcomes.

3. TREATMENT OUTCOMES

3.1. Results of vitrectomy for diabetic retinopathy

Complications of proliferative diabetic retinopathy, such as vitreous hemorrhage and extensive fibrovascular proliferation with or without tractional retinal detachment often affect younger patients with an active family life belonging to the working-age population. Visual impairment in these patients, especially, greatly hampers their ability to participate in society: they may no longer be able to do their jobs, provide for their families and participate in social activities. To maintain or restore visual function, patients with the above mentioned sight-threatening complications of diabetic retinopathy can effectively be treated with a vitrectomy. Short term observational studies with follow up durations of less than one year have reported improved visual outcomes in patients undergoing vitrectomy in 53%-78[1]% for tractional retinal detachment and vitreous hemorrhage.[68-72] However, long-term prognosis has not been studies well in this relative young population. Furthermore, since DRP affects both eyes in the majority of cases, the bilateral visual function is also worth considering.

We found a 5-year cumulative incidence rate of low vision (visual acuity worse than 20/40) of 31% in the operated eye and 14% for bilateral low vision (**Chapter 4.1**). For legal blindness, visual acuity of lower than 0.05, the 5-year cumulative incidence was 12% for the operated eye, while only 3% of the patients suffered from legal blindness in both eyes combined. Our results indicate that in the majority of the patients useful visual function could be restored or maintained. Moreover, no new cases of low vision in both eyes occurred after 5 years. Re-vitrectomy was required in 21% of the patients, while 40% required vitrectomy of their fellow eye. From the results of this study, we can conclude that good medical care can stabilize visual function in the long term in most but not all cases.

Within the context of personalized risk stratification, we also aimed to identify biomarkers indicative of visual prognosis that can be assessed pre-operatively. For low vision in both eyes, only poor baseline visual acuity in the fellow eye and higher age could be identified as predictive factors. In other studies, poor visual acuity in the study eye and the presence of iris neovascularization have been identified as consistent predictive factors.[73-75] Intra-operative characteristics, the severity of the proliferative pathology, defined by the difficulty of fibrovascular tissue dissection and the use of long-acting intraocular tamponade have also been associated with visual outcome in previous studies.[76, 77] However, the combination of pre-, and intra-operative prognostic factors still only accounts for a small proportion of the observed variation.[77] Even though proliferative diabetic eye disease requiring

vitrectomy already represents a small subgroup within the diabetes population, the disease severity at the time of surgery can greatly vary, resulting in a heterogeneous study group. Further stratification based on preoperative visual acuity or disease severity may result in higher predictive accuracy of potential biomarkers. Because this will impact the statistical power of a study, large (multicenter) datasets are required.

3.2. Treatment response to anti-VEGF

Despite the favorable results of anti-VEGF therapy for diabetic macular edema and its successful implementation in clinical practice, one in three patients fails to show sufficient functional and anatomical response.[78, 79] In real-world clinical practice, the proportion of poor responders may even be higher, as patients differ from those in a carefully preselected cohort. In addition, it has been shown that patients in the real world often undergo less frequent monitoring and receive less intravitreal injections than patients in controlled clinical trials.[80] In refractory diabetic macular edema, alternative treatment strategies include switching to a different anti-VEGF agent or to corticosteroids. However, evidence for the best strategy in such cases, let alone individual patients, is currently unavailable as prospective randomized controlled trials that address this question are lacking. The trial-and-error protocol used in current clinical practice leads to delays in the administration of the most effective treatment, which may cause irreversible visual impairment.

There is currently no way of knowing on first presentation which patients will respond best to which type of treatment. In our ongoing attempt to identify new biomarkers associated with treatment response to anti-VEGF, we evaluated the relation between hyperreflective foci on OCT and treatment outcome of intravitreal injection with bevacizumab for diabetic macular edema (**Chapter 4.2**). We found that higher numbers of hyperreflective foci at baseline were associated with an adequate treatment response to bevacizumab, defined by a decrease in central retinal thickness and visual acuity improvement. Hyperreflective foci may be a promising prognostic biomarker, however, this single parameter cannot fully account for the variance we found in treatment response. This is not surprising; it is altogether unlikely that a single biomarker will adequately predict treatment response.

