Chapter

Microvascular Complications of Diabetes Mellitus: Focus on Diabetic Retinopathy (DR) and Diabetic Foot Ulcer (DFU)

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Abstract

Diabetic retinopathy and diabetic foot ulcer are the most frequent, but also the most disabling complications of diabetes mellitus, with a sinister impact on patients' quality of life. Microvascular changes related to the deleterious effect of chronic hyperglycemia play an important role in the pathophysiology of both clinical entities by multiple molecular pathways. Vision-threating diabetic retinopathy may be treated by laser photocoagulation, anti-vascular endothelial growth factor (VEGF) agents and vitreoretinal surgery. Diabetic foot lesions are best treated by revascularization if needed, off-loading, infection control and therapeutic adjuncts (e.g. special dressings). Treatment should ideally be offered by a multidisciplinary expert team. Prevention and early detection, along with adequate control of glucose, lipids and arterial hypertension are of paramount importance to avoid and mitigate these fearful complications.

Keywords: microvascular complications, diabetic retinopathy, risk factors, ophthalmoscopy, angiography, laser photocoagulation, diabetic foot, diabetic neuropathy, treatment

1. Introduction

In diabetes (DM), chronic complications related to the direct or indirect effects of prolonged hyperglycemia on the vasculature have been classified into macrovascular and microvascular complications, depending on the size of affected vessels and the pathophysiological mechanisms involved. Microvascular disease includes retinopathy, nephropathy and neuropathy.

Diabetic retinopathy, one of the first manifestations of microvascular disease, remains today, despite improvements in monitoring and treatment, one of the leading causes of blindness worldwide. Epidemiological studies estimate that approximately 40% of subjects with DM type I over 40 years of age have retinal microvascular changes, of which 8.2% exhibit impaired visual acuity [1, 2]. Both DM types are associated with impaired retinal microcirculation. After 20 years

from the onset of DM, almost all patients with type 1 DM (T1DM) and over 60% of those with type 2 DM (T2DM) will be affected [3]. Furthermore, decreased vision as a result of diabetic retinopathy has a negative impact on the quality of life of patients and their ability to successfully manage DM [4].

Diabetic foot results from diabetic neuropathy and/or peripheral arterial disease and affects annually between 9.1 to 26.1 million [5]. It is a chronic disabling and progressive complication, with potential deformities, chronic ulcerations and infections. Diabetic foot ulcers (DFUs) are encountered in 15% of DM patients, of whom 15-20% reach amputations. The latter lead to increased morbidity and decreased quality of life, but also an important burden on national healthcare systems, with increased health costs and hospitalization [6, 7].

2. Diabetic retinopathy

2.1 Risk factors

2.1.1 DM duration and poor glycemic control

Diabetic retinopathy (DR) is a chronic complication associated with long DM duration and poor glycemic control, the overall incidence of DR and of vision-threatening forms of DR (VTDR) being higher in T1DM than in T2DM [8].
The United Kingdom Prospective Diabetes Study (UKPDS) showed that both the incidence and progression of DR correlate with elevated HbA_{1c}, emphasizing the importance of good glycemic control to prevent visual impairment [9]. Every 1% decrease in HbA_{1c} leads to a 40% reduction in the risk of developing retinopathy, a 25% reduction in the risk of progression to vision-threatening retinopathy, and a 15% reduction in the risk of blindness [10].

2.1.2 Arterial hypertension

The correlations between cardiovascular risk factors and the occurrence and evolution of DR are still a subject of study. However, there is clear evidence that the processes of arteriolosclerosis and the mechanical trauma to the vascular endothelium caused by elevated systolic and diastolic blood pressure are both cofactors in worsening DR. Some but not all studies have shown a negative impact of high blood pressure on DR [9, 11, 12]. The UKPDS has demonstrated a significant correlation between systolic arterial hypertension and DR incidence in T2DM. Thus, patients with blood pressure (BP) > 140 mmHg have a 2.8 times higher risk of developing DR than those with BP <125 mmHg. In the study of Lurbe et al. [13], in a cumulative exposure model, HbA_{1c} and elevated diastolic BP values are predictive factors for the occurrence and progression of RD. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), diastolic BP emerged as a significant risk factor for DR progression in T1DM, but no correlations were found for systolic BP or T2DM [14, 15]. Therapeutic lowering BP was found to have a protective role on retinal lesions in several studies supporting the recommendations for tight blood pressure control to further prevent visual loss n T2DM [9, 14, 16].

