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DIABETIC RETINOPATHY SCREENING AND TREATMENT

BY
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Diabetic retinopathy screening and treatment

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The printed rendition of this thesis is dedicated to my cherished friends, beloved family, and esteemed colleagues who have attentively hearkened to my multitude of musings.

"In the midst of chaos, there is also opportunity." – Sun Tzu

1 Contents

2 Acknowledgements

This PhD-thesis is based on work done at the Department of Ophthalmology, Aalborg University Hospital, in collaboration with the Steno Diabetes Center North Jutland between May 2019 and December 2022. It has been a journey that has involved the disciplines of epidemiology, statistics and clinical medicine. I have received great support from my colleagues, friends and family.

Like any other battle, the real struggles are mostly forgotten when victory is near. The future looks brighter and translucent now. This thesis has come into being through the COVID-19 pandemic, battles with GPDR and the growth of concepts. It is the result of three and a half years of work. Obtaining legal approvals for my work caused delays, which resulted in a challenging period and a sense of being enveloped by an aura reminiscent of the Dementors from Harry Potter. These entities revel in decay and despair, sapping the surrounding air of its tranquillity, hope, and joy through their mere presence and exhibit no genuine allegiance. Approaching a Dementor entails the depletion of every positive emotion and joyful recollection. [1].

Luckily, I was not alone in this fight, and my supervisors have been my Patronus Charm. There is no doubt that the process of writing this PhD thesis has pushed me out of my comfort zone several times. The process is definitely challenging, and I am grateful to have people to share and discuss this experience with.

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3 List of papers

Paper I:
Nissen TPH, Vestergaard P, Vorum H, Torp-Pedersen C, Aasbjerg K. **A cohort follow-up study for diabetic retinopathy screening incidence in the North Denmark Region.** Acta Diabetologica.; accepted 19/6-23.

Paper II:
Nissen TPH, Nørgaard TL, Schielke KC, Vestergaard P, Nikontovic A, Dawidowicz M, Grauslund J, Vorum H, Aasbjerg K. **Performance of a vector machine learning tool for diagnosing diabetic retinopathy in clinical practice.** .(Journal of Personalized Medicine, 2023, in review.

Paper III:
Nissen TPH, Vorum H, Aasbjerg K. **Biologic Therapy and Treatment Options in Diabetic Retinopathy with Diabetic Macular Edema.** *Current Drug Safety* 2020, 15, 1-06. [2] .

4 Abbreviations

AI – Artificial Intelligence
AMD – Age-related Macular Degeneration
AUC – Area Under the Curve
BCVA – Best Corrected Visual Acuity
CI – Confidence Interval (95% CI)
CSMO/(E) – Clinical Significant Macular Oedema
DKK – Danish Kroners
DM – Diabetes Mellitus
DME – Diabetic Macular Edema/Oedema
DOS – Danish Ophthalmological Society
DR – Diabetic Retinopathy
EMA – European Medicines Agency
ETDRS – Early Treatment Diabetic Retinopathy Study
FDA – United States Food and Drug Administration
HbA1c – Haemoglobin A1c
HR – Hazard Ratio
ICO – International Council of Ophthalmology
ICDR – International Classification of Diabetic Retinopathy Severity Scale
ICD-10 – International Classification of Diseases version 10
mmol/L – millimole per Litre
NPV – Negative Predictive Value
NPDR – Non-Proliferative Diabetic Retinopathy
OCT – Optical Coherence Tomography
PDR –Proliferative Diabetic Retinopathy
P.O. – Private Ophthalmologist
PPV – Positive Predictive Value
QOL – Quality of life
R – software for statistical analysis
ROC – Receiver Operating Characteristics
SAS – software for statistical analysis
T1D – Type 1 Diabetes
T2D – Type 2 Diabetes
USD – United States of America Dollars
VTDR – vision threatening diabetic retinopathy
WHO – World Health Organization

5 Introduction

The world is recovering from the first airborne viral pandemic the likes of which have not been seen in magnitude and deaths since the Spanish Influenza (1918-1920) [3]. Nonetheless, several other diseases are currently present in societies worldwide which are on the scale of a pandemic. Diabetes is one of these diseases[4]. According to recent data, this condition affects 537 million people [5] and incidence is rising in cultures that adapt a westernised lifestyle.

Most people in the western world have a degree of free access to health care and can be properly treated for diabetes with medicine. Even though there are great treatment options for diabetes, compliance is either not always optimal or the prescribed medicine is expensive. In the developing world, lack of health care options and pricing deeply affects adherence to treatment of diabetes, and the perfect treatment option has still not been established. [6–11]. Whether or not a patient is treated appropriately, complications of diabetes will eventually effect the patient and each complication will need to be treated.

Medication compliance and the optimal achievement of HbA1c blood levels in diabetes management are highly correlated. The accumulated time with diabetes and the level of HbA1c can to some extent predict the risk of complications which can be highly costly for the patient or society to treat; moreover, these complications can disable patients or even shorten their life [12–14]. Ongoing research and development aimed at improving medication formulations to enhance ease of administration and achieve better glycaemic control are tools being used to address these challenges [15,16].

Despite the general availability of effective and affordable medications, screening for complications remains crucial for the detection and management of diabetes, as it is vital that the condition is promptly identified in order to prevent progression to disabling or life-threatening stages. Among the complications associated with diabetes, diabetic retinopathy (DR) screening programmes have demonstrated cost-effectiveness in identifying and treating retinal pathology in a timely manner [17,18].

When considering the progression of diabetic retinopathy, factors beyond HbA1c, e.g., good blood pressure control, play a significant role. Studies focusing on eyes treated with panretinal photocoagulation (PRP) have shown that effective blood pressure management is associated with a reduced risk of diabetic retinopathy progression [19]. Hypertension, a well-recognised comorbidity of diabetes,

contributes to the development and exacerbation of DR [20–22]. By effectively managing blood pressure, the underlying pathogenic mechanisms driving retinal microvascular damage can be attenuated.

Furthermore, a clinical correlation exists between DR and other diabetic complications such as nephropathy and neuropathy [23]. The pathogenesis of these complications share common metabolic and vascular factors, including insulin resistance, chronic inflammation and endothelial dysfunction [24]. Addressing these factors through comprehensive management strategies, including optimal blood pressure control, can contribute to the prevention or deceleration of DR progression.

In summary, while medication compliance and optimal HbA1c levels are crucial in diabetes management, other risk factors, e.g., good blood pressure control, significantly influence the progression of DR. Efforts to effectively manage blood pressure and address the multifactorial nature of the metabolic syndrome are essential in preventing or attenuating retinal complications. Regular screening programmes for DR play a pivotal role in early detection and intervention, thereby reducing the risk of visual impairment and disability.

5.1 Summary of aims

The aims of this thesis were to investigate the DR screening attendance in the national Danish screening programme for DR in a regional cohort (Paper I), to evaluate a current used software for automatic detection of diabetic retinopathy in a clinical screening setting (Paper II) and to give an overview of the ophthalmological biological treatment options for diabetic retinopathy and macular oedema with focus on drug safety and adverse effects (Paper III).

6 Background

6.1 Diabetes

Diabetes is a chronic, lifelong disease caused by a dysregulation in the blood glucose level [25]. The regulation of the anabolic hormone insulin [26] is the most important hormone for keeping the blood-level glucose on a scale between 4-7 millimole per Litre (mmol/L) [27,28]. Insulin is produced in the 1 million Langerhans islets in the pancreas – specifically by the beta-cells – which converts pre-pro-insulin to proinsulin which then becomes insulin and C-peptide [29]. This mechanism is controlled by a feedback mechanism where rise in blood glucose increases the intake in the beta-cell and by an intracellular pathway which releases insulin into the bloodstream.

Insulin serves as a regulator for cell-growth, cellular membrane-transport of electrolytes and nutrients. A decrease in P-insulin or decrease in functional activity through insulin resistance will induce a rise in the blood-glucose due to a continued production of glucose in the liver and reduced turnover in the muscle tissue. The two major groups of people with diabetes are those with type 1 diabetes (T1D) and type 2 diabetes (T2D) [29,30]. T1D is, simply put, characterised by absence or near absence of secretion of insulin from the pancreas. This is usually caused by autoimmune destruction of insulin producing beta-cells[31,32] and typically appears in younger individuals. T2D is characterised by the development of insulin resistance in the liver and peripheral tissue and beta-cell dysfunction in the pancreas which comes by multifactorial input. Age and lifestyle are important factors.

Insulin resistance and beta-cell dysfunction are interrelated and can contribute to the development of T2D. Insulin resistance can lead to an increase in insulin secretion by the beta-cells as it attempts to maintain glucose homeostasis. Over time, this increased demand on the beta-cells can lead to beta-cell dysfunction and a decrease in insulin production and secretion [33–36]. Several phenotypes exist [37] based on age, obesity and insulin resistance. T2D is the most prevalent form of diabetes with T1D being the next [38,39]. Rare forms of diabetes exist but will not be discussed further in this thesis.

6.1.1.1 Diagnosis

Diagnosis for diabetes in Denmark is defined as Hba1c > 48 mmol/L for T1D and T2D; or a random blood sample with blood sugar > 11.1 mmol/L for T2D; fasting blood

glucose > 7 mmol/L on two separate blood samples or an oral blood glucose tolerance test with a blood sugar > 11.1 mmol/L for T2D [40,41].

6.1.2 Epidemiology

Data from the International Diabetes Federation [42] shows a progressive increase of both prevalence and incidence in each new report on the development of diabetes [43]. The 2021 report estimated prevalence of diabetes to be 537 million worldwide with the possibility of this number rising to 643 million in 2030 [5,38]. Diabetes prevalence varies around the globe with the highest prevalence being in western high-income countries; nonetheless, this prevalence is rising the most in low and middle-income countries in Africa, the Middle East and Southeast Asia. This increase seems to be connected to obesity [44–46]. Other factors such as ethnicity, increasing urbanisation, lifestyle in general and a more westernised lifestyle in particular also increase diabetes risk [47–49]. Diabetes has reached epidemic proportions and is characterised as such by the World Health Organization (WHO) [4]. T1D accounts for approximately 10% and T2D roughly accounts for the remaining prevalence of 90% [50]. The Diabetes Atlas reports up till 45% of people with diabetes are undiagnosed [38], but variations of this number can be seen in different nations. A 2020 Danish study estimates that 24% of patients with T2D were undiagnosed in 2011 [51], and nearly 300,000 were estimated to have prediabetes.

6.1.3 Symptoms

The age of diabetes onset differs between T1D to T2D. The incidences rates for patients with T1D, which is the most prevalent type of diabetes in children and young adults, peaks around the ages of 10-14 and declines to a lower level around the age of 34 [52,53]. This type of diabetes is most prevalent in children and young adults. It is important to note that this type of diabetes can develop throughout a person's life [39,54–57]. Patients with T1D typically have very manifest onset symptoms as the needed production of insulin in the beta cells of the Langerhans islets decreases to around 50% [29,58–60]. The symptoms of T1D can occur for days to months and include excessive water intake and polyuria, weight loss, hunger and dermatological symptoms such as pyoderma, furuncles and carbuncles as well as transitory blurred vision [61,62]. Clinical signs can be acetone smell ex ore, Kussmaul breathing and affected sensorium [28,61,63]. Some patients, usually children and elderly people (12-80% depending on country), come to the emergency department with diabetic ketoacidosis at the debut [64]. Paraclinical signs are glucosuria with ketonuria,

elevated blood glucose, triglycerides, FFA and glycerol. T1D patients are usually diagnosed in the early stages of the disease [61].

Patients with T2D are in many cases asymptomatic and may be diagnosed late in their disease at routine screenings at a family doctor, eye doctor or optometrist [65–68]. Usually these patients are fatigued, have neuropathy or can experience intermittent blurred vision due to fluctuations in the blood sugar and corneal and lens swelling which alters the refraction [69–71]. The more the disease has progressed without being treated, the more the symptoms look like hyperglycaemia T1D symptoms with excess thirst and diuresis.

6.1.4 Complications

The long-term detrimental effects of having diabetes are well described in the literature. These effects correspond to treatment compliance, levels of HbA1c and total time a patient has had diabetes. [72,73]. Comorbidities typically appear after years or even decades in this chronic patient group. The most typical comorbidities are nephropathy, neuropathy, diabetic retinopathy, microangiopathy, soft tissue and bone changes, skin changes, diabetic foot wounds, hyperosmolar hypoglycaemia and hypoglycaemia. This patient group may also have an increased risk of acute corona syndrome [74–77]. New emerging complications, as reported in a recent review by Tomic et al. [74] also include cancer, infectious diseases, affective disorders, liver disease, functional and cognitive disabilities, and bone fragility [78]. Socioeconomic costs are an issue especially in low-income countries [79,80]. As there are a plethora of complications that come with diabetes, looking at the diabetes population size is appropriate.

6.2 Diabetic retinopathy

6.2.1 Epidemiology

Diabetic retinopathy is a condition that arises as a complication of diabetes. The condition impacts the blood vessels within the retina and is one of the primary causes of vision loss. This condition develops gradually over time and often without symptoms being evident to the patient during its initial stages. The condition results from the damaging effects of high blood sugar levels on the delicate blood vessels in the retina. Timely detection and management are crucial in preserving vision for individuals with diabetes. The grading of DR can be divided into five categories

according to the International Classification of Diabetic Retinopathy Severity Scale (Table 1).

Incidence and prevalence of DR have been on the rise over the last decades. A large systematic review and meta-analysis [81] found that 22% of people with diabetes have DR. Of those who were older than 50 years in 2020, DR was the fifth most common disease for moderate to severe vision impairment (2.9 million worldwide in 2020). From 1990 to 2020, there was an 129% increase in cases and a 3.3% increase in age standardised prevalence. Blindness in particular affected 0.86 million people in 2020 [82]. DR saw a global increase of 150% and an age-standardised prevalence increase of 14.9% from 1990 to 2020 [82]. The highest increases in vision impairment and blindness due to DR were in Asia, Oceania, Southern sub-Sahara Africa and North America. A meta-analysis comparing thirty-five papers found the overall prevalence of any DR to be 34.6% with 95% confidence intervals (CI) of (34.5–34.8), for proliferative DR 6.81% (95% CI 6.74–6.89), for diabetic macular oedema (DME) 6.96% (95% CI 6.87–7.04) and for VTDR 10.2% (95% CI 10.1–10.3) [83].

6.2.2 Symptoms of the diabetic retinopathy

Diabetic retinopathy is the most severe diabetes eye complication, as DR is unnoticed and asymptomatic in the initial stages. This is because patients cannot initially observe or register any sign of the DR eye-disease. This is also sometimes the case in more severe stages [84–86]. This is exemplified in the image below of a patient who has no subjective visual complaints but has severe DR (Fig. 1). The recognition and pursuit of healthcare services by patients with DR only occurs when the condition reaches an advanced stage which is characterised by the occurrence of intraocular bleeding within the vitreous humor, extensive intra-retinal haemorrhages involving the macula or the development of macular oedema.

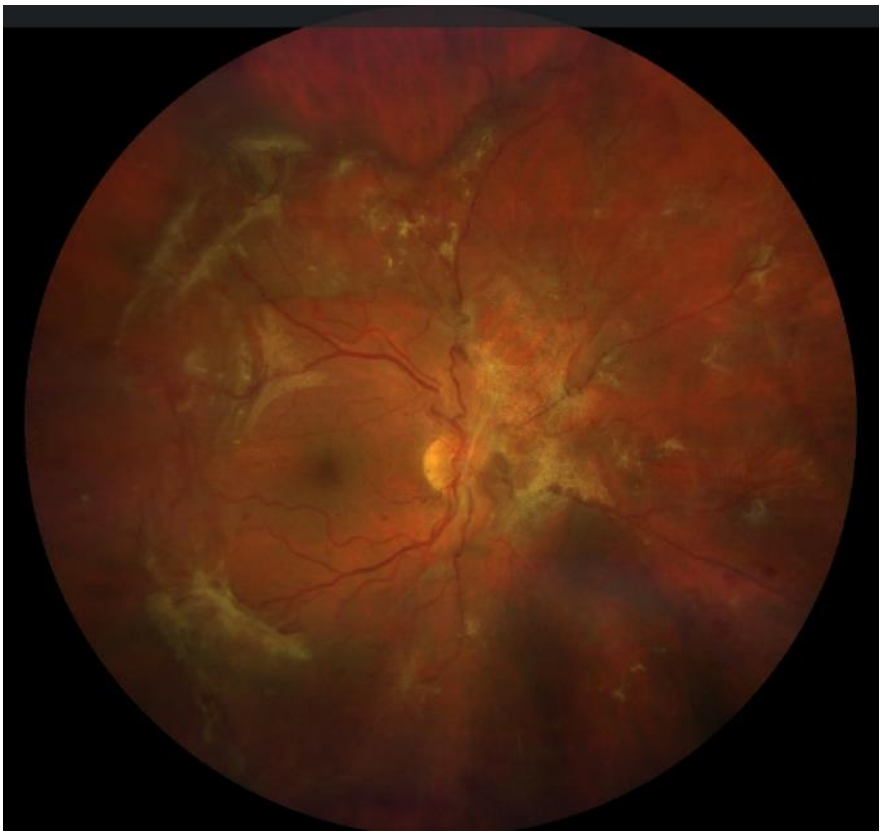

Figure 1 *Right eye of a patient with severe DR. Patient did not have any subjective visual complaints. Best Corrected Visual Acuity (BCVA) 1.0. Clinical photo from Department of Ophthalmology, Aalborg.*

6.2.3 Diagnostic approach

The diagnosis of DR is made by either a trained health professional or an ophthalmologist. It can be made by an ophthalmologist performing an ophthalmoscopy, a trained technician or a doctor assessing fundus images from an eye or by automated software analyses [87]. The specific criteria are described further below (Table 1).

6.2.4 Treatment of diabetic retinopathy

The treatment of DR can be divided into two major targets: Treatment of the underlying disease (diabetes) and ophthalmological treatment of the DR itself (Paper III). The treatment of diabetes relies upon patient compliance and optimisation of medicine. Strong glycaemic control is important for reducing the risk of progression in DR and the need for ophthalmological interventions [88]. The ophthalmological medical treatment can be divided into three groups: Laser therapy, anti-vascular endothelial growth factor and corticosteroids.

Different laser options are available, depending on technology and location of the treated area, such as panretinal photocoagulation (PRP) for proliferative DR, macular focal/grid laser or subthreshold micropulse diode laser therapy for DME. The macular oedema can either be focal with distinct leakage points or diffuse with a diffuse leakage of fluids which reflects a more extensive breakdown of the blood-retina barrier. The principle is the absorption of light in different areas of the retina being retinal pigment epithelium, choroid melanin and/or haemoglobin [89,90] . This absorption of light destroys layers in the retina and especially affect the photoreceptors which are highly metabolic and require vast quantities of oxygen. Thus, oxidative stress of the retina is reduced [91,92] and the derived effect is downregulation of vascular endothelial growth factor which results in reduced vascular permeability, leading to decreased retinal oedema and the potential preservation of vision. [93,94]. Panretinal photocoagulation can be applied to non-proliferative DR and proliferative DR [95].

Several different laser modalities exist, using different gases like Xenon and Argon to produce light of a specific nm-wavelength and varying pulse duration, spot size and power output, as well as computer assisted pattern scanning, navigated laser or operator controlled [94,96–98]. The subthreshold diode micropulse laser is a novel laser therapy where a subthreshold laser is applied in micro pulses. This type of laser is in theory only applied to the retinal pigment epithelium and thus the destruction of the neurosensory retina is avoided. The laser is set at a low energy level and have

scheduled breaks in the treatment so the tissue can return to base temperature. Subthreshold diode micropulse laser does not leave any visible burns on the retina. [99–103]. DME is the most extensively employed application of subthreshold diode micropulse laser [104]. Furthermore, a study has demonstrated its positive impact on best corrected visual acuity (BCVA) during a 3-year follow-up [105]. When compared to ETDRS focal laser protocols DME subthreshold diode micropulse laser has exhibited comparable or superior efficacy while minimizing retinal pigment epithelium damage [106,107].

In summary, the different laser modalities used to treat diabetic macular oedema or DR work by either sealing leaking blood vessels or decreasing oxidative stress by tissue destruction and thus reduces the amount of VEGF expressed in the retina. The specific laser modality used will depend on the severity and location of the DME or DR and the patient's individual needs. (Referred combined from now on as 'laser' when nothing else is specified).

Anti-vascular endothelial growth factor (anti-VEGF) therapy functions by specifically targeting VEGF and impeding its binding to its respective receptors. VEGF stimulates the proliferation of endothelial cells and promotes angiogenesis (a process in which new blood vessels are formed). Anti-VEGF in turn inhibits the growth of new blood vessels and reduces the permeability of existing vessels [108,109]. In DR, VEGF is upregulated in response to ischemia, hypoxia and inflammation, leading to neovascularization [110], capillary non-perfusion, and vascular leakage. In DME, VEGF causes the breakdown of the blood-retinal barrier and the accumulation of fluid in the macula, resulting in visual impairment. The reduced permeability leads to decreased fluid leakage into the retina which may improve vision and prevent further damage to the retina and macula oedema [108,109].

Intraocular corticosteroid treatment is employed to alleviate macular oedema through the modulation of intricate cellular pathways, thereby reducing inflammation [111,112].

Operative treatment of vision threatening diabetic retinopathy (VTDR) can be done by a vitrectomy where a blood-filled vitreous is surgically removed. Patients can also experience diabetic tractional retinal detachments which left untreated will progress to complete loss of vision in the effected eye, resulting in the need for vitreoretinal surgery to save the vision of the affected eye which can result in a high chance of severe impacted BCVA [88,113,114].

6.2.5 Quality of life

If BCVA is effected in persons with DR, quality of life (QOL) as described by several studies is affected [115–124]. Not only are daily tasks affected by the loss of vision, patients with vision loss also become socially and emotionally affected in their daily living. For example, the BCVA may be close to or below the requirements for a driving license, and patients can worry about a further loss of vision. Social life activities such as visiting friends and family may be also affected, and thereby indirectly lead to depression. Commuting to work and maintaining a job can be difficult with decreasing visual acuity [119]. Some evidence points to people with vision loss due to DR being as strongly affected as people with Age-related Macular Degeneration (AMD) [116] regarding loss in QOL. As a result, patients often feel their independence has been strongly limited [118]. BCVA is correlated to the outcome in question regarding QOL, to the length of disease and disease severity [117].

6.2.6 Social and economic costs

As a result of the significant negative effects DR has on patients QOL, much of the measurements addressed in the QOL also have social and economic costs for society. In Denmark, an older report (Bek et al. 1998) found that there were up to 130 new blind persons in Denmark per year. These patients were found to be blind due to diabetes which directly impacted the workforce [125]. The estimated costs for a newly blind work-active person are one million DKK the first year. This amount is 450.000 DKK for persons outside the workforce [125]. A newer systematic review by Köberlein et al. (2013) describes the economic costs of blind and visually impaired people in the United States being considerable for both the society and patient [126] with total annual costs (2011 USD purchasing power parities) being 16,321 USD for visually impaired individuals and 24,180 USD for people who are blind. Furthermore, there is also a significant relative reduction in employment for visually impaired and blind people of 32% in high income regions [127]. The indirect costs of visual impairment or blindness are borne by the employer, worker, government, society, extra carer costs, aid related to visually impairment and indirect deadweight loss for the non-visually impaired through increases in taxes and administrative tasks [128]. To prevent this costly disease for both patient and society, DR screening has been established. The economic benefits of a screening programme have been demonstrated to be cost-effective [129], but in some settings this may not be the case [130,131].

6.3 Diabetic retinopathy screening

Fundus photographs have been available since the first fundus photo in 1886 [132], and practitioners have been able to distinguish retinal abnormalities increasingly well due to improvements in technology in the fields of optics, cameras, photographic film and now digital fundus photos. The introduction of modern colour photographic film and digital film have lowered the cost and threshold for screening. Screening for DR was initially introduced in 1980 in Iceland [133–135] and Sweden [136]. It was first introduced for T1D patients and later for T2D patients. Later other European countries, Singapore [21,136–138], the USA and Australia introduced screening programmes [139–142]. A Danish database for DR screening was established in 2007 [143,144].

6.3.1 Development of diabetic retinopathy screening

The history of a classification system for DR began in 1968 when The Airlie House Symposium made a standardised classification system for DR [145]. Since then, there has been a continuous development with contributions from the Early Treatment Diabetic Retinopathy Study (ETDRS) where clinically significant macular oedema (CSMO) was later included (see Table 1) [146] in the classification scheme. The classification used in ETDRS was the standard for many years but is a bit complex. It is excellent for research but time-consuming and complex to handle in clinical practice where trained personnel are needed. Due to the complexity in clinical practice, the International Classification of Diabetic Retinopathy Severity Scale (ICDR) was introduced to clinical practice which simplified grading DR (Table 1). No worldwide standardised classifications system for screening for DR exists today, but there are several national and regional screening schemes for the condition. The screening scheme used in Denmark is the ICDR. This scale rates the degree of DR; however, the degree of macular oedema is rated separately as the ICDR was invented when Optical Coherence Tomography (OCT) was not available. Typically, macular oedema is graded on a three-grade system from 0 to 2 as seen in Table 1 [147,148]. The introduction of OCT has made it a lot easier to diagnose macular oedema in the retina where subtle oedemas can be very difficult to detect by ophthalmoscopy. Since the middle of the 2010s, computer technology has advanced considerably to a point where it is possible to grade fundus photos from DR screening using software on a computer [87].