Other OCT biomarkers that have shown associations with visual outcomes include ellipsoid zone and external limiting membrane integrity, DRIL and the presence of subretinal fluid. To date however, studies applying multivariable approaches have not resulted in consistent outcomes.[81-84] This is most likely due to heterogeneity of study design: treatment regimen, follow up, the definition of treatment response

and grading protocols have all not been standardized. Additionally, different combinations of morphological characteristics were studied, and none of these groups included hyperreflective foci, despite their potentially important role in the prediction of treatment response to anti-VEGF. In addition to imaging characteristics, clinical variables could be used as prognostic biomarkers for treatment response. In post-hoc analyses of large randomized controlled trials, clinical characteristics such as younger age, male sex, and higher baseline visual acuity were repeatedly, but not consistently associated with higher final visual outcomes.[85] Other areas that could be further explored are the field of (epi)genetics and new imaging modalities such as OCTA. For example, a genetic variant in the *VEGF* gene was found to be associated with better treatment response to bevacizumab treatment, but replication is needed to validate this finding.[86] Most likely, a combination of different types of characteristics is necessary to yield the most robust predictive ability. Therefore, multivariable prediction models should be developed, including multiple imaging-derived biomarkers as well as patient characteristics, environmental and genetic factors.

Besides improving the accuracy of prediction by the identification of new prognostic biomarkers, we need to consider what it is exactly that we aim to predict. Ideally, a prediction model should indicate the most effective treatment option for a patient, based on expected good outcome of this treatment. It is also possible however, that a prediction model indicates that neither of the possible treatment options will be effective. Withholding patients from any treatment can ethically only be considered if the predictive accuracy of the model is nearly perfect. With the currently available prognostic biomarkers it is not reasonable to expect that we can develop a model that predicts treatment response anywhere near perfection. More likely, a model will predict a chance of treatment success for the available therapeutic options, and will give an indication of the maximum effect that can be achieved. Given these computed chances, the ophthalmologist and patient will together be able to make a better informed choice on to whether and what type of treatment to commence. This furthermore empowers us to take the circumstances and wishes of the patient into account, in order to get to a truly tailored approach. Combinations of therapies could also be considered, for example the consecutive administration of both a corticosteroid and an anti-VEGF agent. Although this combination has not resulted in better visual outcomes compared to monotherapy on a population level, it may benefit selected individuals.

4. FUTURE PERSPECTIVES FOR RESEARCH AND CLINICAL PRACTICE

The large projected increase in prevalence of diabetes in the near future, coupled with the growing number of diagnostic and therapeutic options indicates a need for a more cost-effective approach of diabetic eye care. The preferred management strategy may differ inter-individually, and personalized risk stratification can assist in delivering the right care to the right person at the right moment. The development of risk stratification starts with the identification of relevant biomarkers. In this thesis we have identified hyperreflective foci as a predictive biomarker for treatment response to anti-VEGF in diabetic macular edema. Future research should be targeted at improving the accuracy of prediction of treatment response, by the identification of other independently associated biomarkers, for example on OCTA. Subsequently, these findings need to be validated in prospective cohorts. These efforts should ultimately result in a prediction model that uses a combination of relevant clinical, phenotypic and genotypic characteristics to determine the best treatment strategy for a specific individual.

For diabetic macular edema, the current treatment paradigm could be further improved by the exploration of new alternatives. For example, long-term anti-VEGF delivery, such as sustained-release implants or designed ankyrin repeat proteints (DARPins) may hold promise in reducing treatment burden as less intravitreal injections are needed.[87] The need for intravitreal therapy may even be diminished with topical delivery of anti-VEGF or longer acting agents.[88] Besides VEGF, other pathways involved in the pathogenesis of diabetic macular edema may be targeted, such as the kallikrein-kinin system or the angiopoietin pathway.[89] The exploration of new pathways may pave the way for treatment strategies that intervene at an earlier stage of the disease – preferably before macular edema or neovascularization develops. In addition, it is likely that the position of corticosteroids will be further substantiated in the current treatment armamentarium, especially for longstanding diabetic macular edema.