2.1.3 Lipidic disorders and serum LDL

Unlike glycemic control, the role of serum lipids in DR pathogenesis is less clear. There is no parameter in the lipid profile that is strictly associated with the incidence or progression of DR. However, elevated total cholesterol, LDL-cholesterol, Apo B

and Apo B/Apo A ratio are correlated with the appearance of hard exudates, these being lipoprotein extravasations in the retinal capillaries. Hadjadj et al. showed that serum triglyceride levels are correlated with the occurrence of nephropathy and retinopathy in patients with T1DM [17]. Several randomized trials confirmed that the treatment of dyslipidemia can prevent the development of DR [14, 15, 18–21].

2.1.4 Genetic predisposition

Several studies have revealed that in young adults with T1DM, genetic predisposition for the development of DR is connected with the presence of HLA DR3/DR4 antigens. Furthermore, different alleles for codification of cytokines and chemokines, as well as vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)beta1 were characterized, explaining different predisposition for DR in diabetic patients. VEGF plays a key role in increased microvascular permeability and neovascularization in proliferative diabetic retinopathy. The VEGF gene is located on chromosome 6 (6p21.3) and is highly polymorphic in the promoter region, correlated with VEGF expression and activity. In a study by Buranczynska et al., the presence of the D allele at −2549 in the promoter region of the VEGF gene enhanced gene expression [22]. The DD genotype was associated with DR but not with nephropathy, suggesting a cell-specific target of the VEGF isoform. In a study of Jalal and Kalia, regarding the polymorphism of VEGF genes in India, allele A and AA genotype of rs2146323 were significantly correlated both with incidence and with severity of DR [23]. Awata et al. described C (−634) G polymorphism of VEGF gene to be related to macular edema as well as diabetic retinopathy [24]. Ray et al. identified VEGF −460 C genotype to increase VEGF basal promoter activity by 71%, leading to a 2.5 increased risk of proliferative DR [25]. TGF beta signaling is considered to have an immunosuppressive role in the retina. Disorders affecting this pathway lead, at least in experimental animal models, to loss of pericytes, microaneurysms and leakage, finding resembling diabetic retinopathy [26]. Beránek et al. found a more frequent incidence of 915G/C (R25P) polymorphism of TGF beta gene in patients with DR compared to control subjects [27].

2.1.5 Pregnancy

Hormonal alterations during pregnancy were found to be an independent risk factor both for onset and for progression of DR, especially PDR, posing many challenges regarding the management of these patients [28–30]. Another mechanism is pregnancy-induced hypertension and pre-eclampsia [31].

2.1.6 Ocular and systemic inflammation

Recent studies focus on the significance of inflammation in the developments of DR [32–36]. There is strong evidence that ischemia and retinal hypoxia induce release of VEGF and inflammatory molecules at the level of endothelial and glial cells. Furthermore, several studies support the idea that ocular inflammatory disorders, such as prolonged post cataract surgery healing, uveitis, keratitis, are related to DR progression [32–36].

There are increased evidences that chronic systemic inflammation is also related to increased risk of DR onset and progression. In an experimental animal model, recurrent exposure to systemic LPS leads to injury of capillary endothelium and in vivo thinning of the retina in hyperglycemic mice, but not in healthy controls [37]. There are clinical evidences of increased incidence of DR and PDR in long standing non-healing foot ulcer, that could be explained due to the associated chronic low-grade systemic inflammation [38–42].

2.1.7 Antidiabetic treatment and macular edema

The correlations between antidiabetic mellitus medication and the risk of macular edema is still a subject of research. In a comprehensive systematic review and meta-analyses, Zhu and col. found that insulin use, as well as thiazolinedione (TZD) and meglitinide might increase the risk of macular edema, metformin has no statistically significant effect, while the use of sulfonylureas seems to have a protective role [43]. The physiopathological mechanisms are not completely understood, but experimental studies indicate that insulin and TZD may induce changes in retinal flow and increased expression of VEGF and breakdown of retinal-vascular barrier [43–46].