International Classification of Diabetic Retinopathy Severity Scale

Severity level	Findings
0 = No DR	Normal eye
1 = Mild NPDR	Microaneurysms only
2 = Moderate NPDR	More than just microaneurysms. Less than severe NPDR
3 = Severe NPDR	> 20 intraretinal haemorrhages in each of the four quadrants OR Definite venous binding in ≥2 quadrants OR Prominent IRMA in ≥1 quadrant and no PDR
4 = PDR	One or more: Neovascularization Vitreous/preretinal haemorrhage

Clinically Significant Diabetic Macular Oedema

Grade	Criterion	
0	No exudates	Normal
1	Distance between macula and exudate/thickening > one optic disc diameter	Non-CSMO
2	Distance between macula and exudate/thickening ≤ one optic disc diameter	CSMO

Diabetic macular oedema OCT based on ICO guidelines [149,150]

No DME	No retinal thickening or hard exudates† in the macula
Non–center-involving DME	Centre-involved DME: Retinal thickening in the macula that involves the central subfield zone (1 mm in diameter)
Center-involving DME	Non-centre involved DME: Retinal thickening in the macula that does not involve the central subfield zone (1 mm in diameter)

DR = Diabetic Retinopathy. NPDR = Non-Proliferative Diabetic Retinopathy. PDR = Proliferative Diabetic Retinopathy. IRMA = Intraretinal Microvascular Abnormalities. CSMO = Clinically Significant Macular Oedema

Table 1 *International Classification of Diabetic Retinopathy Severity Scale and Diabetic Macular Oedema grading [150–152].*

6.3.2 Screening schemes

The general purpose of all screening is to ensure that unrecognised disease is detected in patients at higher risk of developing a disease in an asymptomatic population [153]. As the research field in ophthalmology is influenced by national traditions and structure of healthcare systems, different DR screening

methodologies and limits for referral to treatment exist in different countries. As golden standard, the clinical classification system ETDRS [154], can be used to grade DR with reproducibility and validity at satisfactory levels. Due to the cumbersome and time-consuming nature of the ETDRS system, which includes more grading levels than necessary for clinical care [148], local and regional screening systems have emerged to address these limitations. [155–158].

In 2003, the Global Diabetic Retinopathy Project group proposed [148] to standardise clinical screening using a simpler method than ETDRS. Their proposal was to adopt ICDR (Table 1) which is used in many countries ,including Denmark, [159] and has some national variations such as the National Screening Committee Classification in the UK [160,161]. The screenings intervals may vary depending on the severity of the disease according to different national guidelines. For a patient without DR, the screening intervals are usually until 24 months [162,163], but can be as low as 3 months with severe PDR and as long as 48 months for patients with no DR [164]. New research has investigated prolongation of the screening intervals, especially for ICDR grade 0 or 1, as the majority of patients with diabetes belongs to these two categories [162]. Several studies have been performed to develop an algorithm based on epidemiological data to increase screening intervals. These studies have focused on transitioning time from no-DR and mild DR to severe DR and PDR which will thereby reduce the load on the healthcare system while still giving the patient sufficient care [165–171].

A challenge with long screening intervals is the retention of patients in the screening programme [172], as length between screenings may lead to loss of contact with the patient and difficulties in generalisation of results and practices from one regional screening programme to another. A more conservative approach based on paraclinical data may be preferred with individual deviations. Another challenge is general screening attendance of which we made a regional evaluation in a 10-year cohort follow-up study based on the population of the Region North Denmark (Paper I).

A Cochrane systematic review by Lawrenson et al. (2018) of interventions to increase screening attendance for DR screening in the USA found association between quality improvement strategies of different kinds and screening attendance compared to usual care but no statistical difference between interventions in general diabetes care compared to specific interventions in DR screening care [173]. Another systematic review included 16 studies by Kashim et al. (2018) [174] and looked at non-attendants (based on all reasons given) and the basis for non-attendance. This review found that patients who belong to “ethnic minorities” or

"socio-economic deprived" groups were less likely to attend DR screening and repeated non-attendance was associated with a higher risk of VTDR [174]. This has been confirmed in the Danish population in two recent register studies [175,176]. Many studies which focus on screening attendance or non-attendance are register based, and do not specify whether non-attendants included are drop-outs or never attendants. Few studies focus on questionnaires regarding patient awareness. A Hong Kong based study (2018) focused on low screening attendance found that this may be due to insufficient patient awareness of available treatment, symptomatology and general education on the disease [177]. Other factors may apply as well, such as weather [178], cultural considerations [179] or younger age and house moving [180].

With the increasing prevalence of diabetes there is a greater need for national screening programs to accommodate the growing number of patients requiring screening for DR. According to a 2003 estimate in England during the introduction of DR screening, around 1.4 million individuals with diabetes were projected to require screening. [181]; however, in the National Health Service annual report showed that from 2019 to 2020 2.3 million were screened out of 2.8 million offered a screening. This was in spite of the fact that 3.5 million were known to be in the programme [182]. These figures indicate a doubling in the demand for DR screening within a 17-year period (2003 to 2019). This is also the case elsewhere as described earlier in section 6.1.2 and is a result of diabetes being on the rise which has led to DR creating an increasing demand for ophthalmologists [183,184].

6.3.3 Automation of screening

As a result of rising demand for ophthalmologists, researchers have investigated innovative technology to find a way to decrease the need for the manual labour and tedious tasks necessary to screen for DR. An early system to distinguish between no-DR and DR was a machine learning algorithm which counts red lesions (microaneurysms, dot bleedings) [185–187]. Since then, development has been focused on machine learning and deep learning, in particular, which is a specialised field of machine learning. In 2012, a deep learning network called AlexNet [188] had an impressively low error rate of 15.4% when it came to recognising general images. This was 10% points better than the second-place tool used in the ImageNet Large Scale Visual Recognition Challenge [189]. This challenge led to a cascade of deep learning algorithms for detecting DR being developed based on the general experiences generated by AlexNet. Gulshan et al. (2016) showed a remarkably high Area under the Curve (AUC) of up to an AUC, i.e., 99.1% (95% CI: 98.8%-99.3%) for

detecting referrable diabetic retinopathy [190]. In 2018, an algorithm (IDx-DR) for detecting referrable DR was approved by United States Food and Drug Administration (FDA) [87]. Deep learning has many use cases in ophthalmology such as automation of screening and biomarker detection on the retina [191], estimation of refractive errors [192], referral in retinal disease [193], cardiovascular risk factors [194], macular oedema in fundus photos [195], DR progression in individual patients [196] and even gender identification [197].

Even though the above-mentioned results are quite impressive, integration into the clinic is still lacking as IDx-DR is still the only FDA approved artificial intelligence (AI). The threshold for referral for different AI systems is moderate NPDR for the IDx-DR, but in Denmark the threshold for referral is sight threatening DR [198]. A multicentre study by Lee et al. compared seven AI DR screening systems, including IDx-DR, on datasets from two screening centres in USA. Most algorithms performed no better than human graders, and Lee et al. argued "for rigorous testing on real-world data before clinical implementation" [199]. In this present thesis (paper II), the subject of validation and performance evaluation of a machine learning software on real world clinical data is explored. The algorithm was provided by RetinaLyze® (Hellerup, Denmark). Even though it's an older algorithm, it has been updated since the original papers [185,186], and today's use (one fundus image vs five fundus images today) in clinical care is different compared to the initial use and has thus led to the need for evaluation.

7 Aims

As described in the introduction and background, patients with DR need to be screened. Even so, there will be a potential deficit of ophthalmologists in the future due to the increasing number of patients expected to develop DR. This is of concern as patients who develop sight threatening DR need to be treated. Herein lies the basis of this thesis.

The aims of this thesis are as follows:

1. To estimate the DR screening attendance by cumulative incidence for patients with diabetes in the North Denmark Region, at private ophthalmologist and at hospitals through official statistics.
2. To assess a software currently in clinical use, for diagnosing the presence or absence of DR in a real-life screening population.
3. To make an overview of the common medical treatment options for diabetic macular oedema with focus on their safety profile.

The aims (as listed above) were divided into three papers. One based on the Danish National Registries (paper I, aim 1), one as a software performance study (paper II, aim 2) and one based on literature review (paper III, aim 3).

Each of these papers are described in the following.

8 Study design

8.1 Paper I: DR screening attendance in a regional population

Paper I was a regional cohort follow-up study based on registries at Statistics Denmark [200]. Specifically, we used The Danish National Prescription Registry [201] for finding people with diabetes, as the registry holds all information regarding dispensed prescriptions in Denmark from 1995 and is registered by a person's civil registration number. Reimbursement of up to 85% of medical prescription costs are provided by the Danish government. The National Health Service Registry [202] was used to find information from private ophthalmologists, and the Danish National Patient Register [203] was used to find information regarding out-patient clinics. The information is mergeable with other registries based on the civil registration number of the patients [204–206].

Patients with diabetes living in the North Denmark Region were identified based on prescriptions for diabetes related medications. Patients with diabetes who consulted either a private ophthalmologist or had had a retinal photo at a hospital were identified. A local database was used for verification of visits at the hospital and linked to the civil registration number.

The aim of the study was to identify the DR screening proportion per year and cumulative incidence DR screening for the diabetes population at private ophthalmologists and hospitals in the North Denmark Region during a 10-year period, i.e., from 2009 – 2018. Additionally, the study aimed to verify methods of assessing screening attendance in the general health registries compared to a high-quality regional database.

8.2 Paper II: The application of screening software on a DR population

This paper was a cohort follow-up study based on patient visits to the DR screening clinic in the region of North Jutland in Denmark. The fundus images for the study were gathered using a list of patients with diabetes attending screening in a hospital setting. A statistician performed a power calculation (a minimum of 960 eyes for power of 0.9 and an alpha of 0.05). The power calculation was based on data from a small pilot study which was made in advance of introducing the software to the Department of Ophthalmology, Aalborg University Hospital.

Three experienced retinal specialists, i.e., two internal ophthalmologists and one external ophthalmologist, were hired to make a reference dataset based on the ICDR. Final grades were decided by majority vote. We wanted to be able to accurately measure the intragrader variability and to measure the performance of the three graders regarding routine grading. We also wanted to measure the intergrader variability between the two internal graders and their previously grades at the routine grading.

The aim of the study was to evaluate a software while only being able to detect the presence or absence of DR via red lesion recognition and compare the results from the software to a reference dataset (graded by three retinal specialists). After this, we compared the reference dataset vs software performance with the performance between the reference dataset and the daily routine screening. The daily routine DR screening was performed by a wide variety of doctors with varying experience. It is important to note that a software which is used clinically should be assessed in the clinical setting in which it is used in order to assess its feasibility.

8.3 Paper III: Treatment options for diabetic macular oedema

Paper III was a retrospective literature review regarding treatment options for DME with a focus on drug safety and efficacy. In order to encompass all non-surgical therapeutic modalities (for DME) within the scope of the review, laser treatment was incorporated despite its non-pharmaceutical nature. The study identified papers, databases and product summaries from the US and EU medical agencies with focus on anti-VEGF, laser and corticosteroids used for treatment of DME. The literature was reviewed for both European Medicines Agency (EMA) and FDA approval and off-label medicine use described in the literature. Surgical treatment for DME is mostly used for mechanical issues such as posterior hyaloid, ERM peeling or retinal detachment [207–209] and was not inside the scope of the journal that published the paper.

The aims of the study were to identify the current available drugs and treatment therapies for DME, to give an overview of both the common and severe adverse effects as well as safety and efficacy of the treatment regarding improvement of BCVA (in patients with DME) with a focus on anti-VEGF.

9 Material

9.1 Paper I: DR screening attendance in a regional population

Inclusion criteria: 1) Prescription of diabetes treatment either as A10A (T1D) or A10B (T2D) drugs [210] with at least two dispensed prescriptions within 180 days where the last prescription must have been collected in 2009. 2) Living in the North Denmark Region in 2009.

Exclusion criteria: 1) Women age <40 who received Metformin and no insulin to avoid the risk of including women with polycystic ovary syndrome or endometriosis [211,212]. 2) Individuals with only 1 prescription redeemed within 180 days.

A total of 171,408 individuals were identified nationwide (in Denmark) as having redeemed two prescriptions on glucose lowering drugs with the latter being redeemed in 2009. The last prescription was set as inclusion date. 2725 patients were excluded due to exclusion criterion. A total of 18,832 individuals with diabetes lived in the North Denmark Region at the beginning of 2009. The end date was December 31, 2018.

9.2 Paper II: The application of screening software on a DR population

Inclusion criteria: We chose to only include patients who had visited the DR screening prior to the software being introduced to the department of ophthalmology. Each patient had to have exactly five fundus images taken by a medical professional with the following presentation: one foveal, one papillary, one nasal, one inferior and one temporal. We included patients from the first of January 2019 onward until the threshold made by the power calculation was met. We included only one eye from each patient. A majority of two or three retinal experts was needed before a grade was accepted as part of the reference dataset.

Exclusion criteria: We chose not to exclude patients with other eye disease such as glaucoma or age-related macular degeneration, as such patients also attend the screening clinic and are assessed by the software. Patients with either more or less fundus images than five were excluded. If the retinal experts did not come to a majority vote, the eye was excluded from the reference dataset.

A total of 1,001 eyes were included for assessment performed by the retinal specialists creating the reference standard. The reasoning behind having few

inclusion and exclusion criteria was to get an as real-world sample as possible, as a highly selected population can potentially bias the outcome.

9.3 Paper III: Treatment options for diabetic macular oedema

Inclusion criteria: Papers describing drugs used for treatment of DME used in western medicine. Papers must have been published in peer reviewed journals. Information on anti-VEGFS's used for AMD was included if the anti-VEGF was used off-label to treat DME or was in a phase III trial. Information regarding safety was extracted from clinical trials and from reports by the FDA or EMA.

Exclusion criteria: Non-peer reviewed papers. If a treatment was not released and approved for clinical use by FDA or EMA or not in phase III trial. Surgical treatment for DME and its complications.

A total of 148 clinical trials and papers were identified. 21 major trials and papers were included by name, as these were assessed as having high importance due to methodology and size of their included population. As this was a study of adverse events, information regarding safety and efficacy was extracted from all available sources. Seven anti-VEGF were identified as relevant for the study. General laser treatment was included without designation subtypes of laser treatment, as the journals focus is drug safety. Three drugs derived from corticosteroids were included.

10 Methods

10.1 Paper I: DR screening attendance in a regional population

The Danish National Prescription Registry [213,214], The National Health Service Registry [202] and The Danish National Patient Register [203] at Statistic Denmark were used to define diabetes population on a regional level in the North Denmark Region. A high-quality local database was merged with the registries to provide additional information on patients screened at hospitals. The mean positive predictive value of the method for finding screening attendant at hospitals was calculated for the entire period, and the high-quality local database was used as ground truth.

All data were examined by using SAS Enterprise Guide version 7.15 for Windows with SAS Statistical Software package for Windows, version 9.4 (SAS Institute Inc., Cary, NC, USA). Time from start (2009) to screening for DR at private ophthalmologists, hospitals or local database was analysed by using survival models and plotted using cumulative incidence curves. Hazard Ratios (HR) (Fine-Gray analyses) were analysed by the PHREG macro in SAS. PHREG macro was used for Chi^2 and Cochran-Armitage estimates.

10.2 Paper II: The application of screening software on a DR population

All fundus images were extracted from Topcon ImageNet i-base. The eyes were graded individually by three retinal experts who prior to the start of the study had taken an e-learning course [215] in DR screening to insure uniformity among the graders. The eyes were screened using the academic version of Labelbox [216]. Statistics analysis and comparisons were performed in RStudio version 1.4.1717 [217] for Windows (RStudio, PBC), R version 4.1.1, and by the guidance of a statistician. Graphs were made in Excel and RStudio. Conger's Exact Kappa was calculated for intergrader variability, and intraclass correlation coefficients was calculated for intragrader variability.

10.3 Paper III: Treatment options for diabetic macular oedema

In paper III, the focus was on adverse effects in pharmacological and medical treatment of diabetic macular oedema. As a result of this focus, three major treatment strategies for diabetic macular oedema were identified: laser, anti-VEGF,

and corticosteroids. For each treatment type the literature was thoroughly investigated by using the public databases PubMed.gov, Cochrane.org and clinicaltrials.gov. The available information on the drugs in the anti-VEGF group and the corticosteroids group was found on the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) online databases (fda.gov and ema.europa.eu) [2]. Furthermore, the DrugBank [218] database was searched for adverse effects of the found drugs included in the review. The Flockhart [219] table for interactions with Cytochrome P450 was also searched, as Cythochrome P450 is a major pathway for clearance of drugs. Only peer-reviewed papers were included in the review.

As anti-VEGF is also used for AMD, papers related to AMD were also included in the review, and despite AMD being a different disease, adverse effects, interactions, and bioavailability should be the same. Some anti-VEGF drugs are used off-label in the clinic for treatment for DME and were included in the study.

11 Results

11.1 Paper I: DR screening attendance in a regional population

The data showed that nearly all patients with diabetes were screened during the period of 2009-2018, with the majority being screened in the first two years with a cumulative screening incidence of 74.2% (95% CI: 73.6 – 74.8) (Fig. 2). There was a statistically significant increase in the cumulative proportion of the screened per year during the period (Cochran-Armitage Trend Test *Z* -32.8, *One-sided P < Z* .0001). Hazard Ratios were shown to be statistically significant with patients with T1D HR: 1.157 (95% CI: 1.100-1.217) and females HR: 1.084 (95% CI: 1.051-1.120). Patients screened at hospitals HR: 1.573 (95% CI: 1.510-1.639) were more likely to be screened compared to patients with T2D, males and patients screened at private ophthalmologists (Table 2). Screening proportions per year, two years, and five years showed a statistically significant increasing trend (Fig. 3). The mean positive predictive value for finding screening visits at hospitals was 86.78% (95% CI: 86.76 – 86.81).

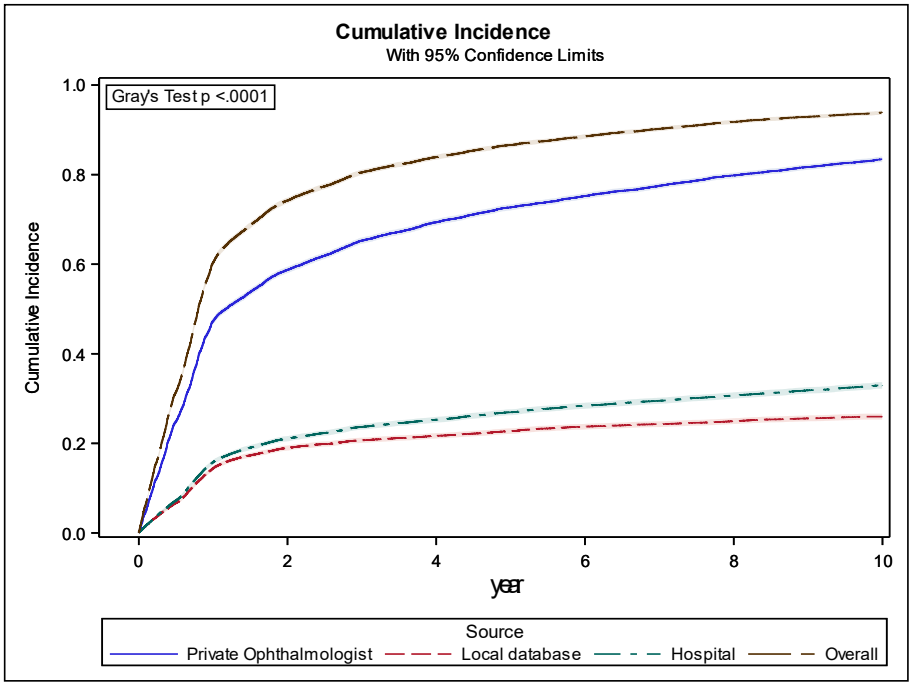

Figure. 2 *X-axis: Years from 1 Jan 2009 and onward. Y-axis: Cumulative Incidence of patients who have seen an ophthalmologist. 'Overall' is the overall cumulative incidence for all data sources. 'Hospital' is the cumulative visits at a hospital. 'Private Ophthalmologist' is the cumulative number of visits at a private ophthalmologist. 'Local database' is the cumulative known screening visits at a hospital. Reproduced from paper I.*

Variable	Inclined towards	*Wald Chi²: p*	HR	HR 95% CI	
Diabetes	T2D<T1D	<.0001	<.0001	1.157	1.100
Sex (T1D and T2D)	Male<Female	<.0001	<.0001	1.084	1.051
Sex (T1D)	Male<Female	0.03	0.03	1.093	1.008
Sex (T2D)	Male<Female	<.0001	<.0001	1.087	1.051
Age/decade (T1D, T2D)	Increasing age	<.0001	<.0001	1.023	1.012
Age/decade (T1D)	Increasing age	<.0001	<.0001	1.074	1.052
Age/decade (T2D)	Increasing age	0.25	0.25	1.007	0.995
DR location (T1D, T2D)	P.O.<Hospital	<.0001	<.0001	1.573	1.510
DR location (T1D)	P.O.<Hospital	<0.001	<0.001	1.642	1.504
DR location (T2D)	P.O.<Hospital	<.0001	<.0001	1.560	1.490

Table 2 *Hazard ratio (SHR) on covariates were calculated using the PSHREG macro in SAS for the incidence curve (Fig. 1 and Fig. 2) to estimate covariate effect on DR screening. For the variable 'Diabetes' more patients with T1D than T2D are screened. CI: Confidence Interval. P.O.: Private Ophthalmologists. [220]. Reproduced from paper I.*

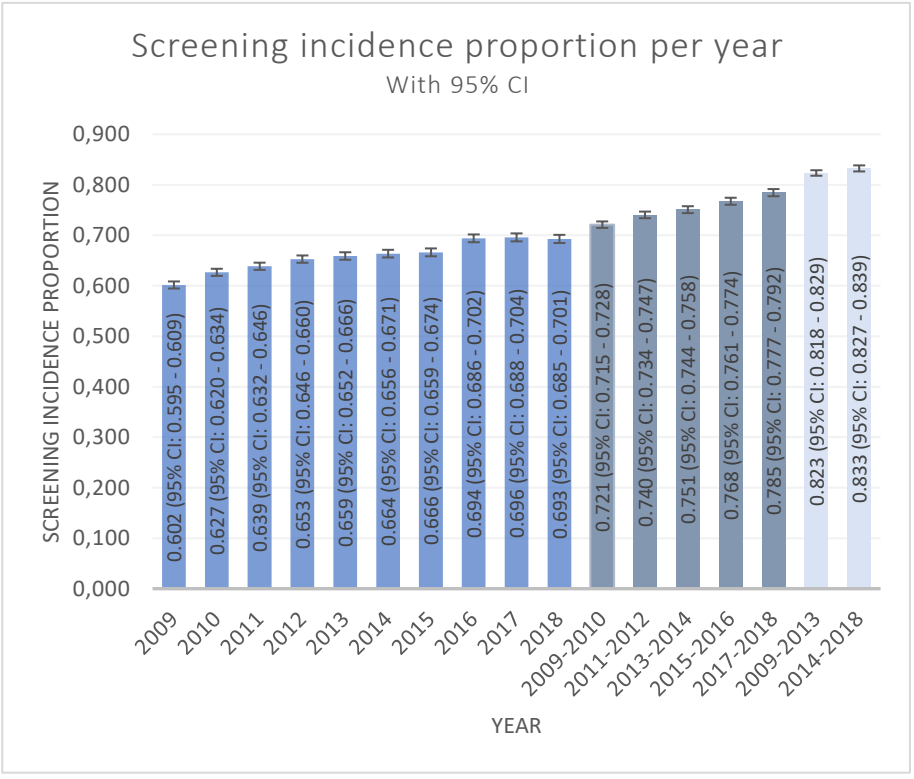

Figure 3 *Cumulative screening incidence proportion of the eligible population from year 2009-2018 (medium blue), by two-year intervals (dark blue) and by five-year intervals (light blue). Reproduced from paper I.*

11.2 Paper II: The application of screening software on a DR population

Of the 1001 included eyes, thirty-four were excluded due to either non-majority among the retinal experts gradings or insufficient image quality rated by the software or retinal experts. 967 eyes were eligible for further analysis. The major findings are described in Figure 4 with the blue colour highlighting the software vs the retinal experts (Combined red lesion threshold: 3) and the orange colour highlighting the routine screening vs the software (Reference vs Routine).

Results from 'Combined red lesion threshold: 3': Sensitivity 84.9% (95% CI: 81.8-87.9), specificity of 89.9% (95% CI: 86.8-92.7), positive predictive value (PPV) of 92.1% (95% CI: 89.7-94.4), negative predictive value (NPV) 81.0% (95% CI: 77.2-84.7) and accuracy 87.0% (95% CI: 84.8-89.0).

Results from 'Reference vs Routine': Sensitivity 87.0% (95% CI: 84.2-89.7), specificity 85.3% (95% CI: 81.8-88.6), positive predictive value 89.2% (95% CI: 86.3-91.7), negative predictive value 82.5% (95% CI: 78.5-86.0) with an accuracy of 86.3% (95% CI: 84.1-88.4).

The confidence intervals of the two methods overlap each other for all five measurements. For the Receiver Operating Characteristics (ROC), we found an Area Under the Curve (AUC) for the overall performance of the software at 93.4%.

We chose to make an inter- and intragrader variability test to verify the quality of the retinal experts grading. We used this test to also validate two of the retinal specialists' consistency as they had also graded some of the eyes in the routine screening. For intergrader variability among the five stage ICDR, we calculated a Conger's Exact Kappa for three graders at 0.731 (95% CI: 0.705-0.757). For a binary outcome of the ICDR scale, the Kappa value was 0.827 (95% CI: 0.798-0.856) among the three graders. For intragrader variability, we found an intraclass correlation coefficient of 0.81 (95% CI: 0.72-0.88) for grader Y and 0.90 (95% CI: 0.86-0.92) for grader X.