These potential improvements of therapeutic efficacy are however no solution for the oncoming diabetes epidemic. Especially in low- and middle- income countries, the financial burden of these expensive treatment options is simply too high. Therefore, effective prevention strategies are needed now more than ever. Good control of modifiable risk factors such as blood glucose and blood pressure is essential, and may be achieved by encouraging good health practices among patients. These include for example lifestyle changes such as improving dietary habits and physical exercise. Prevention of vision loss as a consequence of diabetic

retinopathy can furthermore be achieved by early detection of retinopathy and early intervention. For that purpose, the clinical application of personalized screening programs, like the one developed by Aspelund et al., is key. The translation of research to clinical practice is a complicated process that requires investments at multiple levels: sufficient resources should be allocated and the screening program should ideally be integrated within an electronical medical system. But even more important is the mindset of the health care practitioners: we should be amenable to change and actively share positive experiences. In this, the words spoken by Benjamin Franklin still hold true today: "An ounce of prevention is worth a pound of cure."

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6

Epilogue

6.1

Summary | Samenvatting

SUMMARY

Retinal abnormalities as a consequence of diabetes are a potential threat for visual acuity. The main cause of vision loss as a consequence of diabetes is diabetic macular edema: an accumulation of fluid in the central retina. In addition, abnormal new blood vessels (neovascularizations) can cause vision loss, for example when they rupture and cause a hemorrhage, or when they pull the retina away from its original position and result in a retinal detachment. Under the yoke of the current diabetes epidemic, it is expected that globally 51 million people are suffering from sight-threatening complications by 2040. In order to sustain accessible and affordable diabetic eye care, more efficient allocation of available resources is needed. Due to the multifactorial origin of the disease, patients do not share the same risk profile, for example: not everyone with diabetes will develop diabetic retinopathy, and not all patients with macular edema respond equally well to the same treatment. Stratification of patients into subgroups based on a specific risk profile and translating this into a personalized management strategy will ultimately make medical care for patients with diabetic eye disease more efficient. In this thesis, we aim to contribute to this process by investigating the risk stratification for disease progression and treatment response, as well as by exploring the biomarkers that associate with these risks.

Chapter 1 serves as a general introduction to diabetic retinopathy. It provides an overview of the history, pathophysiology, epidemiology and risk factors for the disease, and outlines the available diagnostic and therapeutic options.

Chapter 2 is focused on the risk stratification for development and progression of diabetic retinopathy. In *Chapter 2.1* we studied the risk factors that associate with the hazard of development and progression of diabetic retinopathy in patients with type 1 diabetes. Besides the previously reported risk factors mean HbA1c, cholesterol and age of diabetes onset, we identified glucose (defined as HbA1c) variability as an independent risk factor for development of diabetic retinopathy. We therefore concluded that optimization of glycemic control should not only include lowering glucose levels, but also minimizing glucose variability on the long term. For progression of diabetic retinopathy to a sight-threatening stage, we found mean HbA1c and albuminuria to be associated with an increased hazard. This knowledge can be used to optimize prediction models that stratify patients according to their risk of developing diabetic retinopathy and progression of the disease.

In *Chapter 2.2* we validated a model developed by Aspelund and colleagues to calculate personalized eye screening intervals using clinical variables for persons with diabetes. The model was originally built and validated in populations with a vast majority of persons with type 2 diabetes, and our current study validated the model in persons with type 1 diabetes. W**e** showed that Aspelund's model is also safe and effective in a population with persons with type 1 diabetes. Using the model, the eye screening frequency can be reduced to 61% compared to annual screening, and by 21% compared to biennial screening.

Phenotypic characteristics also play an important role in meaningful risk assessment of diabetic eye disease. In **Chapter 3** we focus on various imaging modalities and characteristics that can be used in the ophthalmic examination of persons with diabetes. In *Chapter 3.1* we studied the distribution of hyperreflective foci on OCT in a population of patients with type 1 diabetes mellitus. Higher numbers of hyperreflective foci were significantly associated with the presence and severity of diabetic retinal disease. We observed that there is a strong link between the presence of hyperreflective foci and exudative maculopathy. This knowledge forms the foundation for further research, testing hyperreflective foci as a biomarker for disease progression and treatment response. The labor-intensive process of manual hyperreflective foci grading currently limits the evaluation of large datasets. In *Chapter 3.2* we therefore described the development of a computer-assisted approach for the automated detection of hyperreflective foci, reaching a similar performance as human graders. This enables fast and replicable assessment of whole OCT-volumes, instead of single scans.