2.2 Pathophysiology

The pathological changes that lead to diabetic retinopathy are attributable to 3 main factors:

- small vessel wall damage:
- changes in blood flow
- alterations in platelet function

2.2.1 Lesions in small vessel wall

Microvascular changes in the retinal capillaries are due to chronic hyperglycemia by different mechanisms, such as:

2.2.1.1 Aldose-reductase and intracellular polyol pathway

Aldose reductase is an enzyme that converts glucose to sorbitol, which induces osmotic stress by intracellular accumulation. In animal models, this phenomenon leads to microaneurysmal dilatations of the vascular wall, basal membrane thickening and loss of the pericytes [47]. However, experimental studies of treatment with aldose reductase inhibitors have not obtained satisfactory clinical results.

2.2.1.2 Advanced glycosylated end products (AGEs)

Chronic hyperglycemia leads to non-enzymatic glycation or glycoxidation of proteins, resulting in accumulation of AGEs. This process affects both intra- and extracellular proteins, resulting in functional impairment. Deposits of AGEs in the extracellular matrix and subendothelial space lead to permanent alterations of intercellular junctions, monocyte migration and activation of nuclear factor (NF)-κB along with activation of pro-inflammatory pathways [48, 49]. In experimental models, increased AGEs accumulation is associated with loss of pericytes and microaneurysm formation in retinal capillaries [50].

2.2.1.3 Oxidative stress and ROS

Hyperglycemia induces mitochondrial dysfunction and endoplasmic reticulum stress, with increased production of free radicals and reactive oxygen species (ROS) accumulation [49]. These degrade lipids, proteins and ribonucleic acid (RNA) chains.

Furthermore, experimental studies have proved a "hyperglycemic memory": in subjects with long periods of poor glycemic control, reversal of hyperglycemia fails to normalize increased oxidative activity in the retina [51]. Treatment with antioxidants and vitamin E alleviates endothelial dysfunction, but does not prevent the onset and progression of DR and other microvascular complications. Isolated experimental blockade of each of these pathways does not stop retinal microvascular damage, suggesting that the effects of hyperglycemia are manifested at the cellular and extracellular levels. Recently, experimental and clinical studies have demonstrated that inflammation biomarkers and pathways play a significant role in the aggravation of lesions and the evolution towards retinal neovascularization. A large array of cytokines and chemokines were found in increased concentrations in patients with DM, both in ocular samples and in serum: interleukin (Il)1beta, Il 2, 4, 6, 8, TNFalfa and (monocyte chemoattractant protein) MCP-1 [38, 42, 52–54]. Recent works have revealed that the TXNIP/NLRP3 Inflammasome activation pathways may contribute to pathologic neovascularization encountered in advanced stages of PDR [50, 54, 55].

2.2.1.4 Nitric oxide (NO) deficiency

Hyperglycemia induces decreased synthesis and increased consumption of NO by multiple pathways: activation of protein kinase C (PKC) in endothelial cells, oxidation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) via aldose reductase pathway, and non-enzymatic production of superoxide by AGEs. NO plays key roles in microcirculation, by regulation of arteriolar tone, platelet stabilization and preventing leukocyte adherence at the vascular wall. Decreased local levels of NO promotes vasoconstriction, microvascular occlusions and secondary retinal ischemia.

2.2.2 Changes in blood flow and platelet function

General changes in blood favor small vessel obstructions with secondary retinal ischemia:

- increased hematocrit and blood viscosity related to high liver synthesis of fibrinogen and alfa2 globulins
- more rigid erythrocytes, with increased tendency to thrombosis
- increased platelet adhesion and aggregation
- activation of peripheral leukocytes, increased adherence to endothelial cells via beta-2 integrin expression and synthesis of mediators of inflammation

2.2.2.1 Pigmented Epitelium Derived Factor (PEDF) decrease and retinal neurodegeneration

PEDF is a trophic factor expressed by a multitude of retinal cells, an antagonist of VEGF. It decreases vascular permeability and plays an antioxidant role, protecting retinal cells from ROS. In the experimental setting, PEDF is decreased in aqueous and vitreous humor, early in preclinical stages of DR. The pathogenic mechanisms are supposed to be related with decreased insulin, as well as increased toxic mediators, such as glutamate. These early changes may induce mild changes in color vision, contrast sensitivity, visual field and electroretinogram oscillatory potentials [56–60].