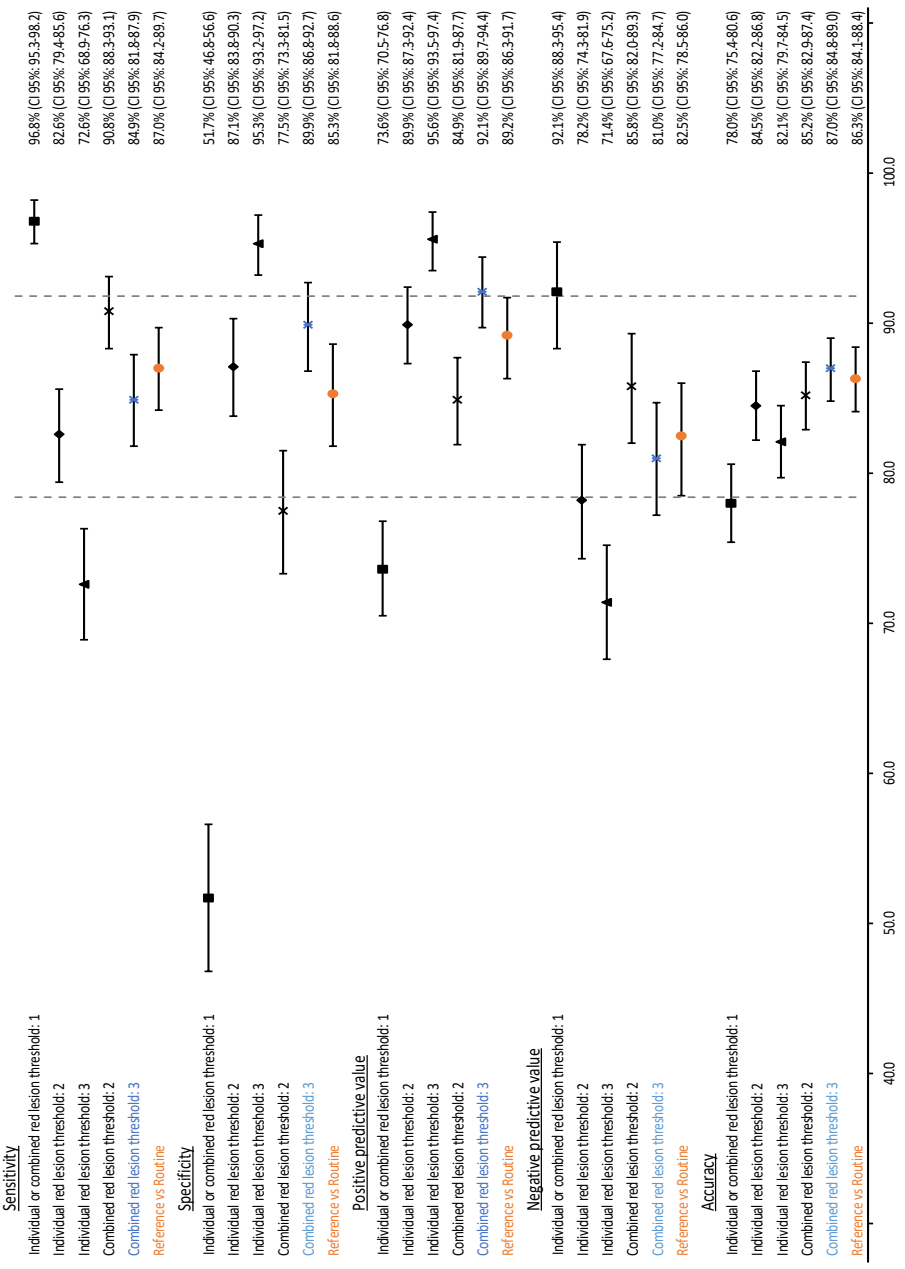

Figure 4 *Forest plot of the performance of the software with different red lesion thresholds and the routine screening. The results are compared to the three retinal experts ground truth. CI: Confidence Interval. Blue text: Best weighted performance of the software. Orange text: Performance of the routine screening. Vertical dotted line: minimum and maximum of the CI range of the routine screening. Reproduced from paper II.*

11.3 Paper III: Treatment options for diabetic macular oedema

Three modalities for treating DME were identified: anti-VEGF, laser and corticosteroids. Seven anti-VEGFs were identified as being used or being in a clinical trial. Laser as a general treatment option and three corticosteroids were identified (Table 3). Anti-VEGF was identified as being first line of treatment due to its generally low rate of adverse effects, being non-destructive and being quite potent for treatment of DME in terms of both decreased vision loss and potential gain of vision [2]. The development of anti-VEGFs has the primary attention of the pharmaceutical companies due to the aforementioned properties. We categorised laser and corticosteroids as second line of treatment in case of treatment failure with anti-VEGF. Corticosteroids have some unwanted adverse effects such as patients getting high intra ocular pressure and developing cataracts. The benefits of corticosteroids are mostly seen in the slow-releasing corticosteroid (Ozurdex) which can come in handy if patients have poor compliance with frequent anti-VEGF injections. Laser is cheap but was also destructive in its nature and did not show superiority compared to anti-VEGF. Laser in combination therapy with corticosteroids (Ozurdex) – if measured against gain in BCVA ETDRS visus – did not show superiority compared to anti-VEGF. If low threshold laser treatment is to be considered a primary treatment option for DME, it could be particularly relevant in developing countries. This is due to the significant impact that healthcare costs can have on treatment accessibility for the general population in such settings. Nonetheless, further research in this area is needed.

Table 3 has been updated since Brolucizumab and Faricimab completed their trials, published papers regarding the results [221,222] and have been FDA approved for treatment of DME [223,224]. Tarcocimab (Kodiak Sciences Inc., Palo Alto, California) was found to have completed Phase 3 trials for both Gleam and Glimmer for DME [225]. Tarcocimab is a high molecular weight (950 kDa) monoclonal antibody biopolymer conjugate intended to prolong the effect of the molecule in the eye [226,227] The Phase 1 trial for Tarcocimab has met its safety requirements. Tarcocimab has an ocular tissue half-life of 10+ days in the retina and 12.5+ days in the choroid [228]. The Phase 3 prospective, randomised, double-masked, two-arm, multi-centre non-inferiority studies (GLEAM and GLIMMER) evaluate the efficacy and safety with intraocular injections every 4 weeks for 3 months followed by 8-24 weeks individualised injection intervals [229–231]. The actual primary completion was in April and May 2023. Final results on efficacy and safety are yet to be published. Interim results are not reported in this thesis.

Name (Commercial Name)	Structure/Technology	Primary Target/Mechanism of Action	FDA and/or EMA approved for DME	Route, Dose, Frequency of Administration
Pegaptanib *(Macugen)* (outdated)	RNA aptamer	VEGF-A	NO, off-label use, outdated	IO, off-label use.
Bevacizumab (*Avastin*)	Humanized IgG1	VEGF, FcRn [232–234]	NO, off-label use	IO, off-label, 1.25 mg 0.05 ml every 4 weeks or by clinical status
Ranibizumab (*Lucentis*)	Humanized Fab/monoclonal antibody (mAb)	VEGF-A	YES	IO, 0.3 mg, 0.05 ml, every 4 weeks or by clinical status.
Aflibercept (*Eylea*)	r-fusion protein	VEGF-A, B, PlGF, FcRN [235–237]	YES	IO, 2mg, 0.05 ml every 4 weeks or by clinical status.
Brolucizumab *(Beovu)*	Single-chain Variable fragment (ScVf) [238]	VEGF-A	YES	IO, 6 mg, 0,05 ml every 6 weeks for 5 doses, and then every 8-12 weeks.
Faricimab (Vabysmo)	CrossMAB	VEGF-A, Ang-2	YES	IO, 6 mg, 0,05 ml every 4 weeks for 6 doses, and then every 4-8 weeks.
Conbercept	r-fusion protein	VEGF-A, VEGF-B, VEGF-C, PlGF	NO, study terminated for AMD (desired primary endpoint was not met) [239]	IO, study terminated for AMD (desired primary endpoint was not met) [239]. Current status unknown.
Tarcocimab (KSI-301)	high molecular weight monoclonal (950 kDa) antibody biopolymer conjugate	VEGF-A	NO, Primary Phase 3 completed	IO, 5mg, every 4 weeks for 3 months, and then individualized 8-24 weeks intervals.
Laser	Argon green (514 nm), dye yellow (577 nm), Krypton red (647 nm), and diode (810 nm) laser.	Peripheral retina, minimum distance to fovea is 2 papil size distance.	YES	Laser machine, individual choices, grid/focal.
(Ozurdex)	Dexamethasone	Down regulating of cytokines	YES	IO 700 microgram implant, release over 1-3 months.
(Iluvien)	Fluocinoloneacetonide	Down regulating of cytokines	YES	IO, 0.2 ug/day or 0.5 ug/day.
(Kenalog)	Triamcinolone Acetonid	Down regulating of cytokines	NO, off-label use	Next to the eye, subtenon, retro bulbar.
Abbreviations: DME: Diabetic Macular Edema. AMD: Age-related Macular Degeneration. RVO: Retinal Vein Occlusion. FDA: U. S. Food and Drug Administration. EMA: European Medicines Agency				

Table 3 *List of most relevant treatments (anti-VEGFs, laser types and corticosteroids) used in ophthalmology for treating diabetic macular oedema. All drugs are dispensed as intravitreal injection except laser photocoagulation which is applied to the retina and Kenalog which is given subtenon or retro bulbar. The dosage and frequency of administration of some drugs varies slightly between the FDA (US) and EMEA (EU) recommendations, and therefore local guidelines should be used for treatment. Reproduced from paper III [2] Brolucizumab has been approved by FDA for DME since the publication . [224]. The description for Brolucizumab in the table has been corrected compared to the published paper. It is the Fc region that binds FcRn, while the Fabs binds VEGF (and not anti-VEGF).*

12 Discussion

12.1 Registry based study (Paper I) DR screening attendance in a regional population.

In paper I, we used registries at Statistic Denmark to demonstrate that nearly all patients with diabetes in the North Denmark Region were screened from 2009 to 2018. During this time, an increasing proportion of patients were also screened which was demonstrated by a significant Chi^2 Likelihood test and through a Cochran-Armitage Trend Test estimate. Hazard Ratios were computed to examine the relationship between diabetes type, screening location, sex and screening frequency. The analysis revealed that patients with T1D, females, and those screened at hospitals had a higher screening frequency compared to patients with T2D, males, and those screened at private ophthalmologists. [240]. Ideally, every patient with diabetes should be screened at a maximum of two-year intervals according to the International Council of Ophthalmology (ICO) [241] The exception would be T1D patients who initially have a five-year interval from onset of diagnosis. We demonstrated increasing DR screening proportions by one- and two-year intervals where 78.5% (95% CI: 77.7 – 79.2) of the eligible screening population from 2017-2018 went for DR screening. Preferably this proportion should be even higher, and one can hope the positive trend continues in the years to come. We do not establish reasons for patients not attending DR screening, as there are several studies investigating this. One reason that should be considered is that patient awareness of the disease is associated with screening attendance [177]. We can thus speculate whether the high SHR for patients who attend screening at hospitals are related to patient awareness due to more a more thorough clinical set-up and follow-up. A generally high positive predictive value for estimating screening visits at hospitals was reported compared to the local database. As a local database was used for ground truth, care should be applied if the same method for finding screening visits at hospitals by using registries is used in other regions in Denmark.

12.1.1 Limitations

As we made our decisions about how to specify our population, some presumptions were made. We used an indirect measure to establish our diabetes population by using dispensed prescriptions and assuming that the medicine was only used for treatment of diabetes even though a drug like Metformin can be used off-label for other diseases such as polycystic ovarian disease or endometriosis [212]. We tried to counter this by sorting-out females age <40 who were only dispensed prescriptions

for Metformin. Defining people with diabetes by using dispensed prescriptions in the Danish registries is a well-established method in epidemiology and is a minor limitation in this particular study.

A more influential limitation is the way screening is defined. The assumption was that a patient with diabetes who saw an ophthalmologist was screened by the ophthalmologist. The major issue with this assumption was that we cannot be sure that the patient was screened for DR and did not just received treatment for something else such as macular hole, glaucoma, cataract, keratitis or dry eye disease. At hospitals, this limitation is the International Classification of Diseases version 10 (ICD-10) code which is used for finding screening visits in the registries. This code is a photo code, but we cannot be sure that diabetes was the reason for the photo being taken as described above. As of October 2015, a specific code has been used for registration of patients screened for DR at private ophthalmologists. Until October 2015, a more general medical retina code was used with the general code for a visit being chosen due to change in coding practices during the study. Even though this is a potential issue for defining DR screening, there was an assumption that ophthalmologists would screen a patient with diabetes for DR .We cannot however be sure, that a diabetes patient only saw an ophthalmologist for another issue and that the diabetes patient was not screened for DR.

12.1.2 Concluding remarks

A reference standard for assessing DR screening incidence would be a EMR high quality registry with input both from private ophthalmologists and hospitals. Such a registry exists [144], but the general issue with registries is the human factor, as the person behind the screen is responsible for correct medical coding and inclusion of all diabetes patients. The Danish Registry of Diabetic Retinopathy reported 220,000 patients included in the database by 2020 but estimates the total diabetes population in Denmark to be 310,000 as of 2021 [143]. This estimate only accounts for about 70% of the total diabetes population.

Registries may be a valid source as they can be the best and easiest available information on large patient cohorts but may also under- or overreport the incidence of screening. Further improvements such as validation of validity and reliability of the method can be examined by assessing positive predictive values on the methodology of how patients are screened at private ophthalmologists and hospitals according to our method.

12.2 Software study (Paper II) The application of screening software on a DR population

In paper II, we demonstrated how a software algorithm performs with different threshold values, and how this affects specificity, sensitivity, PPV, NPV and accuracy. We furthermore compared the results with how routine screening performs compared to a reference standard. The decision to measure the performance of the software versus routine screening grades was made because of software being used in daily clinical care. The software needed to be validated on five fundus images per eye instead of one fundus photo per eye as the first papers which described the software were based on.

As the specific metrics for sensitivity, NPV and accuracy for 'Combined red lesion threshold: 3' are included in the confidence intervals for 'Reference vs Routine' and vice versa, we cannot deny the performance metrics being the same. For specificity and PPV, we could not be sure that the software was significantly better than the routine screening due to overlapping confidence intervals without the specific metrics being included in the other confidence intervals. As we were comparing both the software to a reference standard (established by three retinal specialists) and the routine screening to a reference standard, we got an impression of how the software performs relative to the reference standard and routine screening. This indirect comparison was made due to the prerequisite of the study that a software should at least be non-inferior to the routine screening when both are compared to a reference standard. This is important if the software is to be used clinically.

We observed that an individual fundus photo screening strategy (described in paper II) is inferior to a combined strategy and should preferably not be used. We made this comparison due to how the software reported internally in the hospitals' systems. The software should preferably report per eye and not per image, as it is the eyes which are interesting when screening in daily clinical care. The software performed quite similarly when compared to the results from the papers describing the algorithm by Larsen et al., (strategy: combined red lesion threshold: 3). In summary, while the software may offer valuable guidance to clinicians, its utilisation in clinical practice should be approached with caution. Although it may assist in decision-making, the software's AUC does not reach a sufficiently high level to justify its standalone use without human supervision and intervention.

We found the inter- and intragrader were within acceptable levels compared to other studies [199,242], which is both an assurance of the reference graders used in

this study and of standard of the reference dataset. We cannot say if the DR screening course the three graders took prior to the reference grading influenced how the three reference graders performed in this study or if the course bolstered the intergrader performance.

12.2.1.1 Software vs reference standard and the meaning of performance metrics

By strictly comparing the software to the reference standard, the performance metrics vary significantly depending on the threshold used (Fig 4). Sensitivity refers to the proportion of true positive cases of DR which are correctly identified, and specificity refers to the proportion of true negative cases of DR which are correctly identified by the software when compared to the reference standard and based on the presence or absence of characteristic signs of DR by the human graders such as microaneurysms, haemorrhages, exudates and neovascularisation and by the software by red dots.

A high sensitivity (Fig 4. Individual + combined red lesion threshold: 1) indicates that the model is able to detect most cases of DR – even those with subtle or early signs of the disease and minimize false negatives. These are cases of DR that can be missed or misclassified as normal. A high specificity (Fig 4. Individual red lesion threshold: 3), on the other hand, indicates that the model is able to accurately exclude non-DR cases and minimise false positives, which could lead to cases being mistakenly diagnosed as DR.

Sensitivity and specificity are often inversely related, making it difficult to achieve high scores on both metrics. This means that increasing one metric may lead to decreasing the other. Known as the sensitivity-specificity trade-off, the increase is particularly relevant in the context of AI models for DR where the threshold for classifying a retinal image as positive or negative can affect the balance between sensitivity and specificity.

For example, setting a low threshold may increase sensitivity by detecting more cases of DR but may also increase false positives (Fig 4. 'Individual + combined red lesion threshold: 1'). Moreover, setting a high threshold may increase specificity by reducing false positives but may also increase false negatives (Fig.4. 'Individual red lesion threshold: 3').

Both low sensitivity and low specificity can have negative impacts on the clinical utility and validity of an AI model for DR, as they may reduce the accuracy, reliability, and efficiency of DR screening and management. It is therefore important to optimise the sensitivity and specificity of AI models for DR by balancing the trade-

offs such as our 'Combined red lesion threshold: 3' (Fig 4) which balances the trade-offs of sensitivity and specificity between them. This is the best level of balanced performance where the sensitivity and specificity are both relatively high.

By testing software against a reference golden standard, high sensitivity and specificity in the lab may be demonstrated, but real-world performance can be lower. Having a reference standard allowed us to compare the real-world performance of the daily DR screening at the hospital department. The results from this comparison gives an indication of what may be a clinically acceptable threshold for a software [199].

It can be discussed how clinically important high sensitivity is with a trade-off on specificity. Some would argue that a certain amount of DR screenings being false negative (high specificity, low sensitivity, Fig 4. 'Individual red lesion threshold: 3') is acceptable due to the patients most certainly being screened again in 1-2 years. Others may argue that a high sensitivity compared to a low specificity is most important which may lead to unnecessary follow-up and treatment of non-diseased eyes.

12.2.2 Limitations

A clear limitation of the study is the absence of comparisons to other AI systems, such as IDx-DR [243,244] or EyeArt [245,246]. Additionally, a multicentre analysis, similar to the one conducted by Lee et al. [199] , which incorporated a more diverse range of patients in the reference dataset, was not performed. Due to the limitations of the study timeframe, a direct comparison with other systems was not feasible. This study would probably have benefited from comparison to other software in daily clinical care, as real-world performance is important if a software tool should be applied and trusted by the users. Another major limitation is the software is only limited to binary grading: i.e., DR or no-DR, but in daily clinical care the ICDR is used for grading DR and consists of five grades (Table 1). A software that could be rated according to the ICDR with high metrics would be truly valuable in daily clinical care. It may have been useful to add a sub-analysis of the performance of the model based on disease severity. This would especially be the case if the software were to be approved for making its own clinical decisions without humans; however, it could be difficult to achieve acceptable clinical results even with more advanced software [199].

Another drawback of the software is its lack of ability to detect macular oedema. This is considered a major limitation of the software and is of clinical relevance as

macular oedema is not easily recognized on 2D fundus photos. We could have specified how many of patients had macular oedema and were rated as DR or no-DR. We chose not to do so since the software cannot distinguish macular oedema from fundus photos, and the software does not analyse OCT-scans. As images with a positive DR diagnosis based on the software would have to be manually reviewed by a clinician, DME could be found in this context. False negatives cannot be ruled out as not having DME if they are not manually reviewed by a clinician.

The sample size employed in this study is comparatively smaller than that of other studies utilising deep learning on more extensive and diverse populations. The limited sample size was determined by the available resources and was based on a pre-study power calculation. Therefore, the impact of a larger sample size on the results remains unknown. It can be challenging to compare algorithm performance when they are not evaluated on similar datasets.

12.2.3 Concluding remarks

In paper II, we observed how an algorithm performs differently based on how the results are analysed (individual vs combined with varying red lesion threshold) compared to a reference dataset. Compared to the reference screening, we could not conclude if there was a significant difference due to the confidence intervals overlapping with the central metrics in all but two metrics (specificity and PPV). For a software to be applied for clinical use without human intervention or supervision, the performance metrics should be considerably higher. Based on the current level of performance described, it is recommended that the application of the software be accompanied by human supervision and intervention, rather than allowing the software to make grading decisions independently.

Future studies should ideally compare several algorithms, including the analysis of OCT-scans, in order to assess their performance relative to each other and the reference dataset. To have a substantial clinical impact, a software should be able to distinguish all categories in the ICDR including OCT regarding macula oedema with acceptable clinical metrics.

12.3 Review study (Paper III) Treatment options for diabetic macular oedema

In paper III, we included the drugs available (to the best of our knowledge) for treatment of DME at the given time. Of the three groups, we concluded that anti-

VEGF might be the best general treatment option for patients with DME from an ophthalmological perspective. Anti-VEGFs in general have a similar safety profile, but newer drugs such as Brolucizumab can potentially benefit from a longer dosing interval impart and thus reduce safety risks related to the intraocular injection such as pan-ophthalmic infection. We chose Aflibercept as the drug of choice, as the largest independent randomised, multi-centre, double masked controlled trial [247,248] with 660 participants showed Aflibercept to have a possible advantage over Bevacizumab and Ranibizumab. Additionally, a Cochrane review [249] suggested a slight advantage of using Aflibercept compared to Bevacizumab and Ranibizumab. To our knowledge, no meta-analysis or Cochrane review compares the current FDA and EMA approved anti-VEGFs which may otherwise lead to another anti-VEGF being superior to Aflibercept. The review was written without price considerations for either society or the patient. Price and compliance in the real world have a substantial impact on the choice of medicine. In a first world country that has a taxpayer funded healthcare system, price is not of major importance in the decision for choosing a treatment for a disease. On the other hand, patient compliance can affect the choice. Long travel distances can have a massive impact on patient behaviour to attend the hospital, and in such cases laser or depot corticosteroids can be preferrable. A notable constraint of a review is its susceptibility to becoming outdated as a result of recent advancements in drug development.

12.3.1 Limitations

A review is a more general method of synthesising the evidence in a research field and providing an overview. The search for literature in paper III was comprehensive but may not have been exhaustive. We cannot be sure that all relevant literature was included, and it can be difficult to identify quality sources due to the high number of papers published concerning treatment for DME. In the literature published, finding information regarding drugs structure, kilodalton size, systemic and ocular half-life can be a challenge. Most half-life times of molecules are measured in rodents and are reported by small sample sizes that do not necessarily directly translate to half-life in homo sapiens. There is a possibility that the findings presented in this thesis may be influenced by the authors' own bias, which can pose challenges in critically evaluating the included results. This may limit ability to provide objective analysis and instead result in a more descriptive and summarising approach to the findings of others.

12.3.2 Concluding remarks

In paper III, we provided an overview of the current treatment medical modalities for DME which do not require surgical intervention and defined three areas of interest: anti-VEGF, laser and corticosteroid treatment. Overall, we found that anti-VEGF to be the all-round best treatment for DME if price is not an issue. A study comparing the current FDA and EMA approved anti-VEGFs is needed to suggest the safest and best anti-VEGF drug for treatment of DME. Laser still has its place due to its low costs for patients who cannot afford more expensive treatment such as anti-VEGF. The reference standard for making a review of the current drug treatment of DME would be a systematic review following PRISMA guidelines [250] where two reviewers follow an evidence-based flow diagram which includes searches of databases, registers and other sources [251]. Undertaking this task is quite substantial, as it entails two individuals conducting independent literature reviews and assessing bias. Furthermore, the process is even more time-consuming compared to conducting a regular review. One of the primary goals of a systematic review is to ensure reproducibility and address potential biases in the studies included.

12.4 Reflections

The incidence of diabetes and thereby diabetic retinopathy has been on the rise yearly for the last decades and the trend seems to be continuing. More patients need to be screened for DR, but the availability of ophthalmologists on a global level is not sufficient to meet the expected increasing demand for DR screening [252]. This could affect the ability of health care systems to screen and refer patients for treatment of DME in a timely manner in order to prevent irreversible loss of vision and thereby loss of quality of life and increased expenditures for government, society and patient.

From paper I, we know that the regional screening attendance (Fig 2 and 3) is not as high as recommended by the ICO guidelines or by the Danish Ophthalmological Society (DOS) [164,241,253]. The newest version of DOS recommendations [152] prescribe intervals of up to 48 months for well-regulated patients with diabetes and no DR. This in in contrast to former intervals in the Danish screening programme which prescribed a maximum of 24 months screening intervals from 2010-2017 [164]. We know that patients screened at hospitals who have either T1D or T2D are associated with higher screening attendance than patients who attend screening at private ophthalmologists. We also know that patients with T1D are most inclined to

be screened. The obvious limitation to a registered based study is contamination of other diseases, and the establishment of a proper diagnosis for diabetes based on dispensed descriptions. Furthermore, establishing a more sufficient indirect measure for screening at hospitals based on ICD-10 photo codes and visits would need to be implemented and be generalisable to other regions as, the method in our paper to some extent overestimates DR screening at hospitals as regional differences and interhospital differences in ICD-10 reporting can apply. Estimating the number of patients screened at private ophthalmologists can be difficult as coding for DR screening changes over time; consequently, we assumed a patient visit to a private ophthalmologist triggered a screening for DR which might not be the case. We could have thus both overestimated and underestimated the number of patients being screened at private ophthalmologists. Nonetheless, it is possible to speculate that this finding may represent an overestimation considering that private ophthalmologists receive payment for each registered visit. A Danish registry for keeping track of DR screening at hospitals and private ophthalmologists exists, i.e., The Danish Registry of Diabetic Retinopathy (DiaBase), and was established in 2007 [254]. This is a registry for reported screening and does include non-attendees (never attendants), meaning patients with diabetes who have never seen an ophthalmologist are not included. A recent study by Thykjær et al. reports on attendance and adherence of these patients but only for the population reported to DiaBase [255]. Our study might contribute and give insight regarding patients with diabetes who have never been screened.

In paper II, when comparing the reference standard (the retinal specialists) with the software, we had a relatively small sample of eyes compared to other studies assessing AI software [190,199,256,257]; however, we did a power calculation, made by statisticians, which showed that our results were on par with ophthalmologists in the daily clinic who assess whether DR was present or not. Without an assessment of the reference standard to other algorithms and comparing the algorithms among themselves, it may be difficult to judge our work properly other than through the methodology. Other algorithms can distinguish all five ICDR grades from each other. During the grading process conducted by retinal experts, the utilised software indicated a decline in the performance of one grader when handling a high volume of gradings per day (up to 200 gradings/day) within a relatively short time frame. This was in contrast to the others who consistently performed 25 gradings per day. This observation raised concerns about the adequacy of the chosen time span for completion and prompted consideration of potential measures to establish a more consistent method for grading the eyes. Such introspection prompts further exploration and evaluation to enhance the grading process. One of the challenges

encountered when testing software trained on specific populations is the potential introduction of bias into the algorithm. Clinicians may exhibit a higher proficiency in identifying the less common or more severe cases, such as ICDR grade 3 or 4, among patients of Asian or African origin. [258,259]. Collaborating with a company, even when not directly funded by them, may still introduce bias in the methodology. As researchers, we may have a preference for finding significant results that are favourable for publication and aim to satisfy our cooperation partner who has provided assistance [260].