In *Chapter 3.3* we used a relatively new imaging technique, OCTA, to study microvascular abnormalities in patients with diabetes. We were able to distinguish six different types of microaneurysms according to their morphological appearance: focal bulging, fusiform, saccular, mixed fusiform/saccular, irregular and pedunculated microaneurysms. We also reported that OCTA does not detect all microaneurysms that were depicted on fluorescein angiography, which is the current golden standard.

Chapter 4 contains two studies focusing on the outcomes of treatment for diabetic retinal disease. In *Chapter 4.1* we described the long term outcomes of a vitrectomy for the complications of proliferative diabetic retinopathy, such as vitreous hemorrhage and tractional retinal detachment. We reported a 5-year cumulative incidence of low vision (visual acuity <0.3) of 31% in the operated eye, and of 14% in both eyes. Additionally, after 5 years, 27% of the patients required a re-vitrectomy, while 40% of the patients also underwent a vitrectomy of the contralateral eye.

From this study we can conclude that with sufficient medical care, useful bilateral visual function can be maintained or restored in the vast majority of the patients undergoing a vitrectomy for diabetic retinopathy.

In *Chapter 4.2* we investigated the predictive value of hyperreflective foci on OCT for the treatment response to anti-VEGF in patients with diabetic macular edema. Patients with higher numbers of hyperreflective foci responded better to treatment with anti-VEGF than patients with low numbers of hyperreflective foci. Although hyperreflective foci are a promising imaging biomarker for the prediction of treatment response to anti-VEGF in DME, it is altogether unlikely that a single biomarker yields enough predictive ability for reliable risk stratification. Further research should therefore be aimed at developing prediction models that include multiple variables associating with treatment response. This should ultimately result in a model that determines the most effective treatment option for a selected individual.

In **Chapter 5** we discussed the most important findings of this thesis and place them in a broader perspective. In this thesis we provided building bricks for the development of personalized management strategies for diabetic retinal disease. These strategies are based on risk stratification and are essential to maintain our high standards of medical care, while distributing it more efficiently among the growing number of patients. In addition to developing and optimizing personalized health care, our focus should be on prevention. Visual disability as a consequence of diabetes is often avoidable by tight regulation of the known risk factors, such as blood glucose levels. By investing in prevention now, diabetes-related blindness will reduce, as well as the associated costs.

SAMENVATTING

Netvliesafwijkingen ten gevolge van diabetes mellitus vormen een bedreiging voor het gezichtsvermogen. De meest voorkomende oorzaak van slechtziendheid ten gevolge van diabetes is diabetisch macula oedeem: een vochtophoping in het centraal in het netvlies. Daarnaast kunnen vaatnieuwvormingen problemen geven, bijvoorbeeld wanneer ze scheuren en een bloeding veroorzaken, of wanneer ze aan het netvlies trekken en een netvliesloslating ontstaat. Onder het juk van de huidige diabetesepidemie is de verwachting dat het aantal mensen met een zicht-bedreigende netvliesafwijking stijgt tot 51 miljoen wereldwijd. Om de medische zorg voor deze mensen toegankelijk en betaalbaar te houden, zullen we efficiënter met de beschikbare middelen om moeten gaan. Dit kunnen we bereiken door per persoon te inventariseren welke zorg er op dat moment nodig is. Zo zal bijvoorbeeld niet iedereen met diabetes ook netvliesafwijkingen ontwikkelen en niet alle patiënten met diabetisch macula oedeem reageren even goed op dezelfde behandeling. Op basis van een persoonlijk risicoprofiel kan de behandelstrategie met de grootste succeskans voor een individu worden bepaald. Het doel van dit proefschrift is om deze kansen en de daarmee samenhangende factoren te onderzoeken. Daarmee dragen wij bij aan het vergroten van de efficiëntie van de diabetische oogzorg en het leveren van de juiste zorg op de juiste plek.