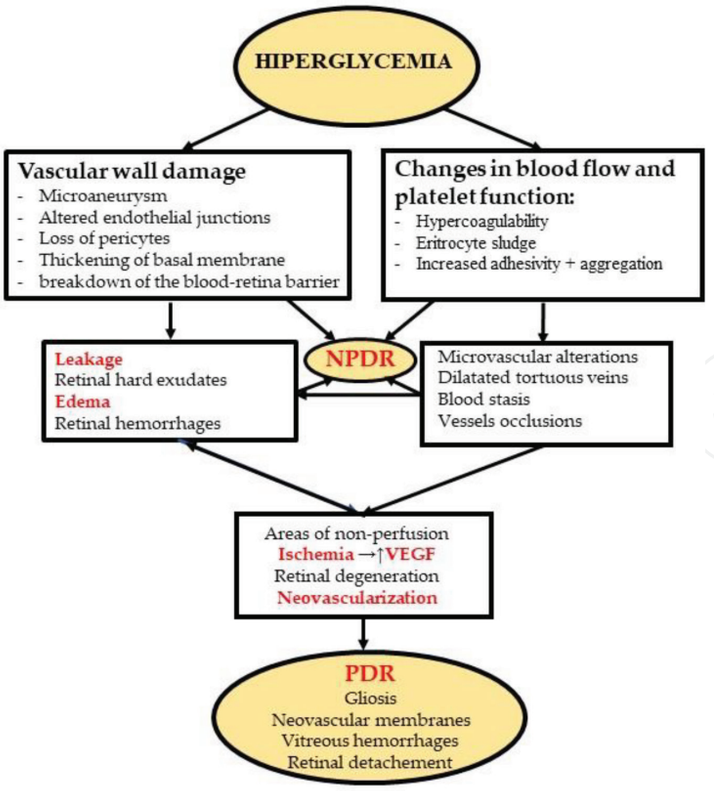

Figure 1.
Pathogenesis of diabetic retinopathy.

All these molecular mechanisms may lead to:

- alteration of tight junctions in endothelial cells, compromising the blood-retinal barrier, increasing extravasation of fluid in the retinal space and the formation of lipoprotein exudates and retinal edema

- loss of pericytes, with the appearance of focal vascular dilations and microaneurysms

- thickening, hyalinization of the basement membranes, with loss of elasticity of the vascular wall and autoregulatory capacity. This, together with the alterations of blood flow and platelets, favors microvascular occlusions, with the appearance of ischemic, hypoxic retinal areas.

The first areas affected by thrombosis and ischemia are in the middle retinal periphery, and the answer is to release a range of mediators, of which the key role is played by VEGF, which promotes retinal neovascularization and interruption of blood flow in many areas (optical disc, macula, iridocorneal angle and iris). The response to retinal hypoperfusion, a maladaptive protective mechanism, leads to the appearance of fragile new vessels, prone to repeated bleeding and leakage, ultimately destroying normal retinal architecture (**Figure 1**).