Paper III summaries our knowledge of the evidence of treatment options for DME, including safety profiles for each drug. Regarding anti-VEGF's considerable overlap in terms of safety, the profile can be observed as all are dispensed by intraocular administration via a needle. It can therefore be speculated that the small variance between the drugs is due to chance or reporter bias such as negative bias, e.g., Brolucizumab which got negative attention after approval [261]. Knowledge of and attention to bias and pitfalls such negative media reports when choosing a treatment should be carefully considered. Additionally, there is a possibility that certain side effects are only related to DME and others to AMD due to different disease aetiology and cellular mechanisms which can be exceedingly difficult to study in vitro human eyes.

12.5 Summarizing discussion

Diabetic retinopathy is a leading cause of visual impairment and blindness worldwide. Effective screening and timely treatment are crucial for managing this condition. A patient with diabetes needs to be screened for DR due the possibility of being unaware of potential progression of DR to sight threatening DR. In this thesis the three papers go from a broad perspective of a regional population-based DR screening (paper I) to focus on the screening process itself (paper II) and treatment of those patients found through the screening process to need treatment (paper III). By juxtaposing these studies, the aim was to provide a comprehensive perspective on DR screening and treatment.

In the first study (Paper I), the attendance of diabetic retinopathy (DR) screening in a regional population was examined. The data revealed that almost all patients with diabetes underwent screening during the study period with the majority being screened within the first two years according to national guidelines. Even so, 6% of the population was never screened. There was a significant increase in the cumulative proportion of patients screened each year. Factors associated

with a higher likelihood of screening included having type 1 diabetes, being female and being screened at hospitals. However, limitations were identified, such as assumptions about diabetes population estimation based on dispensed prescriptions and uncertainties regarding the actual screening for DR at ophthalmologist visits at private ophthalmologists and especially screening at hospitals. However, the positive predictive value for screening visits at hospitals was high.

In the second study (Paper II), the application of SVML screening software for detecting DR in a population was evaluated. 967 eligible eyes were analysed. The study evaluated the performance of a screening software algorithm compared to routine screening and a reference standard. The software exhibited comparable results to previous studies but lacked the ability to detect macular oedema and grade DR according to the International Classification of Diabetic Retinopathy. This lack of capability is a major limitation. The software showed an overall performance with an Area Under the Curve (AUC) of 93.4% in Receiver Operating Characteristics (ROC) analysis. Additionally, the study assessed inter- and intragrader variability, demonstrating good agreement among the graders and high consistency within the retinal specialists. The sensitivity and specificity of the software varied based on different threshold values, indicating a trade-off between sensitivity and specificity. The study concluded that the software should be used with human supervision and intervention and suggested the need for larger multicentre studies to compare different AI systems and evaluate their real-world performance.

The third study (Paper III) focused on treatment options for diabetic macular oedema (DME). Anti-VEGF therapy was identified as the first-line treatment due to its efficacy in reducing vision loss and potential improvement of vision thus highlighting the efficacy and safety profile. The findings suggested that a drug like Aflibercept, in particular, may offer the best treatment option for DME if price is not a consideration. The development of anti-VEGF drugs has received significant attention from pharmaceutical companies with several new products in the last years. However, the study acknowledged the limitations of available treatments and emphasised the importance of considering newer drugs, such as Brolucizumab, with potential benefits like longer dosing intervals which in turn may reduce the safety risks associated with intraocular injections. Though a systematic review and comparison of the FDA and EMA approved anti-VEGF, treatment is needed to come to a conclusion regarding the best anti-VEGF due to recent developments with the approval of Brolucizumab, Faricimab and potentially Tarcocimab. Laser and

corticosteroids were categorised as second-line treatments in case of treatment failure with anti-VEGF. Corticosteroids had unwanted adverse effects such as development of high IOP and cataract in phakic eyes, while laser therapy did not demonstrate superiority compared to anti-VEGF. The use of low threshold lasers as a first-line treatment in resource-limited settings requires further research. The table presenting treatment options was updated to include Brolucizumab and Faricimab which have completed trials, published results and gained FDA approval for DME treatment. Tarcocimab has completed phase 3 trials but awaits publication of results.

By examining these three studies, a comprehensive understanding of the challenges and advancements in DR screening and treatment has been gained. Paper I emphasised the increasing screening attendance, highlighting the limitations of registry-based data and the need for better validation methods. Paper II provided insights into the performance of screening software, indicating the importance of balancing sensitivity and specificity and the necessity for human supervision. Lastly, Paper III shed light on the potential benefits of anti-VEGF therapy for DME treatment, acknowledging the need for further research on newer drugs and treatment options.

Overall, these studies offer insights into the attendance of DR screening and contribute to the ongoing efforts in improving DR screening attendance, the utilisation of screening software and enhancing screening software performance as well as the array of treatment options available for DME and the identification of effective treatment strategies for DME. The findings have implications for healthcare professionals, policymakers and researchers as they emphasise the importance of continuous advancements in DR management to reduce visual impairment and improve patient outcomes. Further studies comparing different algorithms, considering OCT scans, and evaluating real-world performance are warranted to guide clinical decision-making and improve the overall management of DR and DME. Such studies can hopefully thereby enrich the comprehension and adeptness in handling the intricate realm of DR as they span from collective cohorts to individualised approaches, encompassing both population-based strategies and personalised interventions. This would contribute to greater comprehension and skill in effectively dealing with the complexities of diabetic retinopathy.

13 Conclusions

Eye complications in relation to diabetes constitute a significant health risk with a risk of reduced QOL. To avoid late ophthalmological diabetic complications, DR screenings are included as a fixed component in terms of timely intervention. The first preferable treatment option is proper blood-glucose management which reduces the chance of developing DR. The remaining treatment options are the ophthalmological medicines described in paper III. The PhD thesis describes the possibility of establishing a screening procedure using AI software with the aim of limiting resource consumption and finally, based on existing literature, to describe treatment options with a special focus on DME. The main findings of this PhD study are listed below:

- More than 80% of diabetes patients in a regional population were screened over a two-year period with the majority of these being screened at private ophthalmologists.
- A statistically significant rising trend in screening attendance during a 10-year period.
- Screening attendance being significantly higher at hospitals than at private ophthalmologists among both T1D and T2D patients.
- Female patients had a significantly higher screening attendance than male patients with the highest attendance being among T1D female patients.
- Validating screening at hospitals found a high positive predictive value.
- Overall performance of the software was non-inferior to the performance of routine screening when both were compared to a reference standard.
- Selecting a proper threshold and method for reporting performance is important as a too low or too high threshold can change the sensitivity, specificity, PPV, NPV and accuracy of the software and thereby have a clinical negative effect on patient care.
- Proper clinical testing of software assessing DR is a must if introduced to the clinic, and the clinical application should reflect the performance of the software.
- Inter- and intragrader variability were on par with literature.
- Anti-VEGF was the preferred treatment for DME with Aflibercept being the drug-of-choice resulting in few side effects and a clinically acceptable safety profile.
- For corticosteroids used for treating DME, dexamethasone (Ozurdex) is the drug with the fewest side-effects regarding the development of cataract and high intraocular pressure.
- Retinal laser therapy is still a relevant treatment option especially in low-income countries or for patients with few funds.

14 Future perspectives

Diabetic retinopathy is becoming a major challenge in every healthcare system worldwide as incidence of diabetes is rising. Consequently, future perspectives could be:

Registry studies:

- Investigate the reliability of the described DR screening diagnoses at both hospitals and private ophthalmologists in other regions in Denmark and try to enhance the methodology even further.
- A national evaluation of DR screening adherence with focus on long-term DR screening dropouts and never attendants.
- Influence of socio-economic factors difference in patients screened at hospitals versus private ophthalmologists.

AI challenges:

- Additional performance studies comparing the current software with, i.e., EyeArt [246] which does five stage ICDR grading.
- Increasing the size of the reference dataset with additional fundus photos for testing as especially ICDR grade 3 and 4 are sparsely represented.
- Developing a deep learning algorithm for DR screening based on the local database in the North Denmark Region.

Literature studies:

- Expand the current review to a systematic review of current treatment options for DME, side effects and risk of reported bias.
- A systematic review of current performance of commercially available DR AI systems used in clinical care.
- A qualitative study of why T1D patients attend DR screening more frequently than T2D patients.
- A descriptive study of challenges introducing AI for DR screening to a universal healthcare system.

As AI development for assessing DR is progressing quickly, it is becoming more relevant to assess whether AI should be implemented in a national screening programme and to what extent. The challenge with some of the more advanced AI solutions is the threshold for referral of DR the AI has been trained on. In Denmark, the threshold for referral is sight-threatening DR which includes PDR and CSME

[198]. The current AI solutions have not been trained on this threshold but typically on a lower threshold such as moderate NPDR [190,199,257,262]. This probably leads to a significant increase in referred patients. As Denmark has a small relatively homogenous population, a long-term vision would be to establish a common screening database where all fundus photos including OCT and paraclinical data from hospitals and private ophthalmologists are gathered to obtain a large database for collective research and development. This would potentially make the database large enough to train a deep learning AI algorithm which would perform well enough to transfer work from clinicians. This has been proposed by Xie et al., for a DR screening programme in Singapore [263] where a fully automated system is proposed and under development.

The global health cost of nearly 100% screening would be massive due to the workforce needed. Currently deep learning is on the rise and will hopefully relieve clinicians from most of the tedious tasks of DR screening. This would allow for the prioritisation of workforce resources for treating the ophthalmological complications related to the diabetic retinopathy in late stages. If fewer patients are to experience complications related to DR, more patients with diabetes need to be enrolled into the national screening programme in Denmark as perhaps just above 70% known and unknown patients with diabetes were included [143]. This should not only be on confined to single countries but preferably be expanded worldwide.

15 Summary in English

15.1 Aims

The aims of the present thesis were to 1) investigate the diabetic retinopathy (DR) screening incidence in a regional population, 2) assess an AI software for screening of no DR vs DR in a clinical setting and finally to 3) do a literature review of the current medical treatment options of diabetic macula oedema (DME) and their side effects and safety profile.

15.2 Methods

1) Trends in DR screening incidence and covariates at a regional level were examined in an epidemiological study over ten years using data from the Danish National Prescription Registry, the Danish National Patient Registry, the Danish National Health Service Registry and a local database. Validation of screening at hospitals was performed by comparing to a local database.

2) The investigation of an AI software to detect presence or absence of DR was conducted by establishing a reference standard according to an international DR classification by comparing the software with the reference standard at different thresholds and comparing the daily routine screening with the reference standard. Conger's Exact Kappa was calculated for intergrader agreement and intraclass correlation coefficient was calculated for intragrader agreement.

3) For the investigation of treatment options in DME and its side effects, the literature was searched using public research and clinical trial databases as well as national and international medical agencies databases.

15.3 Results

1) An increasing trend in proportion of patients with DR were observed during the ten-year period with a higher proportion of all types of diabetes attending the DR screening if performed at hospitals rather than private ophthalmologist. Mean positive predictive value for screening visits at hospitals was 86.8%.

2) The investigation of the AI software showed comparable results as daily clinical routine screening when both were compared to a reference dataset. The intergrader variability (ICDR grades) for three graders was Kappa: 0.731. Intragrader variability,

and intraclass correlation coefficient: 0.81 and 0.90, respectively, for the two graders (ICDR grades).

3) A table describing making an overview of the treatment available was created. Three medical modalities for treatment of DME exist where anti-VEGF's shows superiority compared to corticosteroids and laser regarding clinical effects and side-effects.

15.4 Conclusions

1) In general, a high DR screening attendance was observed with an increasing trend which suggests growing adherence. Patients with T1D and females are the most adherent patients in the screening programme. By location, hospitals seems to maintain the patients best whether being patients with T1D or T2D. The validation of screening visits at hospitals was showed a high positive predictive value.

2) Software assessing DR can be used in a daily clinical setting if proper clinical testing is performed and limitations of the software are noted and accounted for in implementation. Inter- and intragrader variabilities were at a satisfactory level.

3) The safety profile for the drugs described are generally acceptable especially regarding anti-VEGF's, which also provide the best increase in vision. Corticosteroids have issues with high intraocular pressure and development of cataract. Laser therapy for DME primarily has its place in low-income countries where treatment cost is important.

16 Resume på dansk

16.1 Formål

Formålet med afhandlingen var 1) at undersøge den regionale screenings incidens for diabetisk retinopati (DR), 2) at undersøge hvor god en AI software var til at distingvere DR fra ikke-DR i en klinisk sammenhæng, 3) samt en litteraturgennemgang af den nuværende tilgængelige behandling for diabetisk makulaødem med fokus på behandlingernes bivirkninger og sikkerhedsprofil.

16.2 Metoder

1) Tendenser for screeningsincidenser for diabetisk retinopati og kovariater blev undersøgt i et epidemiologisk studie hen over en tiårig periode med data fra det danske recept-, patient- og sygesikringsregister. Desuden blev validering af screening på sygehuse udført via sammenligning med en lokal database.

2) Der blev etableret en referencestandard i henhold til et internationalt DR klassificeringssystem. Dette blev gjort for at undersøge om en software kan skelne mellem tilstedeværelsen eller fraværet af DR på fundus foto. Softwaren blev undersøgt ved forskellige tærskelværdier via sammenligning med referencestandarden. Resultaterne fra den daglige kliniske screening blev også sammenlignet med referencestandarden. Conger's Exact Kappa blev udregnet for interobservatør og intraclass correlation koefficienter blev udregnet for intraobservatør enighed.

3) Litteraturen vedrørende behandlingsmuligheder for diabetisk makulaødem, bivirkninger og sikkerhedsprofil blev gennemgået via søgninger i kliniske forsøgsdatabaser og forskningsdatabaser samt i nationale og internationale sundhedsmyndigheders databaser.

16.3 Resultater

1) En stigende andel af patienter (både diabetes type 1 og type 2) med DR blev screenet hen over en tiårige periode. Patienter var mest tilbøjelige til at blive screenet, hvis screeningen var på et hospital. Den gennemsnitlige positive prædiktive værdi for screening på et hospital var 86,8% sammenholdt med den lokale database.

2) Undersøgelsen af AI softwaren viste sammenlignelige resultater i forhold til den kliniske screening når begge blev holdt op imod referencestandarden. Interobservatør variabiliteten for tre bedømmere var: Kappa 0,731. Intraobservatør variabiliteten (for ICDR-graderinger) viste intraclass correlation koefficienter på henholdsvis 0,81 og 0,90 for to bedømmere.

3) En oversigt over de tilgængelige behandlingsmuligheder for diabetisk makulaødem blev fremstillet i tabelform. Overordnet er der tre behandlingsmodaliteter, hvor anti-VEGF viser sig bedre end laser og kortikosteroider hvad angår klinisk virkning og bivirkninger.

16.4 Konklusioner

1) Generelt set blev der observeret en høj screeningstilslutning med stigende trend, der beskriver øget tilslutning til screeningsprogrammet. Patienter med T1D eller kvinder er de to grupper med højest tilbøjelighed for at blive screenet. Hospitaler ser ud til at have en højere screeningstilslutning end privat praktiserende øjenlæger. Valideringen af screeningsbesøg på hospitaler viste en høj positiv prædiktiv værdi.

2) Software der kan vurdere DR kan bruges i klinisk praksis, hvis den er klinisk testet og dens begrænsninger er velkendte og taget højde for i implementeringen. Interobservatør og intraobservatør varianserne var tilfredsstillende.

3) Sikkerhedsprofilen var generelt acceptable for de inkluderede lægemidler. Specielt vedrørende anti-VEGF. Anti-VEGF giver generelt set også den bedste øgning i synsstyrke. Kortikosteroider har udfordringer med højt intraokulært tryk og udvikling af katarakt. Laserbehandlings anvendelse som primærbehandling for makulaødem, er bedst bevendt i lavindkomst lande hvor pris er en faktor.

17 Literature

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A cohort follow-up study for diabetic retinopathy screening incidence in the North Denmark Region

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Abstract

Aims:

To evaluate diabetic retinopathy (DR) screening incidence in a universal healthcare system.

Methods:

Registry-based cohort study based on a Danish regional population from 2009-2018. Individuals with diabetes were identified by medication. Screening attendance was estimated by surrogate measures using local and nationwide databases reported by cumulative incidence.

Results:

18,832 patients were included. By the end of the first year, the cumulative incidence of screening for DR was 60.2% and by the end of the second year 74.2%. The cumulative incidence was 93.9% overall, 97.7% for patients with type 1 diabetes (T1D) and 93.4% for patients with type 2 diabetes. Screening proportions per one, two and five years were calculated. Females, patients with T1D, and patients attending screening at hospitals had a higher Hazard Ratio of 1.084, 1.157, and 1.573, respectively. The Cochran-Armitage trend test indicated increased screening frequency from 2009 to 2018. Validation of DR screening was done at hospitals with a mean positive predictive value of 86.78%. Cumulative incidence curves showed a small right shift when censoring the first, second and third screening visits.

Conclusion:

Nearly all patients were screened for DR over a five-year timespan. Female patients with T1D who attended screening at hospitals were significantly more likely to be screened. Validation of screening visits at hospitals was reported with a high mean positive predictive value. Most other studies, to the best of our knowledge, only report screening attendance for patients already enrolled in a DR screening programme. This study describes the overall screening attendance for the total eligible diabetes population.

Keywords:

Screening incidence, diabetic retinopathy, diabetes.

Introduction

The prevalence of diabetes is increasing worldwide [1], and consequently the prevalence of diabetic retinopathy (DR) is also rising [2]. Patients with diabetes should thus have regular checks for DR so that early signs can be identified before the patient experiences any visual disturbances. Early detection and treatment of DR are important as they ensure the prevention of permanent damage occurring in the retina [3]. The global prevalence of DR is just above 20% according to a systematic review from 2021 [4]. In 2019, the worldwide diabetic population worldwide was 537 million adults. This number is expected to rise to 783 million by 2045 according to the International Diabetes Federation [1]. In 2019, the actual number of people with DR was 119 million. If the prevalence of DR remains the same, this number will be around 174 million in 2045 [4, 5].

Screening for DR is a simple procedure involving standard ophthalmological examination techniques such as fundus photo, ophthalmoscopy and/or optical coherence tomography. Signs of retinopathy, such as small dot haemorrhages, microaneurysms, hard exudates, cotton wool spots and neovascularisations are only detected via screening, and patients rarely experience a change in visual acuity. Only severe late-stage DR, such as macula oedema, bleeding due to neovascularization or retinal detachment due to fibrous proliferation impair visual acuity to a degree that the patient will notice, and early stages can therefore only be detected via screening. Despite the simplicity of screening and the benefits of early detection, it is well known that the rate of patients being screened for DR is not very high. The American Academy of Ophthalmology (AAO) reports that only around 60% of patients with diabetes come for screening [6]. Patients who do not adhere to screening recommendations are at high risk of possibly ending up with permanent visual impairment due to severe and proliferative DR [7].

This paper aims to estimate screening attendance by cumulative incidence over a 10-year period (i.e., 2009-2018) for patients who attended a free national DR-screening programme at hospitals and/or at private ophthalmologists in the North Denmark Region. To the best of our knowledge, a study such as this has not been done before. From 2010-2017, patients in Denmark were generally given up to only two-year screening intervals depending on their retinopathy status [8]. Through the utilisation of Danish National Health Registries [9, 10], we identified individuals with diabetes (T1D and T2D) in the Danish National Screening programme for DR and validated the models for detecting screening based on general nationwide registries when compared to a local high-quality DR screening database. We also investigated the likelihood for screening with reference to sex, diabetes type and screening location.

Methods

Study design and population

In Denmark, every resident is assigned a unique personal civil registration number at birth which is linked across nationwide registries at an individual level. [10–12]. The healthcare system is free for all residents, and all service providers are required to report their services to the appropriate registries for reimbursement from the government thus incentivizing and ensuring high-quality data. Diabetes screening is

performed at hospitals (primarily patients with T1D) and private clinics (primarily patients with T2D).

Population:

The study was designed as a cohort study from 2009-2018. The North Denmark Region was chosen due to the presence of a high-quality database that addressed screening for DR which covered the region's screened diabetes population.

Registries used:

The Danish National Prescription Registry [13, 14] holds all information about the type of dispensed medicine, date that medicine is dispensed and number of prescriptions.

The Danish National Health Service Registry [15] contains records of all services performed in the private branch of Danish healthcare that are reimbursed by the government. The number of ophthalmologists not working in connection to the public system in Denmark is extremely low because individuals must pay for an otherwise free service.

The information about out-patient clinics can be found in The Danish National Patient Register which holds all information regarding hospitals visited, departments visited, length of stay, dates of stay, type of visit, diagnosis and procedures performed [10].

The North Denmark Region has a local high-quality DR screening database that contains fundus photos that have date stamps. This database was used for comparing and validating DR screening at hospitals.

Identifying people with diabetes

To identify the Danish diabetes population, the Anatomical Therapeutic Chemical Classification System (ATC) codes [16] for diabetes were used. For anti-diabetics with insulin and insulin analogues, MA10A was used. For anti-diabetics without insulin, MA10B was used. Some patients with T2D were also prescribed insulin or analogues but nearly all patients with T2D had at some point been prescribed a non-insulin. It is not recommended to prescribe hypoglycaemic agents for T1D according to official Danish guidelines [17].

T1D was defined as patients with redeemed prescriptions for insulin or insulin analogues, and those never having redeemed any prescription for non-insulins.

T2D was defined as prescriptions for non-insulin medicine and insulin.

Each patient had to have a minimum of two dispensed prescriptions within 180 days in order to be defined as having diabetes. The second prescription had to be dispensed in 2009 and was used as the inclusion date.

We filtered and discarded women under the age of 40 who only received Metformin (ATC-code A10BA02) to filter out patients with polycystic ovarian syndrome (PCOS) or endometriosis [18, 19].

Screening locations

By default, patients with T2D were screened at private ophthalmologists, and patients with T1D are primarily screened at hospitals. Patients with T2D were only eligible to be screened at hospitals if they were referred by a private ophthalmologist.

Identifying screening in a hospital setting

We defined screening performed at the hospitals as those registered with one ICD-10 code (UCXA). Only patients with diabetes and non-acute contacts were included from hospitals in the North Denmark Region.

Hospital screening - local high-quality DR screening database

At the beginning of 2000, a local high-quality DR screening database created to keep track of patients who require screening for DR was established in the North Denmark Region. The database consists of fundus photos, DR grades, biochemistry, date of visit and visual acuity. The database was linked to other registries by using the patient's civil registration number to compare the above-mentioned population in hospitals. The date of visit was extracted and imported to Statistics Denmark [20] to merge with the above-mentioned registries (4521 patients from the defined diabetes population were in the database). Validation of the screening at hospitals was done by comparing yearly visits in the database to the yearly visits identified through The Danish National Patient Register. This was done due to uncertainty of the definition of patients screened at hospitals in the registers.

Private ophthalmologist

Since 2015, individuals screened by private ophthalmologists have been registered with a specific service code (190112). As data in the registries are still sparse, an indirect estimation of screening by private ophthalmologists was done.

The definition of screening for DR by private ophthalmologists required a visit to a private ophthalmologist and a diabetes diagnosis as defined above (14470 patients had a visit at a private ophthalmologist during this period).

Identifying screening at both hospital and private ophthalmologists, and patients with no screening.

All datasets were merged to combine the information. Diabetes, sex, resident status, birth, death and screening per year were reviewed by one of the three sources of screening. Patients who moved out of the North Denmark Region during the period were censored, and the date of censoring was chosen as 30 June in the year of the move, as no data for the date of the move was provided. Patients who died were censored on the day of death. For calculating the overall cumulative incidence, the first date of screening was selected whether it was from the local database, hospital or private ophthalmologist. When cumulative incidence for a single screening location was calculated, the first date of screening recorded at the respective location was used.

Data analysis

Data management was conducted using the SAS Statistical Software package for Windows, version 9.4 (SAS Institute, Cary, NC, USA). Proc lifetest and the cumulative incidence function were also used. Gray's test was used to test the difference in the cumulative incidence curve (CIC) in multiple groups. The PHREG function was used for the cause-specific hazard ratios (HR) [21] and for calculating both the Chi^2 and Cochran-Armitage estimate. For bar plot, the Exact Binominal function (Clopper-Pearson) was used. Gray's Test for Equality calculated the cumulative incidence curves.

Results

Total population

From 1 January 2009 to 31 December 2018, a total of 580,515 individuals lived in the North Denmark region. Of these, 18,832 individuals (43.9% female) were included, as they redeemed at least two prescriptions related to diabetes with their second prescription being in 2009. Of the 18,832 individuals, 2,627 (13.9%) were diagnosed with T1D (Table 1), and 16,205 individuals (86.1%) were diagnosed as having T2D.

The median age differs in T1D and T2D and is reported in Table 1 along with the Interquartile range Q1-Q3 (IQR).

		Sex		Total
Baseline 2009		**Female**	**Male**	
T1D	Participants	1112	1515	2627
	Percent	13.43%	14.36%	13.95%
	Median age (IQR)	46.15 (30.64)	45.16 (29.02)	45.67 (29.74)
T2D	Participants	7168	9037	16205
	Percent	86.57%	85.64%	86.05%
	Median age (IQR)	67.77 (18.36)	64.72 (16.41)	65.91 (17.33)
Total	Participants	8280	10552	18832
Age at death				
T1D	Median (IQR)	74.4 (22)	69.8 (25.3)	71.62 (23.3)
T2D	Median (IQR)	82.99 (13.35)	77.99 (14.38)	80.19 (14.5)

Table 1 *Epidemiological characteristics of the overall included population. Interquartile range (IQR) Q1-Q3. Kruskal-Wallis test for significant difference in medians between Age at death of T1D vs T2D: p <0.0001*

Cumulative screening incidence

From 1 January 2009 to 31 December 2018, a total of 18,832 participants were eligible for screening. The mean age of death and IQR for T1D and T2D are reported in Table 1.