Hoofdstuk 1 is een algemene introductie op diabetische retinopathie. Er wordt een overzicht gegeven van de historie, ontstaanswijze, epidemiologie en risicofactoren voor deze ziekte en verschillende beschikbare diagnostische en therapeutische opties worden besproken.

Hoofdstuk 2 richt zich op de risico inschatting voor de ontwikkeling en progressie van diabetische retinopathie. In *Hoofdstuk 2.1* onderzochten we de risicofactoren die samenhangen met de snelheid van ontwikkeling en progressie van diabetische retinopathie in patiënten met type 1 diabetes. Naast de risicofactoren die al in eerdere literatuur zijn beschreven (HbA1c, cholesterol en de leeftijd waarop diabetes werd gediagnosticeerd), identificeerden wij de varabiliteit van het suikergehalte in het bloed (uitgedrukt als HbA1c) als een onafhankelijke risicofactor voor het ontwikkelen van diabetische retinopathie. We concludeerden daarom dat er niet alleen gestreefd moet worden naar een zo laag mogelijk suikergehalte, maar ook naar zo min mogelijk fluctuatie in het suikergehalte. Voor de progressie van diabetische retinopathie naar een zicht-bedreigende vorm, vonden wij dat het gemiddelde HbA1c gehalte en albuminurie geassocieerd waren met een verhoogd risico. Deze kennis kan gebruikt worden om huidige predictiemodellen voor de ontwikkeling en progressie van diabetische retinopathie te optimaliseren.

In *Hoofdstuk 2.2* valideerden we het model van Aspelund en collega's, waarmee gepersonaliseerde screeningsintervallen kunnen worden bepaald op basis van de klinische gegevens van patiënten met diabetes. Dit model was oorspronkelijk ontwikkeld bij patiënten met type 2 diabetes en in onze studie werd het model gevalideerd in groep patiënten met type 1 diabetes. We toonden aan dat het model van Aspelund ook veilig en effectief is bij patiënten met type 1 diabetes. Met het model kan de screeningsfrequentie worden teruggebracht tot 61% vergeleken met jaarlijkse screening en tot 21% vergeleken met tweejaarlijkse screening.

In **Hoofdstuk 3** focussen we op verschillende beeldvormende technieken en karakteristieken die gebruikt kunnen worden bij het oogheelkundig onderzoek van patiënten met diabetes. In *Hoofdstuk 3.1* bestudeerden we hyperreflectieve foci op de OCT in een populatie van patiënten met type 1 diabetes. We vonden dat grotere aantallen hyperreflectieve foci op de scan geassocieerd waren met zowel de aanwezigheid als de ernst van diabetische retinopathie. Daarnaast toonden we aan dat er een sterk verband is tussen hyperreflectieve foci en vocht in het netvlies. Deze kennis vormt de fundering voor verder onderzoek naar de waarde van hyperreflectieve foci bij de voorspelling van ziekteprogressie en behandelrespons. Het graden van hyperreflectieve foci is echter een arbeidsintensief proces. Daarom beschrijven we in *Hoofdstuk 3.2* hoe wij een systeem voor de automatische detectie van hyperreflectieve foci hebben ontwikkeld. Hierdoor kunnen nu grote aantallen scans met een simpele muisklik repliceerbaar worden gegraded.

In *Hoofdstuk 3.3* gebruiken we een relatief nieuwe beeldvormende techniek, de OCTA, om microvasculaire afwijkingen te bestuderen bij patiënten met diabetes. We konden zes verschillende typen microaneurysmata onderscheiden op basis van hun morfologische verschijning, namelijk: focale uitstulping, fusiform, sacculair, gemixt fusiform/sacculair, irregulair en gesteeld. Daarnaast vonden wij dat OCTA niet alle microaneurysmata kon detecteren die gezien waren bij fluorescentie angiografie wat nu de gouden standaard is.