By the end of the first year, the cumulative incidence was 60.4% (95% CI: 59.8 - 61.1); and by the end of the second year, the cumulative incidence rose to 74.2% (95% CI: 73.6 – 74.8). At the end of the ninth year, the cumulative incidence was at 93.9%

(95% CI: 93.4 – 94.3), which expresses the fraction of the eligible population that had seen an ophthalmologist (Fig 1, 'Overall') and thereby were defined as having *been screened for DR.*

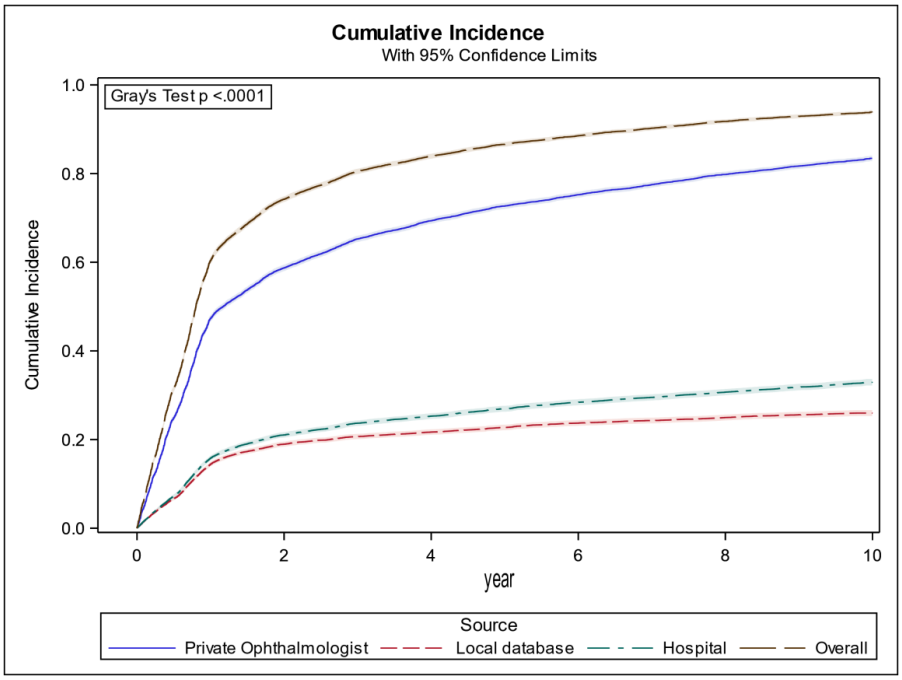

Fig. 1 *X-axis: Years from 1 Jan 2009 and onward. Y-axis: Cumulative Incidence of patients who had seen an ophthalmologist. 'Overall' is the overall cumulative incidence for all data sources. 'Hospital' is the cumulative visits at a hospital. 'Private Ophthalmologist' is cumulative visits with a private ophthalmologist. 'Local database' is the cumulative known screening visits at a hospital.*

Strata by sex and diabetes type

Direct readings from the cumulative incidence function estimates with strata on sex and diabetes for the first and second year and for diabetes for the fourth and ninth year (Fig. 2).

At the end of the first year, 70.0% (95% CI: 67.1 – 72.6) of females and 67.7% (95% CI: 65.2 – 70.0) of males with T1D versus 61.2% (95% CI: 60.0 – 62.4) of females and 57.3% (95% CI: 56.3 – 58.3) of males with T2D had been screened.

At the end of the second year, 85.8% (95% CI: 83.5 – 87.7) of females, 83.9% (95% CI: 81.9 – 85.7) of males with T1D versus 74.2% (95% CI: 73.1 – 75.2) of females, 71.1% (95% CI: 70.2 – 72.1) of males with T2D had been screened.

At the end of the fourth year, the CIC (both females and males) showed 91.5% (95% CI: 90.3 – 92.5) of patients with T1D and 82.7% (95% CI: 82.0 – 83.2) of T2D patients had been screened.

At the end follow-up and the ninth year, (both females and males) 96.8% (95% CI: 96.0 – 97.6) of patients with T1D and 93.4% (95% CI: 92.8 – 93.9) of patients with T2D had been screened.

Females (T1D and T2D) had an HR (Table 2) of 1.084 (95% CI: 1.051-1.119) which means that females in general were more likely to be screened than males. HR for screening at hospitals for both T1D and T2D was 1.157 (95% CI: 1.100-1.217), which translates to patients who were screened at hospitals being more likely to be screened in general than those who went to a private ophthalmologist.

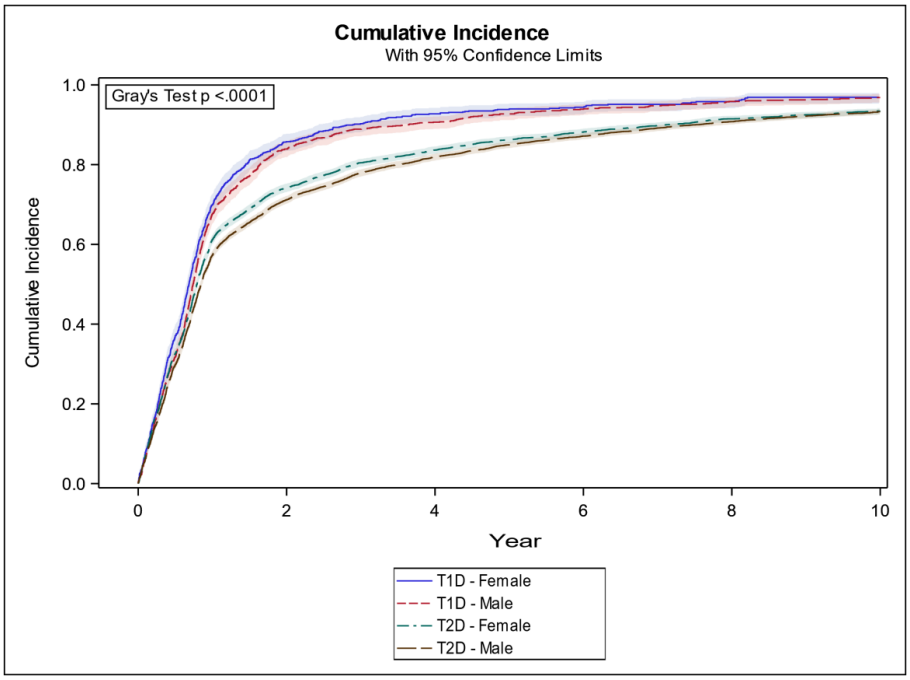

Fig. 2 *X-axis: Years from 1 Jan 2009 and forth. Y-axis: Cumulative Incidence of patients who had seen an ophthalmologist based on all data sources.*

Variable	Inclined towards	*Wald Chi²: p*	HR	HR 95% CI	
Diabetes	T2D<T1D	<.0001	1.157	1.100	1.217
Sex (T1D and T2D)	Male<Female	<.0001	1.084	1.051	1.119
Sex (T1D)	Male<Female	0.03	1.093	1.008	1.186
Sex (T2D)	Male<Female	<.0001	1.087	1.051	1.124
Age/decade (T1D, T2D)	Increasing age	<.0001	1.023	1.012	1.035
Age/decade (T1D)	Increasing age	<.0001	1.074	1.052	1.096
Age/decade (T2D)	Increasing age	0.25	1.007	0.995	1.020
DR location (T1D, T2D)	P.O.<Hospital	<.0001	1.573	1.510	1.639
DR location (T1D)	P.O.<Hospital	<0.001	1.642	1.504	1.792
DR location (T2D)	P.O.<Hospital	<.0001	1.560	1.490	1.634

Table 2 *Hazard ratio (HR) on covariates was calculated for the incidence curve (Fig. 1 and Fig. 2) to estimate the covariate effect on DR screening attendance. For the variable 'Diabetes', more patients with T1D than T2D were screened. CI: Confidence Interval. P.O.: Private Ophthalmologists.*

Strata by data source and diabetes

When stratifying by data source and diabetes (Fig. 3), few patients with T2D were seen at the hospital (T2D – Local database, T2D – Hospital) with a total cumulative incidence of 18.7% (95% CI: 18.1 – 19.4) and 25.9% (95% CI: 25.2 – 26.7), respectively. At the end of the study, 62.4% (95 % CI: 60.4 – 64.4) of the patients with T1D had visited a private ophthalmologist.

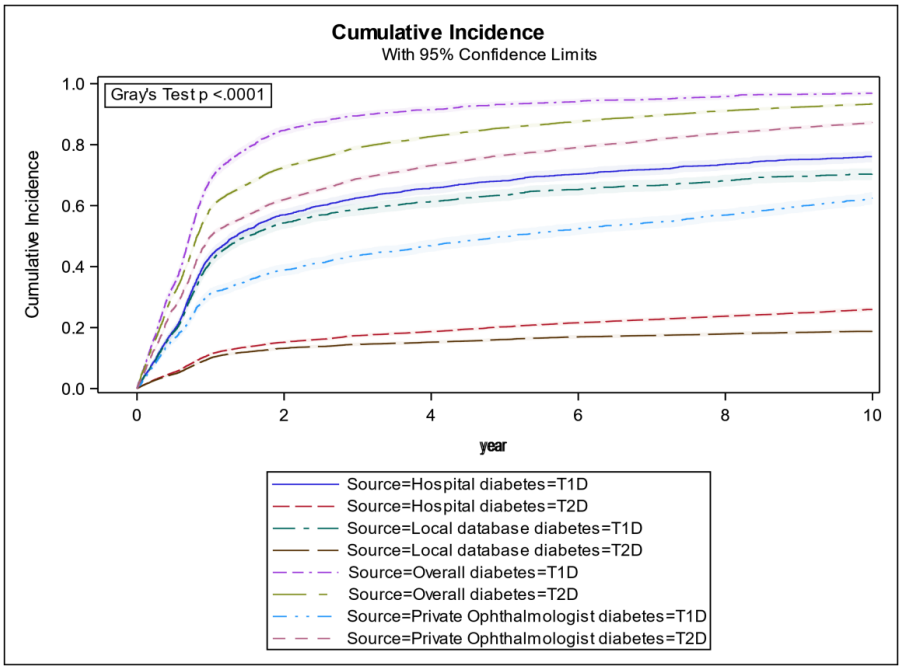

Fig. 3 *X-axis: Years from 1 Jan 2009 and forth. Y-axis: Cumulative Incidence of patients who have seen an ophthalmologist stratified on the respective data sources. 'Overall' is the overall cumulative incidence for all data sources. 'Hospital' is the cumulative visits at a hospital. 'Private Ophthalmologist' is the cumulative number of visits with a private ophthalmologist. 'Local database' is the cumulative known screening visits at a hospital. All data sources are stratified based on diabetes type.*

Screening incidence per year

The incidence proportion of the eligible diabetes population rose from 60.2% (95% CI: 59.5 – 60.9) per year (2009: 11,330/18,832) to 79.3% (95% CI: 68.5 – 70.1) per year (2018: 8,653/12,489)over the 10-year time span (Fig. 4). The Likelihood Ratio Chi^2 was 606.4, p <.0001 with nine degrees of freedom. The Cochran-Armitage Trend Test showed Z -23.8, and *One-sided* $p < Z$ .0001 which indicates a statistically significant positive DR screening trend from 2009 to 2018.

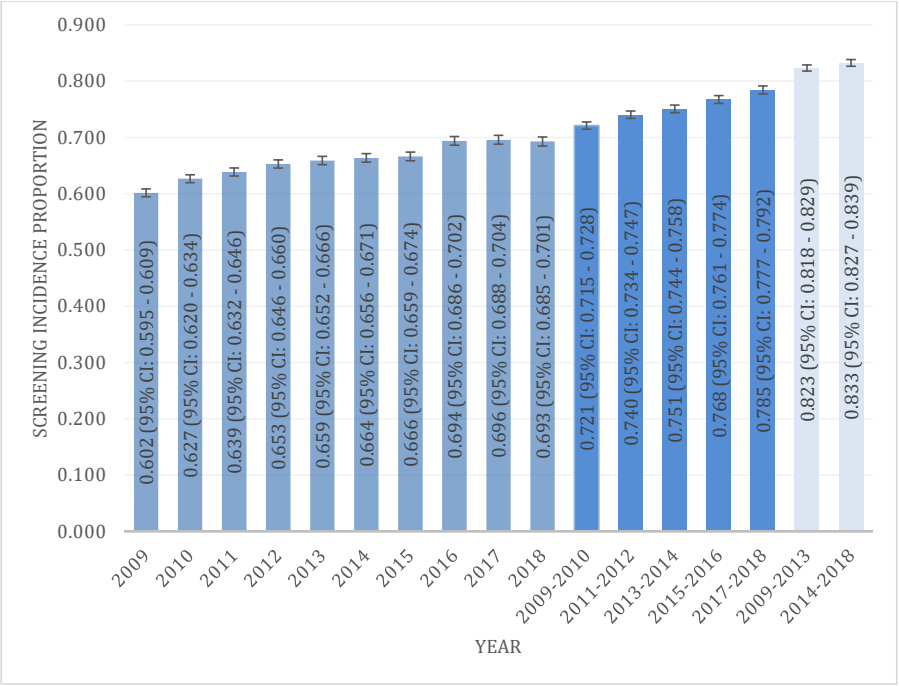

Fig. 4 *Cumulative screening incidence proportion of the screened vs non-screened population. The proportion of the screened population is shown by one-year intervals (medium blue), two-year intervals (dark blue) and five-year intervals (light blue). 95% confidence interval (CI)*

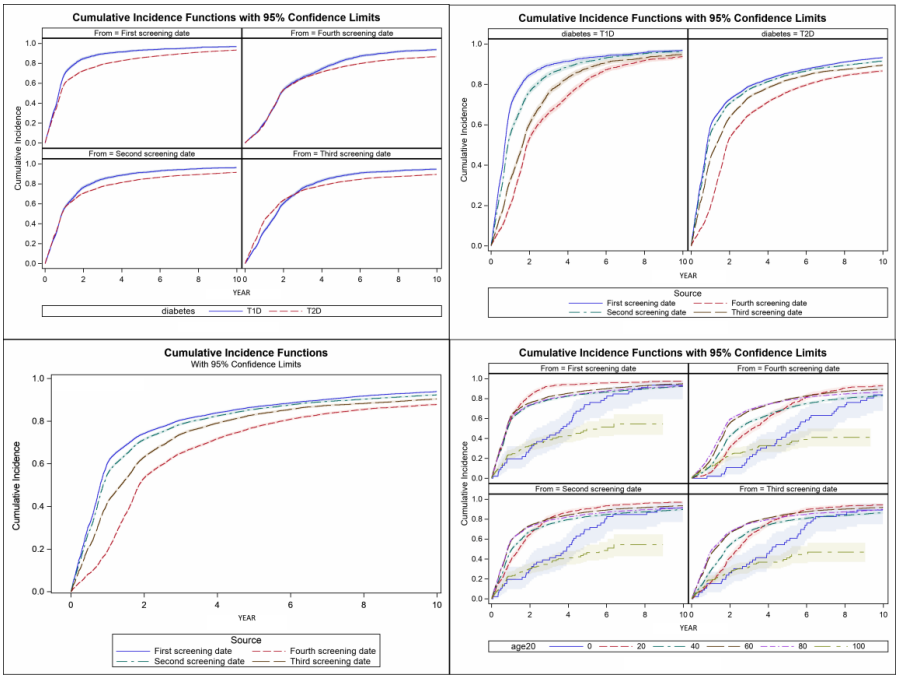

Fig. 5 *Cumulative incidences curves for screening, where the start date is varied from the first to the fourth screening date, whereby censoring up to the first three screening dates. Upper left: Stratified on diabetes type*

and screening date. Upper right: Screening date and diabetes type. Lower left: Screening date and combined diabetes type. Lower right: Age in 20 years intervals (age 20 = 20 years intervals) and screening date. Stratifying screening date (censoring first, second and third screening) shows a minor decline in the overall Cumulative Incidence. This indicates the Cumulative Incidence curve is not driven by only a few individuals. Age 0 (solid blue) containing individuals between age 0 and 19, and age 100 (stippled yellow), containing individuals between age 100 to 119, do have fewer screening visits. Age20 = 20 year interval with starting age marked.

Validation of methodology for finding DR screenings at hospitals

The mean positive predictive value (PPV) was calculated for the diagnosis of DR screening at hospitals versus ground truth, which was the local database, for the years 2009-2018. PPV: 86.78% (95% CI: 86.76 – 86.81).

Discussion

This large registry-based regional cohort-based study, which included more than 18,800 patients with diabetes, found a high cumulative DR screening incidence and increasing trend over 10 years with up to 78% attendance (2017-2018, fig. 4) by a two-year interval (as recommended by national guidelines).

We found an increase in screening attendance by year throughout the study (Table 2). Furthermore, we demonstrated that nearly all patients with diabetes see an ophthalmologist and more than 60% of patients with T1D are at some point seen by a private ophthalmologist. This is being done even though DR screening of patients with T1D is mainly performed at hospitals in Denmark.

Only about 74% of patients had seen an ophthalmologist two years after the study start, with a general discrepancy between patients with T1D and T2D being observed throughout the study. Screening guidelines for DR are outlined in guidelines from the International Council of Ophthalmology [5], but guidelines are not necessarily followed as shown above and across multiple studies in a Cochrane review [22]. This may be due to individualised DR screening intervals or lack of patient compliance.

When adjusting for T1D and T2D, those who are diagnosed with T1D have a higher and steeper incidence curve (Fig. 2). Conversely, the curve for patients with T2D seems to flatten out after year one. We are not aware of other studies describing this phenomenon. Patients with T1D and females, in general, attended DR screening more often than patients with T2D and males in general. This was confirmed by the HR (Table 2) where females were significantly more likely to attend screening with an HR of 1.084 (95% CI: 1.051–1.124). The group with T1D also showed a significantly higher HR of 1.157 (95% CI: 1.100–1.217) compared to patients with T2D. Here it can be speculated that these patients in general with T1D have higher disease awareness than those patients with T2D. As reported by AAO in the introduction, only about 60% of patients with diabetes attend DR screening [6]. However, such data can vary from country to country [23, 24] due to the low-grade quality of evidence which is the result of the inconsistency reported. A Cochrane review from 2018 [22] with 329,164 participants (mainly from USA and Europe) reports 47.2% attendance with usual care and 58.0% attendance with intervention. The National Health Service in England reported 82.4% attendance from 2016 to 2017 [25]; however, it is unclear whether attendance was by the total diabetes population or just by the enrolled population. Our study, on the other

hand, includes the whole diabetes population and not just the population already enrolled in a screening programme.

Age by decade is not deemed a strong estimator of screening attendance in this study but is described as important in other studies [26, 27]. When HR for sex was stratified in T1D and T2D, there was still a slightly higher HR ratio for females/males with T1D than females/males with T2D who attended DR screening. In Fig. 5, we noticed the cumulative incidence rise towards a high endpoint at year 10 when varying from the first to the fourth available screening date. The general tendencies described in the discussion and results seem to be confirmed even when the first screening date is varied by censoring (Fig 5).

The location of the screening appears to influence screening attendance, regardless of whether patients had T1D or T2D. Both groups were significantly more likely to be screened if they were screened at hospitals, which to our knowledge has not been described elsewhere. This may indicate that patients who attend DR screening at hospitals get better patient education, are more informed or are followed up with on more than patients who attend DR screening at private ophthalmologists.

Patient disease awareness could be insufficient as reported by a study from Hong Kong [28]. Several studies [29, 30] focus on reasons for non-attendance (dropouts and never attendance). An overview of the total screening attendance of the Danish diabetes population does not exist, as The Danish Registry of Diabetic Retinopathy only reports patients who have been screened and not the total diabetes population [31]. Additionally, this is the first study describing cumulative screening incidence and proportion for a regional population in Denmark.

Strength and limitations

The strength of this study is the large number of patients, the availability of data from private ophthalmologists and the possibility to have a local database matched by civil registration number in order to get a more accurate estimation of patients who are screened at hospitals. Furthermore, the region is well-covered with screening sites and ophthalmologists. A high PPV for screening at hospitals was found when compared to the local database and could be used for further register studies on a nationwide level. The general trend was confirmed even when varying the first screening date, which implies that the results were not driven by a few individuals.

The limits of the study are the general limitations of using registries like the ones defining the diabetes population where it is known from the clinic that some patients redeem their prescriptions for large amounts of medicine for more than 180 days of use. Patients who do not redeem prescriptions for medication are not included.

A main challenge is how DR screening is defined. We cannot be sure that a visit to an ophthalmologist or hospital effectuates a DR screening. The validation of DR at hospitals may not be generalized at a national level due to the possibility of different coding practices. There is a slight overreporting regarding the way DR screening is defined at hospitals which can probably be explained by false positives due to the methodology.

Conclusion

This study of more than 18,800 patients in Denmark found an overall high DR screening attendance in the diabetic population including never attendants with a statistically significant increasing incidence trend. We found it important to report on patients who have never attended screening, as this might be a less highlighted subject in the literature. T1D patients, patients who attend screening at hospitals and female patients were statistically significantly more likely to be screened for DR. Males with T2D screened at private ophthalmologists were less likely to be screened. The validation of the method to find yearly screening visits at hospitals showed a high mean PPV but should be cautiously used in other regions in Denmark as there is a possibility of different coding practices. Censoring up to the first three screening dates (Fig. 5) did not change the general tendencies of the study.

Authors contribution

Funding

No funding was received to perform this study.

Compliance with ethical standards

Conflict of interests

None.

Ethical standards

The study was approved by the Danish Patient Safety Authority and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent

No need for patient consent is required in this type of study in Denmark. The study was approved by the Danish Patient Safety Authority, reference journal number 31-1521-136.

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Performance of a support vector machine learning tool for diagnosing diabetic retinopathy in clinical practice

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Abstract: Purpose: To examine real world performance of a support vector machine learning software (RetinaLyze) in order to identify possible presence of diabetic retinopathy (DR) in patients with diabetes via software implementation in clinical practice. Methods: 1001 eyes from 1001 patients—one eye per patient—participating in the Danish National Screening Programme were included. Three independent ophthalmologists graded all eyes according to the International Clinical Diabetic Retinopathy Disease Severity Scale with exact level of disease being determined by majority decision. The software detected DR and no-DR and was compared to the ophthalmologists' gradings. Results: At a clinical chosen threshold, the software showed a sensitivity, specificity, positive predictive value and negative predictive value of 84.9% (95% CI: 81.8-87.9), 89.9% (95% CI: 86.8-92.7), 92.1% (95% CI: 89.7-94.4), and 81.0% (95% CI: 77.2-84.7), respectively, when compared to human grading. The results from the routine screening were 87.0% (95% CI: 84.2-89.7), 85.3% (95% CI: 81.8-88.6), 89.2% (95% CI: 86.3-91.7), and 82.5% (95% CI: 78.5-86.0), respectively. AUC was 93.4%. The reference graders Conger's Exact Kappa was 0.827. Conclusion: The software performed similarly to routine grading with overlapping confidence intervals, indicating comparable performance between the two groups. Intergrader agreement was satisfactory. However, evaluating the updated software alongside updated clinical procedures is crucial. It is therefore recommended that further clinical testing before implementation of the software as a decision support tool is conducted.

Keywords: Machine learning, diabetic retinopathy, screening, RetinaLyze

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1. Introduction

Diabetes is a leading cause of severe visual impairment and blindness throughout the world. The prevalence of patients with diabetes has increased rapidly and was estimated to be 463 million in 2019, and it is estimated that this number will be as high as 783 million in 2045 with the highest percentage (79%) of patients living in low- and middle-income countries [1]. DR is reported to be the single most preventable form of blindness in the working-age (20-74 year) population of the United States alone [2,3]. The need for ophthalmologists and trained technicians required for screening is increasing worldwide, and current prognosis cannot be met earlier than 2040 [4,5]. Optimisation of the DR screening through the use of trained technicians and telemedicine with reading centres is still both cost- and labour intensive mostly because of the heavy draw on human resources. Automated computer guided decision support tools may reduce the need for skilled labour in this area.

Decision support tools may be implemented by the use of machine learning software implemented through artificial intelligence (AI) in an automated screening where

software either fully and independently grades images or partly grades or marks DR changes. Several commercial solutions are already available, and AI software for diagnosing or assisting in the diagnosing of DR is a growing industry. IDx-DR was among the first AI tools to be approved by the U.S. Food and Drug Administration (FDA) to automate detection of greater than mild DR [6]. Software analysis of retinal images has seen significant progress recently especially after the introduction of capable hardware and algorithms for applying a subtype of AI called deep learning [7]. The performance of deep learning compared to traditional machine learning is superior when applied to images, but it comes with some challenges. Specifically, deep learning algorithms need to be trained on huge, typically non-public, datasets with a sufficient variety of ethnic phenotypes.

DR scoring systems and equipment can vary from dataset to dataset which creates inconsistencies between lab performance and real-world performance. The differences in lab vs real-world performance are due to algorithms not typically being applied to the identical population as the one on which it was trained. The software may perform differently depending on the ethnicity of a person as this correlates greatly to the retinal pigment epithelium [8–10] as well as the digital fundus camera and number of retinal photos used [11–13] thus possibly causing misinterpretations by the algorithm. Despite these challenges, deep learning systems assessing fundus photos from patients with diabetes have shown high specificity and sensitivity in the laboratory compared to retinal specialists [14] when trained and implemented properly. In real world performance studies, only a few of the commercially available systems performed well (Algorithm G: sensitivity of 80.47% and specificity of 81.28% on a regraded sub dataset). This was probably caused by the difficulties previously mentioned as described by Lee et al [15].

The availability of digital fundus cameras has increased, and it has become more common to take multiple retinal photos [11–13] in DR screening to cover a wider area due to early stage DR changes in the periphery. This is a challenge for the generalisability of DR screening software as it has typically been developed on a dataset with its own characteristics, i.e., camera types and ethnicity, which may introduce bias if applied to another population. Clinical validation of software is therefore important if used in settings other than what the original development and validation intended.

Another subtype of machine learning software is support vector machine learning (SVML) which may be used to determine whether retinopathy is present or absent in an image. First described in 2003 by N Larsen et al and M Larsen et al [16,17], this software was developed based on two datasets with 137 patients with 260 non-photocoagulated eyes and 100 patients with 200 eyes. Both datasets used digitalised 35-mm colour transparency film with one 60-degree foveal fundus image per eye. The software has since been updated and was reintroduced to the market in 2013. Few if any studies have evaluated if the updated software can correctly detect the presence or absence of DR in a multi-image screening setting with five fundus images per eye.

The objective of this study was to evaluate the performance of the updated software from RetinaLyze A/S on a reference labelled dataset in order to compare performance of the routine grading to the reference dataset and to evaluate the inter- and intragrader performance of the reference labelled dataset, as the advantage with SVML is that the analysed results are easily explainable for the clinician as shown below.

2. Materials and Methods

Based on a power calculation, this study was performed on a new larger population than the original study's population. In our study, we deviated from the original studies [16,17] by utilising five images per eye instead of one image per eye. This divergence reflects the change in clinical procedures for DR screening in our current clinical setting where the updated protocol involves capturing five images per eye. We therefore found the software needs to be tested again due to more images per eye used today in the clinic

and because the software has been updated and is being used commercially in different locations.

The fundus photos used in this study were taken retrospectively from Steno Diabetes Center North Jutland's (Denmark) DR screening programme database acquired in a hospital setting from 2019–2020. A total of 1001 patients from the period were included. Each represented with one eye. The vast majority of participants were Caucasian. Other ocular comorbidities were not registered. A power calculation using McNemar's test was performed by two statisticians and showed a minimum sample size of 960 eyes with a power of 90% to detect DR and a delta difference of 5%. Inclusion criteria were a history of any form of diabetes with an ICD-10 DE11* - DE14* diagnosis and a total of five photos per eye—i.e., one fovea centred image, one papillary image and three peripheral images—as per hospital standards for screening. Patients with previous panretinal laser treated eyes were included in this study. In the following, we describe the dataset.

After obtaining the photos, routine grading was performed on the images by doctors. For the purpose of this study, additional steps were taken. First, the software assessed the photos, and then three certified retinal specialists performed the "gold standard" reference grading. Finally, the results from routine, software and reference grading were compared statistically.

More information regarding the photographic technique and software is found in Appendix A

2.1 Routine grading

The eyes were originally graded from 2019 to 2020, by a mix of 10 ophthalmologist consultants and senior registrars who had varying experience in clinical care. The International Clinical Diabetic Retinopathy Severity Scale (ICDR) [18] was used for grading. The scale is derived from the ETDRS study and is one of the most commonly used scales for grading DR [19,20]. Routine grading was performed prior to and without knowledge of the study.

2.2 Reference grading ("gold standard")

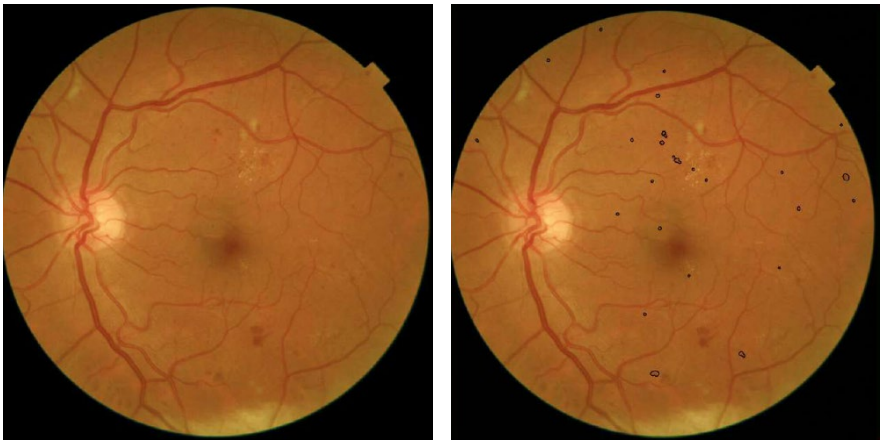
All 1001 eyes were reference graded independently using the free academic version of Labelbox [21] by three experienced ophthalmologist consultants from two different eye departments whose grading was used as a golden reference standard. The eyes were shown in random order, and each ophthalmologist was allowed as much time as needed for grading. All three ophthalmologists had passed a grading course prior to the study to ensure consensus and uniformity [22]. The five stage International Clinical Diabetic Retinopathy Disease Severity Scale [20] was used for grading, and the grading was done on all 5 images available per eye. Each eye was scored according to ICDR. Each eye had one fovea centred image, one papillary image and three peripheral images. The final grade for each eye was determined by majority vote. If there was disagreement between all three graders, the eye was discarded from the analysis.

2.3 Automated grading

The images were analysed by commercially available software from RetinaLyze A/S [23] (henceforth "the software"). The technology behind the software has been described thoroughly by Larsen et al [17]. No performance studies of the software have been performed since the reintroduction of the software to the market in 2013 other than a pilot-study [24,25]. The software has been slightly updated since its reintroduction with improved analysation time and the ability to grade an image as non-gradable.

The software marks the analysed red lesions, as shown on the central fundus image Figure 1b, compared to the original fundus image Figure 1a. It does not detect all red lesions as seen at the paramacular inferior bleeding. Each marking counts as one red lesion

detected on an image. All the red lesions on one image are summed to a single numeric value and summed with the numeric values from the other four images from one eye. The software is only capable of detecting red lesions and not other characteristics of DR such as hard exudates, cottonwool spots or neovascularisations. Further technical explanation is provided in appendix A.



(**a**) (**b**)

Figure 1. (a) No software interpretation present. **(b)** Software interpretation of red lesions marked with black circles.

2.4 Statistical analysis

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for routine grades vs reference grades and software grades vs reference grades. The threshold for the software was chosen according to an individual and a combined image grading strategy described more thoroughly in, Appendix A. The most clinically relevant results are included in this paper. The rest can be found in the Appendix.

As three graders had graded the entire dataset, the intergrader and intragrader variability was calculated using Conger's Exact Kappa for multiple graders and Intraclass Correlation Coefficients (ICC) type 2 for intragrader variability.

3. Results

Of the 1001 screened eyes, a total of 11 eyes were excluded due to lack of a majority decision among the retinal specialists. The retinal specialists further rated 10 eyes as ungradable due to cataract, asteroid hyalosis and insufficient image coverage of the retina.

The software described in this paper was developed for diagnosing any presence of DR and not for DR requiring treatment. The software rated 19 eyes as ungradable. The eye with asteroid hyalosis was rated as having no DR and not as ungradable by the software. One eye with cataract blur was rated ungradable by the software but not by retinal experts.

Six eyes were rated as ungradable by both the specialists and the software. One eye with good image quality, but with insufficient coverage of the retina, was excluded by the retinal specialists. A total of 34 eyes were excluded.

A total set of 967 eyes – 509 right eyes and 458 left eyes – from 967 patients were included for further analysis. Of the included patients, 730 patients had type 1 diabetes, 230 had type 2 diabetes, 9 had gestational diabetes and 29 had ICD-10 [26] diagnosis E13.* (Other specified diabetes mellitus) and E14.* (Unspecified diabetes mellitus).

Average grading time for each ophthalmologist was 44 seconds, 55 seconds, 77 seconds, respectively, with a total average of 58 seconds per eye.

3.1 Results at low threshold

The results with a threshold with ≥ 1 red lesions/eye from the software were compared to the reference graded dataset with 967 included eyes and are included in Table 1a. As the threshold of 1 revealed identical results regardless of individual or combined scoring, the numbers are only represented once.

Of the 18 eyes rated as false negatives by the software, seven were graded by the retinal specialists as mild DR, nine as moderate DR, one as severe DR with a large papillary confined haemorrhage and one as proliferative DR (active).

The software correctly identified 96.8% (95% CI: 95.3-98.2) of the patients with DR and 51.7% (95% CI: 46.8-56.6) without DR. The overall accuracy of the software was 78.0% (95% CI: 75.5-80.6). The positive predictive value (PPV) was 73.6% (95% CI: 70.5-76.8) and the negative predictive value (NPV) was 92.1% (95% CI: 88.3-95.4) (Figure 3). The result was achieved with the standard settings of the software. The area under the curve (AUC) was 93.4% with the ROC curve seen in Figure 2.

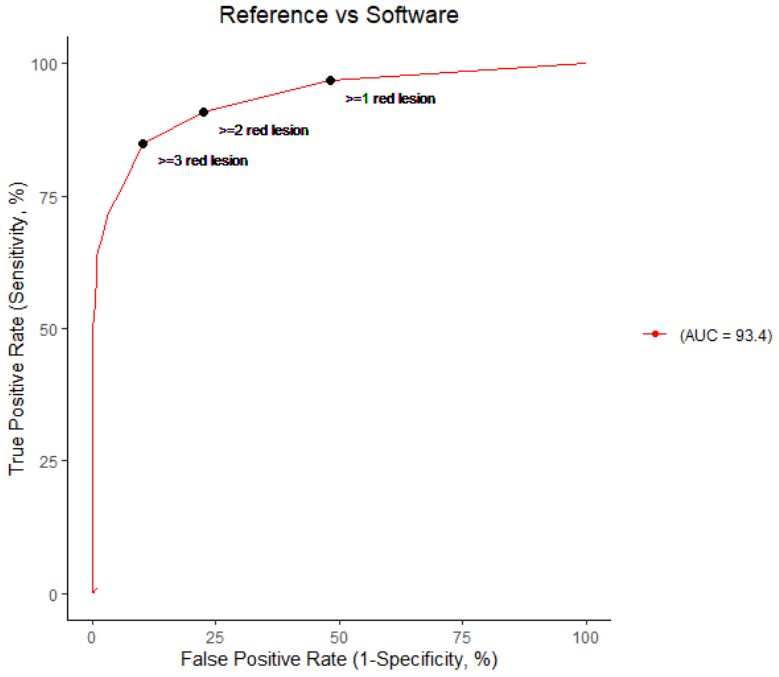


Figure 2. Performance of the software vs the reference labelled dataset illustrated on a Receiver Operating Curve (ROC) (red line) with an Area Under the Curve (AUC) at 93.4%. The three different thresholds for diagnosing DR according to the software are shown by the three filled circles. The combined image grading strategy is used for this ROC as the individual image grading approach would make a different ROC with a lower AUC for each increase in the red lesion threshold.

3.2 Reference grading vs routine grading

The reference graded eyes were compared to the gradings acquired through daily DR routine screening. This showed sensitivity at 87.0% (95% CI: 84.2-89.7), specificity 85.3% (95% CI: 81.8-88.6), positive predictive value 89.2% (95% CI: 86.3-91.7) and negative predictive value 82.5% (95% CI: 78.5-86.0) with an accuracy of 86.3% (95% CI: 84.1-88.4). 964 reference graded eyes were used to compare the routine gradings of the 964 eyes. For three eyes, routine grades were not available. See Table 1b and Figure 3.

Table 1.

	Reference		
Software	**DR**	**No DR**	**Total**
DR	545	195	740
No DR	18	209	227
Total	563	404	967

(a) Individual and combined grading. Error matrix of the reference grading vs automated grading by the software ≥ 1 red lesions per eye. Number of eyes.

	Reference		
Routine	**DR**	**No DR**	**Total**
DR	489	59	548
No DR	73	343	416
Total	562	402	964

(b) Error matrix of the reference grading vs routine grading. Number of eyes.

	Reference		
Software	**DR**	**No DR**	**Total**
DR	465	52	517
No DR	98	352	450
Total	563	404	967

(c) Individual image grading. Error matrix of the reference grading vs automated grading by the software ≥ 2 red lesions per eye. Number of eyes.

	Reference		
Software	**DR**	**No DR**	**Total**
DR	511	91	602
No DR	52	313	365
Total	563	404	967

(d) Combined image grading. Error matrix of the reference grading vs automated grading by the software ≥ 2 red lesions per eye. Number of eyes.

	Reference		
Software	**DR**	**No DR**	**Total**
DR	384	18	402
No DR	179	386	565
Total	563	404	967

(e) Individual image grading. Error matrix of the reference grading vs automated grading by the software ≥ 3 red lesions per eye. Number of eyes.

	Reference		
Software	**DR**	**No DR**	**Total**
DR	478	41	519
No DR	85	363	448
Total	563	404	967

(f) Combined image grading. Error matrix of the reference grading vs automated grading by the software ≥ 3 red lesions per eye. Number of eyes.

3.3 Results with higher thresholds

For individual image grading with a threshold ≥ 2 red lesions per eye, a sensitivity of 82.6% (95% CI: 79.4-85.6), specificity of 87.1% (95% CI: 83.8-90.3), PPV of 89.9% (95% CI: 87.3-92.4) and NPV of 78.2% (95% CI: 74.3-81.9) was shown. The accuracy was 84.5% (95% CI: 82.2-86.8). With a threshold ≥ 3 red lesions per eye, sensitivity dropped to 72.6% (95% CI: 68.9-76.3), but specificity went up to 95.3% (95% CI: 93.2-97.2). PPV and NPV were 95.6% (95% CI: 93.5-97.4) and 71.4% (95% CI: 67.6-75.2), respectively, with an accuracy of 82.1% (95% CI: 79.7-84.5). The results are shown in Table 1c, Table 1d and Figure 3

For combined image grading, a threshold ≥ 2 red lesions per eye showed a sensitivity of 90.8% (95% CI: 88.3-93.1), specificity of 77.5% (95% CI: 73.3-81.5), PPV of 84.9% (95% CI: 81.9-87.7), NPV of 85.8% (95% CI: 82.0-89.3) and accuracy of 85.2% (95% CI: 82.9-87.4). For a threshold of ≥3 red lesions, this setting resulted in slightly lower sensitivity at 84.9% (95% CI: 81.8-87.9), a specificity of 89.9% (95% CI: 86.8-92.7), PPV of 92.1% (95% CI: 89.7-

94.4), and NPV of 81.0% (95% CI: 77.2-84.7) with higher accuracy at 87.0% (95% CI: 84.8-89.0). Please see Table 1d, Table 1f and Figure 3.

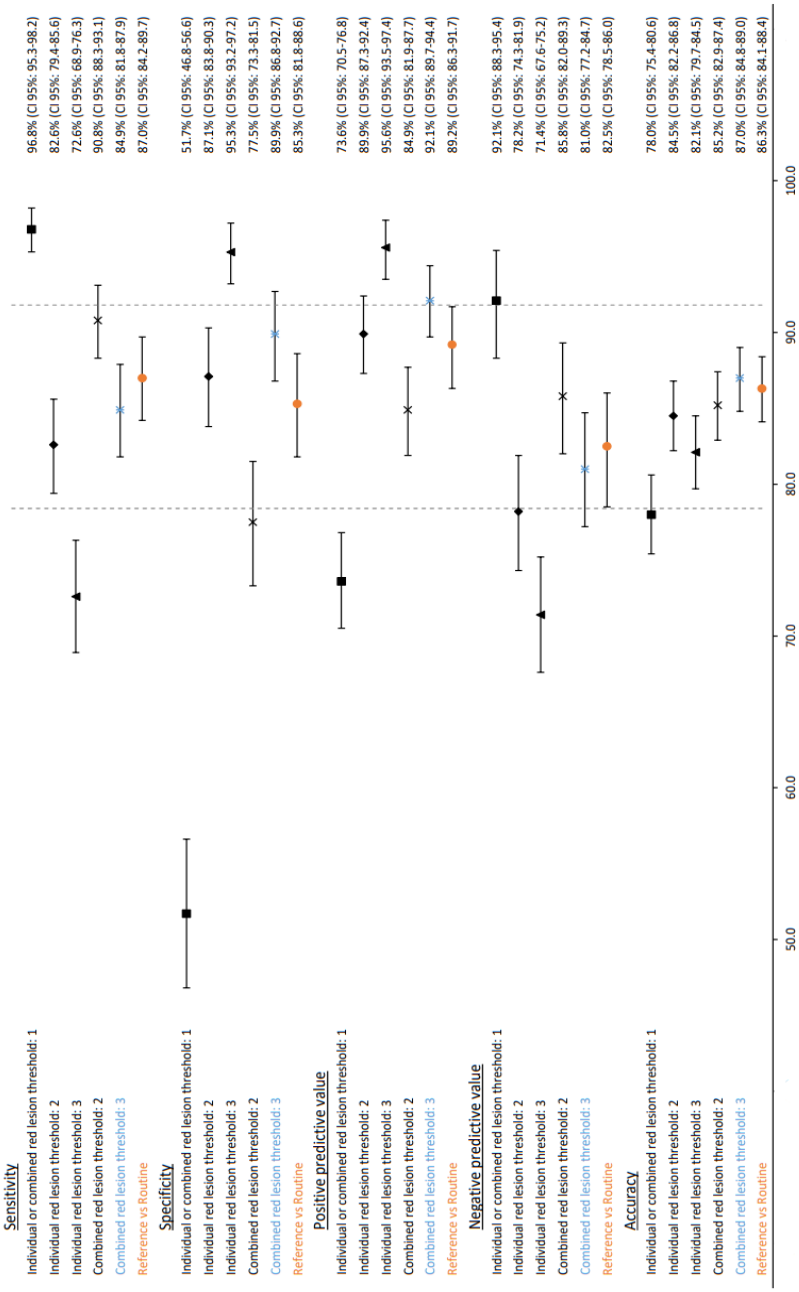


Figure 3. Forest plot of the different sensitivities, specificities, positive predictive values, negative predictive values and accuracy. X-axis is percentage. Individual image grading and combined image grading is as described in the methodology. The dotted vertical lines represent the outermost CI for the results for routine screening (orange). The best comparable threshold and strategy is the combined image grading with a threshold of 3 red dots (blue).

3.4 Grader variability

For the intergrader variability correlation between the three reference graders' gradings, the variability was calculated using the Conger's Exact Kappa [27] method for multiple graders. The Kappa (K) value ranged from 0 (no agreement) to 1 (perfect agreement). Values between 0.8≥ K ≥0.61 show substantial agreement. K>0.8 is almost perfect agreement [28]. For the ICDR grading, this resulted in a Kappa value of 0.731. The binary classification of the ICDR class 0 was defined as no DR and classes 1-4 were defined as DR. This resulted in a Kappa of 0.827.

Intragrader (X compared to Y) variability was available for two of the three reference graders. The two reference graders had previously screened 59 and 132, respectively, of the included eyes as part of the routine grading. The intraclass correlation coefficient (ICC2 (2,1)) was calculated for both graders. Grader Y ICC: 0.81 (95% CI: 0.72-0.88). Grader X ICC: 0.90 (95% CI: 0.86-0.92). ICC between 0.75 and 0.9 indicates good reliability, and ICC greater than 0.90 indicates excellent reliability [29].

4. Discussion

In this study, we demonstrated the ability of a red lesion detection software to detect the presence or absence of DR in a five-field fundus photo screening. We applied two different strategies. 1) Individual image grading where each of the five images' red lesion scores were set individually, and 2) combined image grading where the red lesion scores were summed for all five images. A red lesion threshold was then set to assess how software performance changes as the threshold changed according to the individual and combined image grading strategy. At the software base settings, only one red lesion in one of the five fundus photos per eye with a high sensitivity of 96.8% (CI 95%: 95.3-98.2) but low specificity of 51.7% (CI 95%: 46.8-56.6) was shown to have the same result for both the individual and combined image grading. We utilised a single red lesion as a threshold affects the specificity and thus leads to a higher number of false positives. At a threshold of 2 red lesions, the two strategies diverged. The individual image grading showed better weighted performance with higher sensitivity and specificity, but combined image grading strategy dropped only slightly in sensitivity but increased in specificity (Figure 3).

The most ideal approach would yield results that are comparable to the grading performed in the clinical routine. By using individual image grading as a strategy, we lost information for each ascending threshold. Our observations showed the best-balanced performance of the individual image grading was a threshold of a minimum of ≥ 2 red lesions per eye for categorising the eye as having DR (Figure 3). This strategy of reporting is inferior to the combined image grading strategy and should preferably be avoided as individual image grading strategy results are not all included in or better than the confidence intervals of routine grading as shown in Fig. 3.

In the combined image grading strategy, with a threshold of a minimum of ≥ 2 red lesions per eye for categorising the eye as having DR, the results were more comparable to the results from routine grading. The software had a better NPV compared to the routine grading 85.8% (CI 95%: 82.0-89.3) vs 82.5% (CI 95%: 78.5-86.0) which could be important if the software assists in diagnosing DR. At a threshold of ≥ 3 red lesions per eye, sensitivity was a bit lower than the sensitivity of routine grading, but specificity was greatly improved from 77.5% (95% CI: 73.3-81.5) to 89.9% (95% CI: 86.8-92.7) compared to a threshold of ≥ 2 red lesions per eye both of which were compared to the routine grading and resulted in fewer false positives.

As seen in Figure 3, the confidence intervals of the combined image grading strategy with a threshold ≥ 3 red lesions per eye either overlapped or were superior to routine grading in all of the five categories perhaps making this the best approach for clinical

practice. With an increased threshold to 3 red lesions per eye, the software operated at higher specificity compared to the reference grading. As there was no unambiguous difference in the confidence intervals, we cannot deny the values were the same or that there is a significant difference between the combined image grading strategy with a threshold ≥ 3 red lesions and routine grading. Figure 3 highlights a trade-off between specificity and sensitivity or PPV and NPV as the threshold for red lesions increases.

AUC was 93.4% (Figure 2) compared to reference grading which is decent and comparable to the AUC of the studies by M & N Larsen et. al. [16,17], who reported an AUC of 94.1%.

Compared to the original studies of the software from 2003 [16,17], the software achieved similar specificities, sensitivities and accuracies on a single central fundus photo. This study is however not directly comparable to the ones from 2003 by M Larsen et al. and N Larsen et al. because we used five fundus photos per eye and N Larsen and M Larsen et al. used one central fundus photo per eye. The disadvantage of using five fundus photos is an increased chance of false positives increasing with the number of photos taken and analysed by a red lesion detection tool due to overlap of the images and the possibility of a red lesion being counted twice by the software. Stitching the images together was also considered, but this had its own concerns, i.e., decreased image quality in the periphery and the fact that stitching can potentially cover areas which may not be analysed. Using five images per eye may also be an advantage as minor DR changes can show themselves in the periphery. These changes may not be observed if only a central fundus photo is recorded.

Compared to deep learning software, few larger comparison studies have been made to the best of our knowledge. Software tends to perform a bit under lab performance when evaluated on real-world data. In the study by Lee et al. [15], the best performing software of seven commercially available software included showed a sensitivity of 80.47% and specificity of 81.28%. The software was anonymised. This paper evaluated referrable DR where the threshold for referrable DR may vary from country to country. Lee et al. reported generally high negative predictive values (82.72–93.69%) and a large spread in sensitivity of 50.98–85.90% [15]. A direct comparison of the seven software included in the study by Lee et al. [15] is not feasible as the SVML in the study does not use referrable DR as a threshold but only categorises whether DR is present or not. The SVML software included in this study performs decently with a sensitivity of 84.9%, specificity of 89.9% and NPV of 81.0% (see Figure 3. Combined red lesion threshold: 3)

A strength of this study is that three independent ophthalmologists reviewed the dataset and made a majority decision on the ICDR grade. Furthermore, as the software was not developed on the fundus camera used for making the dataset, the results may be more generalisable to other fundus cameras as well. The explainability of the results is considered good as the software outputs an image with black lines around the red lesions (Figure 1b) which can easily be compared to the original photo (Figure 1a). This is an advantageous form of reporting as both the clinician and patient can easily understand what the software detects and why it scores as it does. Additionally, the software rated only 19 (1.9%) eyes as ungradable which is considered acceptable.

A major limitation of the software is that it lacks the ability to grade according to the ICDR scale. This is important to note as newer deep learning software have been able to accomplish this. Software that is supposed to be used for screening of DR should be able to distinguish DR according to the ICDR classification. Another limitation of this study is the dataset's size compared to other big data studies, and the dataset's heterogeneity with Caucasians as the primary ethnic group due to the heterogeneity of the population of Denmark.

The software has not been tested for performance on multiple photos per eye before this study. We argue that the tested software can be useful in a screening setting to sort between eyes with disease and without disease more easily or to replace specially trained personnel doing the coarse sorting at screening centres. The red lesion threshold for

diagnosing DR should be determined according to local requirements. The software has currently mainly been tested on a primarily Caucasian population and generalisability to other ethnicities is unknown.

The limits of the software are its the ability to detect papillary haemorrhages of both DR and moderate DR and not being able to grade the stages of the ICDR scale or make a cut-off for referable DR. To perform at its best, the software should ideally make a collective grade on all the five images per eye as shown in the combined image grading strategy.

The intergrader agreement was comparable to the literature [30,31] with a Conger's Exact Kappa at 0.731 (95% CI: 0.705-0.757) for three graders at the ICDR scale and at 0.827 (95% CI: 0.798-0.856) at binary grading. For the intragrader variability, the Kappa was calculated using ICC type 2 and showed ICC at 0.81 (95% CI: 0.72-0.88) for grader Y and ICC of 0.90 (95% CI: 0.86-0.92) for grader X which are considered decent scores[29].

5. Conclusions

The software exhibited similar performance to the original studies and demonstrated comparability to routine grading. Acceptable levels of intergrader and intragrader variability were observed. However, it should be noted that the software lacks the capability to grade according to the International Clinical Diabetic Retinopathy severity scale. For the software to be implemented as a screening tool, conducting local clinical validation and establishing regular quality control measures is crucial . The accuracy of software-generated reports should be carefully examined as indicated by the performance differences observed in both individual and combined image grading strategies. With the increasing prevalence and incidence of diabetes worldwide, there is a growing need for diabetic retinopathy screening to preserve visual health. Therefore, it is important to prioritise local ophthalmic resources for individuals most in need. Despite the limitations of software analysis, the progress and implementation of DR software analysis can be valuable. We acknowledge the development of deep learning software that offers higher AUC, specificities, and sensitivities is needed. Nevertheless, it is crucial to thoroughly test all software tools before their clinical implementation. In our opinion, clinicians may find it easier to interpret a mark around a red lesion compared to a heat map generated by deep learning software. Considering the SVML software used in this study, conducting this performance study was necessary for evaluating its clinical relevance.

Supplementary Materials:

Author Contributions: Conceptualization, Tobias Nissen, Henrik Vorum; methodology, Kristian Aasbjerg; software, RetinaLyze, Labelbox; validation, Katja Christina Schielke, Malgorzata Dawidowicz, Jakob Grauslund, Thomas Lohne Nørgaard; formal analysis, Tobias Nissen, Kristian Aasbjerg; investigation, Tobias Nissen; resources, Tobias Nissen, Amar Nikontovic; data curation, Tobias Nissen, Amar Nikontovic.; writing—original draft preparation, Tobias Nissen; writing—review and editing, Kristian Aasbjerg, Henrik Vorum, Peter Vestergaard; visualization, Tobias Nissen; supervision, Kristian Aasbjerg, Henrik Vorum, Peter Vestergaard; project administration, Kristian Aasbjerg, Henrik Vorum, Peter Vestergaard. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Danish Patient Safety Authority, reference journal number 31-1521-136, 6 March 2020.