Hoofdstuk 4 bevat twee studies die zich richten op de uitkomsten van behandeling bij diabetische netvliesziekten. In *Hoofdstuk 4.1* beschrijven we de lange-termijnuitkomsten van een operatie voor complicaties van proliferatieve diabetische retinopathie, zoals een glasvochtbloeding en een netvliesloslating. We rapporteren een 5-jaars cumulatieve incidentie voor slechtziendheid (visus <0.3) van 31% in het geopereerde oog en 14% in het beste oog. Daarnaast moest 27% van de patiënten nog een vitrectomie ondergaan in hun geopereerde oog en 40% moest een vitrectomie ondergaan van hun andere oog. Op basis hiervan kunnen we concluderen dat met de juiste medische zorg, bij een groot deel van de patiënten een bruikbare gezichtsscherpte kan worden behouden.

In *Hoofdstuk 4.2* onderzochten we de voorspellende waarde van hyperreflectieve foci op OCT voor de behandelrespons op injecties in het oog met het anti-VEGF bij patiënten met diabetisch macula oedeem. Patiënten met grote aantallen hyperreflectieve foci reageren beter op behandeling met anti-VEGF dan patiënten met een klein aantal hyperreflectieve foci. Het is echter onwaarschijnlijk dat één enkele biomarker genoeg voorspellende waarde heeft om de kans op behandelsucces te voorspellen. Verder onderzoek moet zich daarom richten op het ontwikkelen van een model waarbij meerdere variabelen gebruikt worden die een mogelijke relatie met de behandelrespons hebben. Uiteindelijk moet dit resulteren in een model dat de meest geschikte behandeloptie kan bepalen voor een individuele patiënt.

In **Hoofdstuk 5** bediscussiëren we de belangrijkste bevindingen van dit proefschrift en plaatsen we deze in een breder perspectief. In dit proefschrift worden de bouwstenen aangereikt waarmee persoonlijke managementstrategieën voor diabetische netvliesziekten verder kunnen worden ontwikkeld. Deze strategieën zijn essentieel om medische zorg in goede kwaliteit te kunnen blijven aanbieden aan het groeiende aantal diabetes patiënten. Naast het ontwikkelen en optimaliseren van gepersonaliseerde zorg, moeten we onze pijlen richten op preventie. Slechtziendheid ten gevolge van diabetes is te voorkomen door goed risicomanagement, zoals een strakke glucoseregulatie. Door nu te investeren in preventie kunnen we de diabetesgerelateerde slechtziendheid en daarmee samenhangende kosten sterk verminderen.

6.2

Data management page

DATA MANAGEMENT PAGE

Type of data	Subject to privacy (yes/no)	Way of anonymization	Storage
Informed consents of patients included in studies in this thesis	Yes	All patients have been assigned a study-ID number and the key is stored in password protected SPSS files	Written informed consents of patients included at the Ophthalmology department in Nijmegen are stored in a locked archive. The key file can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\6 Key Files
Clinical data of patients included in type 1 DM database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Data can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\5 Databases\DRP Database
Images of patients included in type 1 DM database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Color fundus photographs are stored on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\HF in T1DM\Fundusfotos OCT-scans are stored on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\HF in T1DM\OCT-scans
Clinical data of patients included in bevacizumab database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Data can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\Hyperreflective foci
Images of patients included in bevacizumab database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Images are stored on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\Hyperreflective foci\OCT-scans for grading
Clinical data of patients included in vitrectomy database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Data can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\CVH
Clinical data of patients included in OCTA database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Data can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\Angio-OCT\OCT-A data\Data analyse
Images of patients included in OCTA database	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Images are stored on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur\Angio-OCT\OCT-A data\Data
Files for publications presented in this thesis	No	NA	All files can be found on the Ophthalmology H-drive: \\UMCFS030\ohkdata$\Onderzoek\1 Personal folders\Vivian Schreur

6.3

List of publications

LIST OF PUBLICATIONS

Automatically detected hyperreflective foci in optical coherence tomography as a prognostic biomarker.
Venhuizen FG, Schreur V, Schaffhauser S, Altay L, van Ginneken B, Liefers B, Fauser S, Klevering BJ, Hoyng CB, Theelen T, de Jong EK, Sánchez CI.
Manuscript in preparation

Long-term outcomes of vitrectomy for proliferative diabetic retinopathy.
Schreur V, Brouwers J, Smeets S, Phan ML, Hoyng CB, de Jong EK, Klevering BJ
Manuscript submitted