Informed Consent Statement: No need for patient consent is required in this type of study in Denmark.

Data Availability Statement: Data is not available due to restrictions regarding anonymity.

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abstract of the paper was presented 28-30 October at the 2021 EASDec conference, European Association for Diabetic Eye Complications.

Conflicts of Interest: The authors of this paper have not been financially involved with RetinaLyze A/S during this study. Henrik Vorum was a former member of the Advisory Board of RetinaLyze A/S prior to this study.

Appendix A

A.1 Photography

All patients were dilated with phenylephrine 10% and/or tropicamide 1% prior to photographing. Vision was measured by a technician using an auto kerato-refractometer, Topcon KR-800S (Topcon Corporation, Japan), the colour image was recorded by Topcon Maestro 3D-OCT-1 (Topcon Corporation, Japan) with 45º degrees optic field at four different locations and uploaded to the local ImageNet i-Base for grading by an ophthalmologist. Usual time from photo taken to available to the clinician was around 10 seconds depending on local computer hardware and internet connection. The images were analysed in the cloud.

The images had a resolution of either 1960 X 1934 or 2032 X 1934 compressed in JPG format. None of the retinal images in this study were used to develop, test or validate the algorithm used for analysis. No pre-processing was done by the authors.

A.2 The Software

A.2.1 Grading

The software used a three-grade scoring system and graded each image separately giving them a grade dependent on red-lesion count: no DR (green and no red lesion annotations), possible DR (yellow 1-2 red lesion annotations) or DR present (>= 3 red lesion annotations). When comparing the result of the software to human graders, we combined the eyes with images with *possible DR* and *DR present* into one category (presence of DR) and kept the *no DR* category for eyes with no red lesions markings on any of the five images.

We used two different approaches to define DR from the software results: one where each image was assessed individually, and one where the results of all images were summed (combined score; sum of red lesions for all images). For each approach, we used a “DR present” threshold of 1, 2 or 3 red lesions per image (individual) and per eye (combined) and compared the result to the retinal specialists’ reference grading.

A.2.2 Red lesion / technique:

The software used a technique which found seed points in a fundus image and categorised it as a red lesion. A seed point is characterised by being a local minimum in the fundus image. Many local minima are located in the blood vessels. In order to exclude some local minima and thereby avoid classifying the blood vessels as red lesions, the software tracks the blood vessels and removes these local minima. The rest of the local minima are grown until they frame the potential lesion. Some of these potential lesions, are "false" lesions. This is caused by fundus images having a heavily varying backgrounds, e.g., areas with a visible nerve fibre layer or choroidal structures. In order to differentiate these false lesions, a visibility measure is used. The visibility feature is the average of the product between the gradient and the weighted angle between the direction of the centre of mass and local orientation. The remainder of the potential lesions are classified as red lesions. The software is only able to find red lesions and no other characteristics of DR such as neovascularisation, cottonwool spots and hard exudates.

A.3 The Software - Quality control:

The purpose of the Image Quality Measure was to automatically identify images of poor or moderate quality, which should not be diagnosed automatically by the system, without human evaluation. The measure quantified image quality in terms of gradient contrast, as measured by a robust coefficient-of-variation (CV) of the gradient magnitude. The heuristic idea was that visually, as well as in the automatic lesion detection algorithm, visibility of local features in the image was related to gradient magnitude. Large variation in the gradient magnitude, and hence a large CV, indicated that there was a significant difference between sections with small gradients ("background") and sections with large gradients (sections with features such as vessels or the optic nerve head). Images which had a low CV were generally dark or blurred and were therefore marked as ungradable. Eyes rated as ungradable by the software were excluded.

A.4 Thresholds:

A.4.1 Individual image grading

Three thresholds were chosen: ≥ 1, ≥ 2 or ≥ 3 red lesions detected in at least one of the five images in one eye. The software reports per image, and not per eye, which is why we include this approach.

At ≥ 1 red lesion, the software had to have no red lesions marked on all the five fundus images of an eye to mark it as 'no DR'. If just one red lesion was marked in just one of the five images, the whole eye was rated as 'DR'. At ≥ 2 red lesions, the software had to have zero or one red lesions marked on all the five fundus images of an eye to mark it as 'no DR'. If two red lesions were marked in just one of the five images, the whole eye was rated as 'DR'.

A.4.2 Combined image grading

The combined red lesions for the five images were summed. We calculated the sensitivity and specificity for three thresholds of the summed red lesions of ≥ 1, ≥ 2 and ≥ 3, respectively, for red lesions as thresholds. The different thresholds were made to test how they affected the sensitivities and specificities. Image overlap is addressed in the discussion.

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REVIEW ARTICLE

Biologic Therapy and Treatment Options in Diabetic Retinopathy with Diabetic Macular Edema

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Abstract: Proliferative diabetic retinopathy and diabetic macular edema can be a potentially sight-threatening disease if not treated correctly. It is directly correlated to the duration of diabetes and how well managed the patients' diabetes is. In the last 15 years, the treatment of diabetic eye disease has taken a quantum leap in methodology due to the group of biological agents named antivascular endothelial growth factor (anti-VEGF). The introduction of the first biological agent has revolutionized the treatment, not only in diabetic eye disease but also across most inflammatory eye diseases, causing leakage of fluid from the blood vessels *i.e.,* in age-related macular degeneration. The availability of these biological agents, despite their considerable costs, have significantly improved the outcomes measured in visual acuity compared to more traditional treatments of diabetic retinopathy in the form of sole laser treatment and glycemic control. The agents demonstrate a favorable safety profile, but if the rarest and most severe side effects occur, there is a potential total loss of vision.

This review aims to make an overview of the current pharmaceutical therapeutic options in the treatment of diabetic macular edema. This includes laser therapy, intravitreal steroids, and a primary focus on intravitreal antivascular endothelial growth factors.

Keywords: Diabetic macular edema, anti-VEGF, laser, corticosteroids, safety profile, treatment.

1. INTRODUCTION

Diabetes Mellitus presents in two forms; as a partly chronic autoimmune inflammatory disease (type 1 diabetes) and, for the major part, a lifestyle disease (type 2 diabetes) [1-3]. It causes well-known vasculopathies manifesting in nerve-damage, ischemic heart disease, increases the risk of stroke, and is also one of the leading causes of visual impairment in the world [4, 5], which is the focus of this review. In 2017, diabetes affected 430 million people worldwide and is expected to increase up to 629 million by the year 2045 with the major increase in Asia [6-9].

Patients with diabetes have elevated levels of HbA1c, which is a risk factor for diabetic retinopathy [10], one of the top three causes of visual impairment. By 2020, it is estimated that a total number of 237.1 million people will have a moderate or severe visual impairment. Uncorrected refractive errors account for most, about 127.7 million people, followed by cataract (57.1 million), age-related macular degeneration (8.8 million), glaucoma (4.5 million), and diabetic retinopathy (3.2 million). A smaller part is blind, estimated at 38.5 million in the year 2020, with cataract as a primary reason (13.4 million), with uncorrected refractive error (8.0 million), glaucoma (3.2 million), age-related macular degeneration (2.0 million), and diabetic retinopathy (0.4 million). Refractive errors and cataracts are total reversible conditions, if treated, and the top three conditions that are somewhat preventable are glaucoma, age-related macular edema, and diabetic retinopathy [11]. Another study reports that diabetic retinopathy is the single most preventable form of blindness in the working-age (20-74 year) people in the United States [12, 13]. Different studies use different compilation methods, hence the different results found in the literature in the area cover visual impairment and blindness.

The mechanism causing retinopathy is believed to be hyperglycemia, which results in oxidative stress, followed by tissue damage and dysfunction, especially in tissue with high demands of oxygen and nutrition such as the retina. Diabetic retinopathy, in the early non-proliferative form, is characterized by microaneurysms, hard exudates, intraretinal hemorrhages, venous bending, intraretinal microvascular abnormalities, and macular edema. Furthermore, a breakdown of the blood-retinal barrier [14] may result in intra- and/or subretinal edema, known as diabetic macular edema. Ultimately, it may develop into a proliferative form which, in addition to the characteristics of the non-proliferative form, promotes active neovascularization in the retina and thus includes the risk of retinal hemorrhage and edema [15].

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The patients do not experience any changes to their vision in the early stages of diabetic retinopathy, and, therefore, most countries have developed nationwide screening programs for it. In later stages and without treatment, the development of proliferative retinopathy and diabetic macular edema severely affects vision. The best way to avoid the progression of subclinical signs *i.e.,* small bleedings, is strict glycemic control regulated with diet and the right combination of glucose-lowering medicine, typically a combination of short- and long term insulin-like drugs. The prevalence of diabetic retinopathy is around 35.6% of all individuals with diabetes, of which 7.5% of patients have proliferative diabetic retinopathy, and 10.6% have vision threatening diabetic retinopathy, with the mean age being 58.1 years (3-97), median diabetes duration being 7.9 years (interquartile range 3-16), and median HbA1c being 8.0% (6.7%-9.9%) [16]. It is reported that the prevalence of diabetic retinopathy was the highest among African Americans and lowest among Asians. It is not reported which socioeconomic factors were confounders in the analysis. The prevalence of diabetic retinopathy, proliferative diabetic retinopathy, diabetic macular edema, and vision threatening diabetic retinopathy, generally increases with the duration of diabetes, increased levels of HbA1c, higher blood pressure, and type 1 diabetes vs. type 2 diabetes (77.3% vs 25.2%) [6, 16]. A recent (2020) large registry study shows an association of diabetic hereditary on the maternal side, and also second-degree relatives, supporting environmental factors influence on development in both type 1 and type 2 diabetes [17]. Other studies also have similar results [18].

Only diabetic macular edema and proliferative diabetic retinopathy are treated with ophthalmic interventions. With 425 million diabetics worldwide (2017) [19], diabetic retinopathy is one of the leading causes for severe blindness, ranked 7th at 1.06% [11] due to micro and macrovascular complications in the eye. It is the most common microvascular complication in diabetes. 1/3 of all patients with newly discovered type 2 diabetes present with diabetic retinopathy, and practically, all diabetes patients have some stage of diabetic retinopathy when they have had diabetes for 20 years, followed by peripheral nerve damage (30-50% of patients at some point), kidney disease (50% at some point, 1/3 of these progress from microalbinuri to proteinalbinuri, 1/3 remains with microalbinuri, 1/3 reverts to normal function), and vascular diseases understood as cardiovascular with a prevalence of 32.2% in patients with type 2 diabetes [20-24].

Diabetic retinopathy is graded according to the International Clinical Diabetic Retinopathy Disease Severity Scale, which is derived from the ETDRS study [15, 25] and is one of the most used grading scales. Other national grading scales exists as mentioned above, but are not described here. The Scale is divided into five categories, with increasing severity, from small aneurysms and microdots, to prolific diabetic retinopathy. The categories are as follows [26]: No apparent retinopathy, Mild Non-proliferative Diabetic Retinopathy, Moderate Non-proliferative Diabetic Retinopathy, Severe Non-proliferative Diabetic Retinopathy, Proliferative Diabetic Retinopathy. The prevalence of the different diabetic retinopathy types varies considerably from country to country and between patients with type 1 and type 2 diabetes. A large multinational review from 2012 with a total of 35 studies (1980–2008) provided data from 22,896 individuals with diabetes. The overall prevalence was 34.6% (95% CI 34.5–34.8) for any DR, 6.96% (6.87–7.04) for proliferative DR, 6.81% (6.74–6.89) for diabetic macular edema, and 10.2% (10.1–10.3) for VTDR. All DR prevalence endpoints increased with diabetes duration, hemoglobin A1c, and blood pressure levels and were higher in people with type 1 compared with type 2 diabetes [26]. Diabetic macular edema can occur in any stage of diabetic retinopathy and is described below as it can be seen as its' treatable special sub--disease.

1.1. Diabetic Macular Edema

The introduction of anti-VEGF medications has made a paradigm shift in the treatment of diabetic macular edema (DMO or DME) in the last decade, from laser photocoagulation to anti-VEGF. The first anti-VEGF to be approved to treat diabetic macula edema by the U.S. Food and Drug Administration was Ranibizumab in 2012, in the RISE/RIDE trail [27] and Aflibercept in 2012 in the VIVID/VISTA trail [28], although Bevacizumab has been in use off-label at least since 2006, and is not approved to treat ocular diseases by the FDA [29].

In the United States, 4% of patients over 40 with diabetes have diabetic macular edema [30], earlier studies have reported an even higher prevalence [31]. Macular edema occurs in both non-proliferative diabetic retinopathy and in proliferative diabetic retinopathy [7, 32]. In the ETDRS study [33], diabetic macular edema is a binary diagnosis represented as absent or present, along with a diabetic retinopathy grade from 1 to 5. The edema must be within 500 micrometers of the macular or inside the macula itself, to be called diabetic macula edema. It is called a clinically significant macular edema when it affects the vision of the patient. It is nowadays diagnosed *via* Optical Coherence Tomography (OCT), which uses light to make an image of the retina, where it is very easy to diagnose. If OCT is not available, a fundoscopy is used to see the edema in 3D . An edema is by definition present in a 2 dimensional image, if there are hard exudates in the retina within 500 micrometers of the macular [15, 25].

Diabetic macular edema is a consequence of elevated levels of diacylglycerol, which activates protein kinase C leading to fluid leakage from a blood vessel and macular edema [34, 35]. In a two year timespan, the vision of approximately 50% of patients with diabetic macular edema will be decreased with two or more lines >= 10 letters ETDRS [36]. In the treatment of macula edema, the first effective ophthalmologic treatment was laser photocoagulation applied to the retina. It stabilizes visual acuity for 3 years, but an improvement of visual acuity is uncommon (<3%), and 13% of patients lose 15 or more letters [25, 37, 38]. The loss of vision significantly decreases the quality of life and capability of disease management [39]. New treatments for diabetic macu-

lar edema have been developed since the introduction of laser photocoagulation. The two new main groups of treatment being anti-Vascular Endothelial Growth Factor (anti-VEGF) and corticosteroids.

The aim of this review is to summarize the current treatment with anti-VEGF, laser, and corticosteroids for diabetic retinal eye disease with a focus on diabetic macular edema.

2. METHODOLOGY

The literature concerning safety and adverse effects of medicines used in treating diabetic macular edema are described in many different papers. Some papers were only concerned with diabetic macular edema, while some were also concerned with edema caused by age-related macular degeneration, which is the largest disease in a number of individuals in which anti-VEGF is used, and the most research is performed in this field. To the best of our knowledge, only a few, if any, papers compare all the current on-market anti-VEGFs, or compare all steroids, or all anti-VEGF to all steroids. To the best of our knowledge, the most recent meta-analysis of anti-VEGF's was published in 2018, and the literature search for papers included was performed on 26 April 2017 [40], and, therefore, more recent papers as of May 2020 were not included in the review. Typically it was just two to three products that were properly compared in clinical trials, the majority being sponsored by the pharmaceutical industry [40]. Some papers on drug safety and adverse effects for anti-VEGF treatment in age-related macular degeneration are included in this review, due to the lack of papers solely describing the safety in diabetic macular edema patients, and the high similarity in dosage, and administration for these medications. The adverse effects in this review are classified as related to either administration of the drug or related to the drug itself.

The literature search was conducted during February 2020 on PubMed.gov, Cochrane.org, and clinicaltrials.gov with no limitation on the year of publication or language. The United States Food and Drug Administration (FDA) https://www.fda.gov/ and European Medicines Agency (EMA) https://www.ema.europa.eu/en/medicines online databases were also searched for drug information. Only peer-reviewed papers have been included in this review, besides the links to reports and webpages.

The Drug Bank [41] and the Flockhart tables [42] were searched for drug interactions and pharmacogenomic effects for the final included drugs.

The review focuses on clinically used drugs and phase III trials. The major search terms were the following: "anti-VEGF drugs", "VEGF types", "VEGF subtypes", "diabetic macular edema", "steroids treatment", "DME", "DMO", "AMD", "intravitreal injections", "Cochrane review", "corticosteroids", "efficacy", "side effects", "safety profile", "adverse effects", "serious adverse effects". The "anti-VEGF drugs" and "VEGF types" terms produced 3945 hits. This was further sorted by combining the above-mentioned terms mixed in various logical ways and also searched ad-hoc. The AMD term was added because there were not sufficient information solely on DME and efficacy and safety. A total of 148 different clinical trials were identified. Of them, a total of 21 major trials have been assessed as important in this review and are, therefore, mentioned by name. All trials mentioned by name have had power calculations, which have been met sufficiently for the task they have been calculated to fulfill.

3. THERAPEUTIC AGENTS OVERVIEW

An overview of the drugs, method of action, and possible adverse events are listed in Table **1**. Generally, the drugs had a similar safety profile. However, newer upcoming drugs may be given with greater intervals with the same or better clinical outcome, but this is not established knowledge.

Table 1. List of most relevant treatments (anti-VEGFs, laser types, and corticosteroids) used in ophthalmology for treating diabetic macular edema. All drugs are dispensed as intravitreal injections, except laser photocoagulation, which is applied to the retina and Kenalog, and administered to subtenon or retrobulbar. The dosage and frequency of administration of some drugs vary slightly between the FDA (US) and EMEA (EU) recommendations, and therefore, local guidelines should be used for treatment.

Name (*Commercial Name*)	Structure/Technology	Primary Target/Mechanism of Action	FDA and/or EMA approved for DME	Route, Dose, Frequency of Administration
Pegaptanib *(Macugen)* (outdated)	RNA aptamer	VEGF-A	NO, off-label use, outdated	IO, off-label use
Bevacizumab (*Avastin*)	Humanized IgG1	VEGF, FcRn	NO, off-label use	IO, off-label, 1.25 mg 0.05 ml every 4 weeks or by clinical status
Ranibizumab (*Lucentis*)	Humanized Fab/monoclonal antibody (mAb)	VEGF-A	YES	IO, 0.3 mg, 0.05 ml, every 4 weeks or by clinical status
Aflibercept (*Eylea*)	r-fusion protein	VEGF-A, B, PlGF	YES	IO, 2mg, 0.05 ml every 4 weeks or by clinical status
Brolucizumab *(Beovu)*	Monoclonal antibody (mAb)	VEGF-A	NO, pending approval,	IO, Phase-III for DME
Faricimab (Phase III)	CrossMAB	VEGF-A, Ang-2	NO, pending approval	IO, Phase-III for DME

Name (*Commercial Name*)	Structure/Technology	Primary Target/Mechanism of Action	FDA and/or EMA approved for DME	Route, Dose, Frequency of Administration
Conbercept	r-fusion protein	VEGF-A, VEGF-B,VEGF-C, PlGF	NO, pending approval for AMD	IO, approved in China, undergoing clinical trials in the EU for approval for AMD, and later DME and RVO.
Laser	Argon green (514 nm), dye yellow (577 nm), Krypton red (647 nm), and diode (810 nm) laser.	Peripheral retina, minimum distance to fovea is 2 papil size distance.	YES	Laser machine, individual choices, grid/-focal
(*Orzudex*)	Dexamethasone	Down regulating of cytokines	YES	IO 700 microgram implant, release over 1-3 months
(*Iluvien*)	Fluocinoloneacetonide	Down regulating of cytokines	YES	IO, 0.2 ug/day or 0.5 ug/day
(*Kenalog*)	Triamcinolone Acetonid	Down regulating of cytokines	NO, off-label use	Next to the eye, subtenon, retro bulbar

Abbrevations: DME: Diabetic Macular Edema. AMD: Age-related Macular Degeneration. RVO: Retinal Vene Occlusion. FDA: U. S. Food and Drug Administration. EMA: European Medicines Agency.

4. FIRST LINE OF TREATMENT

4.1. Anti-VEGF Therapies.

VEGF (vascular epithelial growth factor) in humans represents a highly potent group of molecules, which has been identified as the most important factor in several eye diseases, the most important ones being age-related macular degeneration and diabetic retinopathy with diabetic macular edema. VEGF is produced by cells that lack oxygen [43], including the cells in the retina, and is a crucial player in angiogenesis. The VEGF system consists of five types: VEGF-A, -B, -C, -D, and PGF (placental growth factor). VEGF-A is the most thoroughly described type of VEGF, with several isoforms [28, 44-46]. VEGF-A functions through VEGFR-1 and VEGFR-2, two high-affinity tyrosine kinase receptors. The ligand-receptors interaction stimulates the proliferation of endothelial cells, prevents apoptosis, stimulates endothelial cell mitigation, and angiogenesis [47-49]. VEGF-B placenta growth factor, and VEGF-A bind to VEGFR1 and VEGF-A, VEGF-C, and VEGF-D bind to VEGFR2 [50]. The receptors work in a close complex relationship with each other, which up and downregulates angiogenesis in complex intracellular pathways. In knock-out mice of either receptor, the embryos die at day 8-9 due to endothelial overgrowth (VEGFR1) or defective blood-island formation and vasculogenesis (VEGFR2) [50-52].

There are five types of commercially available anti-VEGF for intravitreal injections with different indications, including macular edema and proliferative diabetic retinopathy. Anti-VEGF works by blocking of VEGF receptors or VEGF like receptors, so VEGF cannot stimulate the growth of blood vessels. Even though some anti-VEGF drugs are only EMA or FDA approved for age-related macular degeneration, it is very common to use the medications off-label for diabetic or other eye diseases [53-56] with retinal edema or ischemia.

As all anti-VEGF drugs have similar action, *i.e.,* inhibiting the effect of VEGF released by cells undergoing oxidative stress, the administration in the form of intravitreal injection is identical, and overall assessment of common side effects related to either administration or method of action is described for all drugs in general. A more thorough review of each drug, as well as a comparison between different drugs and treatments, is found later in the section. The major difference between the products are their molecular structures and targets, half-life that effects the need for re-treatment, and clinical outcomes. The following sections describe the general contraindications, procedure-related side--effects, and the product, followed by a review of each anti-VEGF product.

General contraindications: Before giving an intravitreal injection, the health personnel must be certain that there are no ocular or periocular infections, *i.e.* any kind of keratitis, VZV infections, blepharitis, or any active intraocular inflammation such as uveitis. Hypersensitivity to the drugs is rare, but manifests as any anaphylaxis; rash, pruritus, urticaria, erythema, or intraocular inflammation [56-60].

General procedure-related side effects: The most common side effects related to the procedure are caused by the penetration of the bulbus with a thin needle, pain, and subconjunctival bleeding. The anesthetic drops are given so that intravitreal injection can be tolerated, which may cause increased tearing and dry eye syndrome, which is why patients should have artificial tears subscribed [61]. Furthermore, blepharitis has been reported more frequently by patients receiving anti-VEGF treatment [55]. The worst side effect that can happen is endophthalmitis, where the whole eye becomes infected, which potentially destroys the retina, leading to a severely impaired vision. The Per-injection rate of endophthalmitis is reported to be as low as 0.06% in one study [27]. Finally, retinal detachment is very rare but can happen due to the penetration of the bulbus. Without surgical intervention, retinal detachment will, in most cases, result in blindness [28, 55-58, 62, 63].

General product-related side effects: The risks related to the medicaments themselves are not very common. Some studies indicate a slightly increased risk of developing an epiretinal membrane, which is a benign condition [64, 65], causing none to mild visual impairment except in rare severe end-stages. Due to the potential systemic anti-angiogenic effect of anti-VEGF, the drug should not be dispensed to people with recent ischemic events in the past 3 months, especially with the smaller kilo Daltons (kDa) anti-VEGF molecules. In general, this is seen as a contraindication.

Smaller kDa anti-VEGF molecules seem to have a shorter half-life in the vitreous, as smaller molecules may easily leak out of the vitreous into the bloodstream. The elimination of the antibodies happens *via* binding to free endogen VEGF, passive renal elimination, and proteolysis. It is not possible to make a statement on the effect on the visual outcome or side effects based solely on the pharmacokinetic parameters [28, 55-57, 59, 62, 63]. In addition, arterial thromboembolic events have been observed in neovascular AMD anti-VEGF phase 3, multi-center, randomized, double--masked clinical trials in the HAWK and HARRIER studies (2019) [58], which is a further reason for cautiousness. The primary outcome for the HAWK and HARRIER trials were the visual outcome, for which the trials where sufficient powered to show. They have not been powered to show certain adverse effects. In prolonged anti-VEGF therapy, a systematic review in JAMA Ophthalmology indicated a possible increased risk for vascular events in patients who were already at high risk for vascular disease when receiving monthly treatment for two years, thus indicating that the cumulative exposure to anti-VEGF may be a risk factor [66]. The systematic review was performed according to the PRISMA guidelines with a predefined protocol, in which the patients should have been treated for a minimum of two years. Four double-blinded RCT studies were included (RISE, RIDE, VISTA, VIVID). The data analysis used Cochrane Collaboration guidelines, and RevMan 5.3 was used for the statistical analysis. The Grade of Recommendations Assessment, Development, and Evaluation system was used to assess the quality of evidence. The studies included were not powered to evaluate safety risks of uncommon events, and many trials excluded potential high risk cardiovascular patients.

In the following, each treatment anti-VEGF is described in detail in chronological order.

Pegaptanib (Macugen, OSI/Eyetech, Melville, NY, USA) is the first approved anti-VEGF for treating ocular diseases. Pegaptanib is an RNA pegylated aptamer. It binds to extracellular VEGF165, inhibiting VEGF165 from its receptors VEGFR-1 and VEGFR-2. Pegaptanib is a 40 kDa large molecule [53, 55, 67, 68]. It was FDA approved in 2004 [69] for neovascular wet age-related macular degeneration, but is now considered obsolete and replaced by non-selective anti-VEGF therapies. Pegaptanib is suggested to be administered with an injection with 0.3 mg every six weeks [55]. The average half-life in plasma in humans is 10 days (+/-) 4 days, in the vitreous 10.4 days [53, 55, 70]. It was approved by the EMA in 2006, but is not authorized any longer [71].