Ophthalmological findings in facioscapulohumeral dystrophy.
Goselink RJM, Schreur V, van Kernebeek CR, Padberg GW, van der Maarel SM, van Engelen BGM, Erasmus CE, Theelen T.
Brain Communications. 2019 Oct 11. Doi: 10.1093/braincomms.fcz023

Retinal hyperreflective foci in type 1 diabetes mellitus.
Schreur V, de Breuk A, Venhuizen FG, Sánchez CI, Tack CJ, Klevering BJ, de Jong EK, Hoyng CB.
RETINA. 2019 Jul 25. Doi: 10.1097/IAE.0000000000002626

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Hyperreflective foci on optical coherence tomography associate with treatment outcome for anti-VEGF in patients with diabetic macular edema.
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Wesdorp M, Schreur V, Beynon AJ, Oostrik J, van de Kamp JM, Elting MW, van den Boogaard MH, Feenstra I, Admiraal RJC, Kunst HPM, Hoyng CB, Kremer H, Yntema HG, Pennings RJE, Schraders M. Clinical Genetics, 2018 Aug;94(2):221-231, *doi: 10.1111/cge.13368*

Morphological and topographical appearance of microaneurysms on optical coherence tomography angiography.
Schreur V, Domanian A, Liefers B, Venhuizen FG, Klevering BJ, Hoyng CB, de Jong EK, Theelen T. *British Journal of Ophthalmology, 2018, Jun 20, doi: 10.1136/bjophthalmol-2018-312258.[Epub ahead of print]*

Deep learning approach for the detection and quantification of intraretinal cystoid fluid in multivendor optical coherence tomography.
Venhuizen FG, van Ginneken B, Liefers B, van Asten F, Schreur V, Fauser S, Hoyng CB, Theelen T, Sánchez CI.
Biomedical Optics Express, 2018 Mar 7;9(4):1545-1569, doi: 10.1364/BOE.9.001545

Automatic detection of the foveal center in optical coherencetomography.
Liefers B, Venhuizen FG, Schreur V, van Ginneken B, Hoyng CB, Fauser S, Theelen T, Sánchez CI.
Biomedical Optics Express. 2017 Oct 23;8(11):5160-5178, doi: 10.1364/BOE.8.005160

6.4

Curriculum vitae

CURRICULUM VITAE

Vivian Schreur was born on the 15th of May in Rheden, The Netherlands. In 2007 she graduated from the Olympus College in Arnhem (gymnasium-certificate, Natuur & Gezondheid). In 2007-2008 she attended Natoma High School in Natoma, Kansas, United States of America. She started her medical study in 2008 at the Radboud University in Nijmegen. During her studies, she worked as a fulltime board member of the Medical Faculty Association Nijmegen and served as chairman for the Medical Faculty Student Council. Her interest in ophthalmology developed in her final year, in which she took part in clinical ophthalmological research on diabetic retinopathy at the Radboud university medical center. In December 2015, she started as a PhD candidate at the Radboud university medical center under the supervision of prof. dr. Carel Hoyng, prof. dr. Jeroen Klevering and dr. Eiko de Jong. She was awarded the Anne Katrin Sjølie Award for the Best Abstract by the Eye Complications Study Group of the European Association for the Study of Diabetes in 2017. During her PhD, she was co-organizer of the Dutch Ophthalmology PhD Students (DOPS) conference in 2017. In February 2019, she started a residency in Ophthalmology at the Radboud university medical center.

6.5

Dankwoord

DANKWOORD

Hoewel mijn naam in alle eenzaamheid op de omslag van dit boekwerk lijkt te prijken, is promoveren een ontegenzeggelijke teamprestatie. Zonder de kennis en kunde van mijn begeleiders, de fijne samenwerking met collega's en de bereidheid van studiedeelnemers, had mijn proefschrift nu niet voor uw neus gelegen. Op deze plaats past dan ook een woord van dank.

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Ook andere co-auteurs Clarisa Sánchez Guitterez, Lebriz Altay, Sascha Fauser, Cees Tack, Amber van der Heijden, Heijan Ng, Einar Stefánsson, Sandra Smeets, Milan Phan, bedankt voor jullie bijdrage aan de artikelen die we samen hebben geschreven.

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