Bevacizumab (Avastin, Genentech, San Francisco, CA, USA) (off-label) Bevacizumab was first registered in 2006 in colon cancer treatment [72]. It is a humanized monoclonal antibody with an Fc region that binds all forms of anti-VEGF and FcRn. It binds to soluble VEGF, and inhibits VEGF to bind to Flt-1 (VEGFR-1) and KDR (VEGFR-2) on endothelial cells, suppressing the stimulation of angiogenesis. Elimination half-life is long, with 18-20 days in the plasma due to the Fc region, up to 50 days are reported, and 3-6.7 days in the vitreous [53, 73]. It is 148 kDa large molecule [53, 74]. As Bevacizumab is not approved by the FDA or EMA for the use in the treatment of ocular diseases, the dosage is based on experience, and the use is off-label. Common dose is 1.25 mg in 0.05 mL. It is, however, used in several trials [75, 76], and is suggested as the most cost-effective anti-VEGF compared to Aflibercept and Ranibizumab [75]. As a cancer drug, the adverse effects are many, and a french analysis of Bevacizumabs safety profile was performed, showing an increase in vascular events and related events such as "gastrointestinal perforation (4.8%), thromboembolic events (4.0%), pulmonary embolism (3.2%), hypertension (2.7%), gastrointestinal hemorrhage (2.7%), and cerebral hemorrhage or vascular accident (2.6%)" [77]. The safety profile of Bevacizumab in the use of ophthalmology is an issue due to the knowledge that the systemic exposure in ocular use is higher than that of Ranibizumab.

Ranibizumab (Lucentis, Genentech, San Francisco, CA USA) is a humanized monoclonal antibody, mAb. It is a recombinant humanized IgG1 kappa isotype fragment, which lacks an Fc region and is produced by an Escherichia coli expression system medium containing tetracycline, which is non-detectable in the final product. It is 48 kDa in size [53, 78]. Ranibizumab binds to VEGF-A and prevents the interaction of VEGF-A with VEGFR-1 and VEGFR-2 on the surface of endothelial cells, reducing neovascularization. Half-life in plasma and vitreous is approximately 9 days [53, 59]. Initially, 0.3 mg is administered for diabetic macular edema in the US, and 0.5 mg in the EU, as monthly injections [27, 79, 80]. It was approved by the EMA in 2011 to treat diabetic macular edema based upon the 12-month, randomized, double-masked, multi-center, laser-controlled phase III study RESTORE study, which compared Ranibizumab monotherapy, combined with laser or mono laser therapy [81]. Actually, Ranibizumab is FDA approved for the treatment of diabetic macula edema and several other intraocular diseases [59]. A recent study in the USA has shown that 5 to 10 years treatment of 0.5 mg Ranibizumab is considered cost-effective in the USA [82]. The 24-month single--masked, randomized, three-arm RETAIN study evaluated, as a part of it, the safety for Ranibizumab 0.5 mg with two cardiovascular deaths suspected to be treatment -related [83]. A meta-analysis of seven RCTs found no difference in the safety profile between Ranibizumab and laser [84].

Aflibercept (Eylea, Regeneron Pharmaceuticals, Tarrytown, NY, USA, and Bayer Healthcare, Berlin, Germany). A recombinant fusion protein. It is a dimeric glycoprotein with a weight of 97 kDa and contains glycosylation bringing it up to 115 kDa [53, 56]. It consists of portions of VEGFR-1 and VEGFR-2 extracellular domains fused to the Fc domain of human IgG1 [85-87]. Aflibercept binds both VEGF-A and placental growth factor, which is also a member of the VEGF family. The half-life in plasma is approximately 5-7 days [53]. The intravitreal half-life is 7.13 days in humans. It binds with a higher affinity to VEGF-A and its subforms than Ranibizumab and Bevacizumab [88]. It has been approved for several indications such neovascular wet

age-related macular degeneration, macular edema following retinal vein occlusion, diabetic macular edema [56, 89]. It is also approved for systemic treatment for metastatic colorectal cancer under the name Zaltrap. For diabetic macula edema, the recommended dose for Aflibercept is 2 mg, 0.05 mL, administered by intravitreal injection. The two major randomized, multi-centre, double-masked, controlled studies for the efficacy and safety of Aflibercept are the VIVID and VISTA studies. The VIVID and VISTA studies measured the efficacy outcome as a change in the Early Treatment Diabetic Retinopathy Study (ETDRS) Diabetic Retinopathy Severity Scale (EDTRS-DRSS), and the efficacy showed Aflibercept to be clearly statistically superior to the control groups which were treated with macular laser photocoagulation. The two arms receiving Aflibercept had the Best Corrected Visual Acuity mean gain from baseline to week 148 at 10.4 and 10.5 letters and 1.4 letters ($P < 0.0001$) gain in the laser control group in the VISTA trail. In the VIVID trail, the corresponding numbers were 10.3 and 11.7 letters for the groups receiving Aflibercept, and 1.6 ($P < 0.0001$) letters for the group receiving laser treatment [28]. Aflibercept has shown greater gains in visual acuity compared to Bevacizumab and Ranibizumab, especially with a poor visual acuity at the beginning of the treatment [75, 90]. It is the drug of choice when cost is not considered in several countries.

4.2. Studies comparing the safety profile among three on-market anti-VEGFs

The three most used anti-VEGF drugs for diabetic macula edema are compared to each other in the multi-center, randomized, interventional, single-masked (participant) Diabetic Retinopathy Clinical Research Network (DRCR.net) Protocol T study where the performance and safety for Bevacizumab, Ranibizumab, and Aflibecept were clarified. Sufficient power calculations were met for the primary outcome of change in the best visual acuity. The participants were a variety of ages, sex, and races. The study was divided into two groups with a visual acuity of less than 69 letters ETDRS or 69 letters ETDRS or more. Aflibercept and Ranibizumab demonstrated superior performance if the initial visual acuity was below 69 letters ETDRS, and if the visual acuity was 69 letters ETDRS or above, Aflibercept, Ranibizumab, and Bevacizumab performed the same [76, 91]. Aflibercepts' performance was significantly better if the baseline visual acuity was below 69 letters ETDRS in the one year follow up. In the 2-year follow-up [90], there was no significant difference in performance between Aflibercept and Ranibizumab regarding the visual outcome in the below 69 letters ETDRS subgroup. The RESTORE extension study, of two-year [92] and three-year [93] safety for Ranibizumab, demonstrated a higher incidence of cardiovascular events for Ranibizumab in the treatment of diabetic macular edema compared to Aflibercept and Bevacizumab, but this has not been reported in phase III, randomized, multi-center, double-masked, 3-year trials, sham injection-controlled for 2 years twin studies RISE and RIDE [27] or in the randomized, controlled, double-masked, multi-center phase II study RESOLVE study [94]. A Cochrane systematic review of only randomized controlled trials from the treatment of age-related macular degeneration comparing only Bevacizumab and Ranibizumab did not find any difference in safety between Bevacizumab and Ranibizumab [95], but due to different etiologies between the diseases (AMD and DME), the conclusion from the study might not also be valid for diabetic macular edema. Further evidence is needed to clarify the long-term safety differences for Ranibizumab, Bevacizumab and Aflibercept for the treatment of diabetic macular edema.

A Cochrane meta-analysis from 2018 found 24 relevant randomized, double-blinded, studies where 14 were industry-sponsored and 10 were non-sponsored [40]. Bevacizumab, Ranibizumab, and Aflibercept was investigated. All three anti-VEGFs showed improved visual outcomes. 3 out of 10 patients improved vision with more than three lines with Ranibizumab, and 1 out of 10 improved vision with more than three lines with Aflibercept. The common and serious adverse side effects were assessed, as there were no significant differences between the three drugs, and reported with moderate to high certainty. The cardiovascular comparison as heart attack and stroke are comparable with only very low-certainty evidence [40].

4.3. Anti-VEGF Products Pending Approval

Brolucizumab (Beovu, Novartis Pharmaceuticals Corp, NJ, USA) is a recombinant human vascular endothelial growth factor inhibitor and A humanized monoclonal single chain Fv antibody fragment. It is a 26-kDa large molecule. It binds with a high affinity for VEGF-A isoforms [58, 96]. Brolucizumab binds to VEGF-A, to the three major isoforms, and prevents binding to VEGFR-1 and VEGFR-2. The half-life in plasma is 6.2 days [97]. In monkey eyes, the mean terminal half-life in vivo is 56.8 h [98]. The safety and efficacy of Brolucizumab in patients with diabetic macula edema is currently being studied in the 2-year, randomized, double-masked, multi-center, active-controlled study phase III KITE [99] and 2-year, randomized, double-masked, multi-center, active controlled study phase III KESTREL [100] trails. It was FDA approved for neovascular wet AMD on October 8 2019, and was also approved by the EMA on February 17 2020 [96, 101]. In the HAWK and HARRIER study, it showed better fluid reduction than Aflibercept and the ability to maintain patients on a 3-month interval after the loading dose, with one injection every month for three months [58]. There are currently undergoing trials for approval for treating diabetic macular edema. 0.05 mL with 6 mg of Brolucizumab is intravitreal administered [96].

Safety and efficacy have been studied in phase III, wet age-related macula degeneration studies, HAWK and HARRIER [58]. The current most reported adverse effects, according to the product information provided by the EMA, are reduced visual acuity (7.3%), cataract (7.0%), conjunctival hemorrhage (6.3%), and vitreous floaters (5.1%). The most serious adverse reactions are blindness non-specified (0.8%), endophthalmitis (0.7%), retinal artery occlusion

(0.8%), and retinal detachment (0.7%) [96]. Immunogenicity is a possibility as 23-25% of patients treated with Brolucizumab for 88-weeks had anti-brolucizumab antibodies in their blood [58, 96].

Faracimab (Roche/Genentech, Inc. San Francisco, CA, USA) is a new promising intraocular anti-VEGF antibody with bispecific targets for both VEGF-A and angiopoitin-2 [102-105]. Faracimab is currently undergoing efficacy and safety trials in the two-year, three-arm, randomized, double--masked, multi-center, phase III study RHINE [100] and the multi-center, randomized, double-masked, active comparator-controlled phase III study YOSEMITE [106, 107] and is compared to Aflibercept for the treatment of patients with DME. It has shoved superiority to Ranibizumab in the gain of visual acuity both for naïve and previously treated patients with diabetic macular edema in the phase II prospective, randomized active comparator-controlled, double--masked, multi-center BOULEVARD trial [104]. The BOULEVARD phase 2 trial main outcome measures were mean change in best visual acuity for Faricimab vs. Ranibizumab, and the power calculations were met to support the efficacy target. It also compared the safety of Faricimab with Ranibizumab as a secondary objective. The study reported dose-dependent reductions in central subfield thickness and in Diabetic Retinopathy Severity Scale, including longer time for re-treatment. Most importantly, the primary outcome reported a statistically significant gain of letters over Ranibizumab. No new or unexpected safety issues occurred [104, 108].

Conbercept (Kanghong Biotech Company, Chengdu, Sichuan, People's Republic of China) has primarily been used in China, and is currently undergoing clinical trials in Europe for the use in age-related macular degeneration [109]. It is a 143 kDa large molecule. It is derived from a full human cDNA sequence in Chinese hamster ovary cells, engineered into a recombinant anti-VEGF fusion protein [110, 111]. Conbercept binds with a high affinity to all isoforms of VEGF-A, VEGF-B, VEGF-C, and PlGF [112]. Conbercept is seen as a direct competitor to Aflibercept, due to a 50-fold higher affinity to VEGF, and a quite similar intravitreous half-life in rabbit eyes (4.2 vs. 3.92 days respectively). No half-life in human eyes has been reported [89, 109, 113]. The drug safety for Conbercept has not been well studied and compared to other anti-VEGF drugs. The randomized, double-masked, multi-center, sham-controlled PHOENIX trial (NCT01436864) was a phase III trial in China with 81 patients in the active group. There are no reports of systemic adverse or serious adverse effects. No comparisons to other anti-VEGF were made [114]. The phase III multi-center, double-masked, randomized, dose-ranging trials PANDA-1 (NCT03577899) [115] and PANDA-2 (NCT03630952) [116] evaluating the efficacy and safety trials in the USA and Europe for the treatment of wet age-related macular degeneration, are currently active, comparing Conbercept and Aflibercept. The safety has to be evaluated in patients with diabetes as well, due to the different etiologies of the diseases.

Other treatments are used for treating DME besides anti-VEGF. In the next section, the second line of treatments, including laser therapy and corticosteroids, are presented.

5. SECOND LINE OF TREATMENT

5.1. Laser Photocoagulation

Laser therapy was the first treatment with a satisfying effect on diabetic macular edema and diabetic retinopathy. Focal and grid laser was the standard treatment for diabetic macular edema for more than three decades [25, 117]. It halved vision loss in patients with macular edema, but few experienced improvements in visual acuity in the following years [27, 118, 119]. Panretinal photocoagulation is standard in proliferative diabetic retinopathy, but it can result in peripheral vision loss or disturbances, and eventual worsening diabetic macular edema [120]. The laser works by sending light with a specific wavelength, duration, and effect into the retina, where it is absorbed, and destroys the tissue. Different lasers produce different wavelengths, and duration and effects can be variable. The rationale to use laser is that i is a technology that delivers a high amount of energy in a very localized area. Laser can thereby occlude leaking blood vessels, destroy ischemic retinal areas, which results in reducing the oxidative stress on the retina thereby reducing the increased VEGF drive in the retina, stopping and decreasing the macular edema, but according to a Cochrane review, the actual mechanism is not known entirely [121].

The primary risks of laser treatment of diabetic macular edema are the forming of epiretinal membrane, growing laser marks, misfire at the retina and the lens, decreased peripheral vision. Due to the destructive nature of the laser applied at the retina, a few studies with a subthreshold laser has been made. A subthreshold laser is laser treatment to the retina, with a much lower energy setting than normal focal laser. The subthreshold laser has not only shown fewer destructive side effects on the retina but also a slower regression of diabetic macular edema compared to standard laser treatment [122, 123].

The last medical treatment group for diabetic macular edema is corticosteroids, which will be described in the following section.

5.2. Intravitreal Steroids

Three types of intravitreal steroids are available; Orzudex and Iluvien are approved by the FDA and EMA for treating DME and Kenalog is used off-label for treating DME [124]. The current recommendation from the European Society of Retina Specialists for the use of corticosteroids is that they should only be considered as first-line choice in patients with cardiovascular risk factors, as these patients are not included in all the major anti-VEGF studies, or in patients with low compliance to monthly anti-VEGF injections [80].

The rational to why corticosteroids might be a suitable drug of choice in the treatment of diabetic macular edema is due to the role of inflammation in the development of diabet-

ic macular edema caused by the elevated levels of diacylglycerol and the activation of protein kinase C, and the accumulation of leukocytes on the endothelium of the retinal capillaries. This causes the blood-retina-barrier to become dysfunctional in a positive feedback loop of the pooling of leukocytes upregulating the intracellular adhesion molecule 1, which in return attracts new leukocytes. In the end, it leads to elevated levels of inflammatory molecules in serum and vitreous and increased vascular permeability [14, 125-127]. The corticosteroids act as immunosuppressants in downregulating the inflammatory pathways, and thereby decrease the immune response, including downregulating of the VEGF synthesis. A study has shown Triamcinolone Acetonid to target multiple objects, including VEGF, whereas anti-VEGFs are a lot more specific in their target [128, 129].

General side effects related to the procedure: The most serious side effect is endophthalmitis, which is rare and vision-threatening; furthermore, there is a small risk of puncturing the eye and stabbing the lens. More common side effects are pain related to the injection, subconjunctival hemorrhage, vitreous hemorrhage inflammation in the anterior chamber, posterior chamber opacity (the patient sees the implant) [130-132].

General side effects related to the drugs: Risk of increased intraocular pressure (IOP) for weeks and months due to the slow release of the drug. All patients should frequently attend intraocular pressure monitoring [80]. There is also a increased progression of cataract in phakic eyes, which is why pseudophakic eyes are preferred for treatment with steroids. Systemic side effects are also reported [132-135].

Pseudofakic patients are preferred for treatment with corticosteroids, as practically all patients will develop cataracts. A thorough description of each drug is included below.

Dexamethasone (Orzudex, Allergan, Inc., Irvine, CA, USA) [136] is delivered as an intravitreal eye implant through a 22-gauge applicator [132]. It is 700 microgram of biodegradable slow-release dexamethasone, which last for about three to six months. It is EMA and FDA approved for treatment for diabetic macular edema [137]. It is listed at the Flockhart tables [42] as a P450 interaction drug, which works through substrate 3A457 and inducer 2D6. The pharmacogenomic effects of Orzudex are not described.

The addition of dexamethasone to continued Ranibizumab therapy does not improve visual acuity among eyes with 24 weeks persistent DME, following anti-VEGF therapy [138]. It was first studied in the randomized, controlled, multi-center, double-masked, parallel-group PLACID trial, where Ozurdex and laser were compared to laser. The combination of laser and Ozurdex showed significantly higher visual acuity outcome than just the laser [139]. Another study lasting 18 months found that Ozurdex improved visual acuity and decreased central macular thickness in patients with diabetic macular edema [136]. An increase in intraocular pressure has been reported. The most important trial for Ozurdex has been the three-year MEAD trial were Ozurdex was compared to sham, and Ozurdex showed a significant effect on visual acuity and treating diabetic macular edema [140]. The prospective, multi-center, open-label CHAMPLAIN study showed a similar effect in treatment-refractory diabetic macular edema in vitrectomized eyes, where one-third of the patients gained over 10 letters in visual acuity [141].

Fluocinoloneacetonide (Iluvien, Alimera Sciences, Alpharetta, GA, USA) is a long lasting agent, up to 36 months. The recommended dose of Iluvien is a 190 microgram IO implant. It is inserted in the vitreous through a 25-gauge needle in topical anesthesia [142]. It is approved by the FDA and the EMA for the treatment of chronic diabetic macular edema and edema resistant to other treatment modalities. Contraindications are a patient history of high IOP, former high IOP steroid responder, and any sign of intra- or periocular inflammation. It has a sustained delivery system, and it is designed to function for up to one year [143]. The randomized, double-masked, parallel group, multi-center dose-finding comparison FAME study has reported better visual acuity for low dose Iluvien compared to higher doses of Iluvien, and nearly every participant got cataract and high intraocular pressure [142, 144-146]. The FAME study showed promising results in treating patients with refractory diabetic macular edema, as the group who gained over 15 letters was significantly higher.

Triamcinolone Acetonid (Kenalog/Triesence, Alcon Laboratories, Inc., Fort Worth, TX, USA) (Off-label) [124]. The first reported intravitreal treatment with Kenalog in intractable diabetic macular edema was reported in 2001 [147]. It has not been approved by the FDA to treat diabetic macular edema, but only to enhance the visualization of the vitreous during vitrectomy and to treat some posterior segment inflammation [148]. It is delivered either by the intravitreal or as a depot behind/next to the eye. No evidence of electroretinography retinotoxic effects is found [149]. Raised IOP has been reported after a single use of Kenalog, and nearly all patients need pressure-lowering eye drops to control the pressure. Also, an overrepresentation of cataract has been reported after injections with Kenalog, therefore, pseudofac patients are preferred [131, 133]. Central Serous Chorioretinopathy has reported idiosyncratic for treatment. Intravitreal treatment with Triamcinolone Acetonid might be associated with a decrease in choroidal thickness in eyes with diabetic macular edema [150]. The effect can last for three months, and repeated injections are necessary. It is also capable of reducing proliferative diabetic retinopathy next to the diabetic macular edema due to its antiangiogenic effects [151]. As for now, it is not a sustained delivery system like Iluvien or Ozurdex. The DRCR.net protocol B, which was a 5-year multi-center, independent randomized, controlled clinical trial, compared Triamcinolone Acetonid to focal/grid laser therapy in diabetic macular edema patients and showed that the mean visual acuity 2 years into the study was better in the laser group, than the 2 mg or 4 mg Triamcinolone Acetonid groups. Ocular hypertension was significantly higher in Triamcinolone Acetonid groups, with up to 40% having high intraocular pressure compared

to 10% in the laser group. Cataract was developed by the majority of the Triamcinolone Acetonid patients. Glaucoma surgery had to be performed in 4 eyes of the 4 mg Triamcinolone Acetonid group [134, 135]. Triamcinolone Acetonid does perform worse compared to Ranibizumab in the DRCR.net trial [152].

Some treatments with steroids can affect the blood glucose levels due to a strong hyperglycemic effect of steroids. This raises the question if diabetic patients should alter their medication in conjunction with intravitreal steroid injections. A retrospective study with 30 patients, in which all had a 4 mg intraocular Triamcinolone acetonide injection in prior vitrectomized eyes, reported a slight (19%) increase in blood glucose levels 4 hours after the injection ($P = 0.05$), with a return to baseline within 24 hours. Glycosylated hemoglobin levels at baseline and after 3 months were similar (7.3±1.1% vs. 7.4±1.1%, respectively). This was done in previously vitrectomized eyes, in which the clearance rate of triamcinolone is much higher than in non-vitrectomized eyes [153]. The same 30 patients' blood glucose curves from their prior vitrectomy were compared with their triamcinolone injection blood glucose curves and were found to be similar [154]. These findings are agreement to those in a similar study with cataract surgery with and without administration of subconjunctival steroid [155]. These two papers suggest in their conclusions that the rise in blood glucose is not related to the steroid injection, but to a general stress response, and ocular steroid injections, therefore, can be performed without active blood glucose control.

6. A GENERAL COMPARISON OF ANTI-VEGFs, LASER AND CORTICOSTEROIDS

In the present paper, we describe the treatment options available for diabetic macular edema and report the scientific evidence for effect and safety. We found that generally, anti-VEGF should be the first line, second line laser, or steroids in accordance with international guidelines and the individual patient.

Anti-VEGF intravitreal injections with Ranibizumab, Aflibercept, and Bevacizumab have shown superiority to focal/grid laser photocoagulation both in decreasing the risk of vision loss and the possible gain of vision [30, 81, 119, 120, 156-158]. Ranibizumab has shown to be non-inferior to panretinal photocoagulation [120]. Phase III randomized, double-blinded, multi-center, interventional DRCR.net study protocol B proved that in phakic patients, focal/grid laser was superior to Triamcinolone Acetonid [134, 135]. One study compared combined laser and Ozurdex treatment vs. laser alone for the treatment of DME in 80 patients, and did not find any clear evidence that combination therapy is better than laser alone in ETDRS score, even though the edema decreases by far more in the combination group. The lack of improvement in ETDRS in the combination group may be due to the development of cataract [159]. High IOP was reported in 20% (8 patients) in the combination group vs. 2.5% (1 patient) in the laser group, 33% (9/27) phakic eyes underwent cataract surgery in the combination group. This illustrates the challenges with treatment with corticosteroids, even with the medication which has the best safety profile compared to the other two in this review. Still as mentioned above, corticosteroids have a place in treatment-refractory DME, as shown in the CHAMPLAIN trial [141].

Laser has, due to the superiority of anti-VEGF treatment, been removed from the first-line treatment strategy for diabetic macular edema in highly developing countries with good healthcare, but maybe subthreshold laser has a place to treat milder or non-central diabetic macular edema, or to be used in third world countries without wide access to anti-VEGF due to the costs [121].

CONCLUSION

There has been considerable development of anti-VEGF drugs in the last couple of decades. Additionally, drugs are under development with Brolucizumab as the latest, currently approved drug for AMD, and is in the process of being approved for the treatment of DME. From China, a locally developed well-tried anti-VEGF, Conbercept, is currently undergoing clinical trial in the EU for approval for AMD treatment and probably hereafter seeking approval for the treatment of DME. The introduction of anti-VEGF has totally changed the way DME is being treated, from extensive destructive laser treatment of the retina with the risks and shortcoming that come with this, to more and more advanced anti-VEGFs and slow-release corticosteroids, and maybe topical administered eye drops in the future. Hopefully, the future drugs will be with even greater safety profiles. Most of the trials of the medicaments are industry-sponsored, which more often have favorable outcomes than non-sponsored trials due to bias [160-162]. We see this as a risk of overestimation of results. Selection bias may be an issue as a part of the studies, in our opinion, having a wide range of exclusion criteria and a narrow range of inclusion criteria. This does not, to our experience, align with the daily clinical practice, where you see a variety of patients, and the compliance, especially in older patients with long travel distances, can be very varying, even though we write from a society where health care is free.

Generally, the safety profile is very acceptable, especially for the anti-VEGFs. The corticosteroids' main issue is the high intraocular pressure, a common adverse effect, and all patients that are phakic have accelerated cataract development and need an operation in a few months to years. Still, laser has its place in the treatment of DME in low-income countries where anti-VEGF is still too expensive but is not comparable to the many time more effectiveness of anti-VEGF and the less destructive nature of such injections. The subthreshold laser might have a place for milder forms of diabetic macular edema in the future, even though more RCT needs to be done to evaluate the efficacy and safety of such.

Future perspectives in drug development would be in new administration forms and delivery systems for anti-VEGF such as topical eye drops, or slow-release anti-VEGF implants. As for now, there is one once-a-day topi-

cal eyedrop selective inhibitor for VEGF-A in a phase II trial [163]. This would be most beneficial to the patients, as the safety risks related to the procedure would be considerably lower than an injection, but still, low dose, locally administered anti-VEGF could lead to possible serious vascular events. Additionally, further experiments with medication treating diabetic macular edema, should address if there are any differences in the safety or efficacy regarding type 1 or type 2 diabetes. Such a distinction is not currently a normal parameter to report by. The majority of studies are performed on patients with type 2 diabetes, as this is the largest group of patients with diabetes. No systematic reporting on differences in efficacy or safety on diabetes type 1 or type 2 has been published, to our knowledge. No systematic reporting in studies, on sex or ethnic relationships as socioeconomic factors have been accounted for, have been made. Most studies include different races, but do not define their inclusion or exclusion criteria, and do not report subsample analysis of the different ethnic groups. Information regarding pharmacogenomics in the treatment of eye disease is currently not available for the drugs mentioned in this review, except the relation between dexamethasone and the cytochrome P450 system, which is mentioned in the Flockhart table [42]. This is an area of research that may have a substantial impact on personalized medicine and outcome for the patient, if the research can show a differentiated effect of the drugs, depending on the gene profile of a patient.

In our opinion, anti-VEGF should be the drug of choice when the cost is not an issue, due to the huge effectiveness and favorable safety profile. The small risk of severe adverse effects is, in our opinion, highly outweighed by the benefits of preserved or even increased visual acuity for the patients.

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