2.3 Clinical manifestations of DR

Fundus examination documents the presence and severity of retinal lesions. Clinical signs include:

- **Microaneurysms:** tiny round red dots, typically located initially temporal to the fovea; sometimes hard to be differentiated from dot hemorrhages in ophthalmoscopy; they are best shown by fluorescein angiography

- **Retinal hemorrhages**: a) "dot-blot" hemorrhages result from the venous end of the capillaries and are located in the middle layer of the retina; b) "flame-shaped" hemorrhages arise from pre-capillary arterioles and are located more superficially in the retinal nerve fiber layer

- **Retinal edema:** elevated, white-greyish appearance of the involved area; may be a consequence of leakage and fluid accumulation or of retinal ischemia (intracellular retinal edema). When foveal region is involved, it may assume a cystoid appearance and fluorescein angiography reveal a flower-petal pattern

- **Hard exudates:** these are well delineated, small, bright yellowish retinal lesions, formed by extravasated lipoproteins and lipid-filled macrophages and are mainly located in the outer plexiform layer. They are considered a sign of current or previous macular edema. When located in the macular region, they tend to organize in a circinate manner. They could resorb spontaneously months after the leakage is stopped, otherwise, chronic leakage leads to enlargement of the exudates and cholesterol accumulation.

- **"Cotton wool" spots**: these are small, whitish, fluffy superficial lesions, that cover the underlying retinal vessels and bear the significance of focal retinal ischemia and infarction. They are composed by neuronal debris and can disappear in time by autolysis and phagocytosis.

- **Venous loops and venous beading (VB):** these frequently occur adjacent to areas of nonperfusion and bear the significance of increasing retinal ischemia

- **Intraretinal microvascular abnormalities (IRMA):** these are arteriolo-venous shunts, bypassing the capillary bed, and are considered an indicator of capillary occlusion and retinal ischemia. Together with VB, they are considered the most significant predictor of progression to PDR [58].

- **Neovessels:** these are thin, with a single cell layer wall, extremely fragile, with a lace-like appearance and may be situated at the surface of the optic disk or elsewhere, in general at the periphery of the areas of non-perfusion. They can be best evidenced by fluorescein angiography and optical coherence tomography (OCT).

2.4 Staging of DR

Dr is classically referred as: non-proliferative (NPDR) and proliferative (PDR). The classification is determined by the presence of retinal neovascularization. Recent Guidelines of International Council of Ophthalmology for Diabetic Eye Care recommends the following staging system, based on findings encountered in ophthalmoscopy, to be used in clinical practice (**Table 1**).

The ICO guidelines also refer to the location, extension of the diabetic macular edema (DME), as it is an important cause of decreased vision in DR, even in the absence of neovessels. Central involved DME is considered to be an area of retinal thickening in the macula that does involve the central subfield zone (of 1 mm in diameter).

DR	Findings on Dilated Ophthalmoscopy
No apparent DR	No abnormalities
Mild NPDR*	Microaneurysms only
Moderate NPDR	Microaneurysms + other signs: • dot and blot hemorrhages • hard exudates cotton wool spots
Severe NPDR	Moderate NPDR with any of the following: • Intraretinal hemorrhages ≥20 in each quadrant; • Definite venous beading (VB) in 2 quadrants; • Intraretinal microvascular abnormalities (IRMA) in 1 quadrant; and no signs of proliferative retinopathy
PDR*	Severe non-proliferative DR + one of the followings: Neovascularization Vitreous/preretinal hemorrhage

**NPDR: non proliferative diabetic retinopathy; *PDR: proliferative diabetic retinopathy*

Table 1.
International Classification of Diabetic Retinopathy [57].

2.5 Clinical forms of DR associated with high risk of vision loss

Diabetic maculopathy is the most frequent cause of decreased vision encountered in patients with T2DM. It can be manifest in every stage of the DR and represents the involvement of the fovea by hard exudates, macular edema due to fluid extravasation or by macular ischemia. In early stages, the loss of vision is mild; however, if untreated, it can may to permanent photoreceptor damage.

DME is considered clinically significant if [61–63]:

- located at or within 500 μm of the center of the macula
- hard exudates at or within 500 μm of the center if associated with thickening of adjacent retina
- the area of retinal thickening is larger than one optic disc area and is located within 1 disc diameter of the center of macula

2.5.1 Advanced diabetic ocular disease

Advanced diabetic disease can remain asymptomatic for a long period of time, due to slow proliferation of the retinal neovessels and their location, usually in mid-periphery. It consists of retinal neovessels that grow into elevated fibrovascular membranes that enter the vitreous body, leading to serious complications: vitreous hemorrhage and retinal detachment [62]. Proliferation of the abnormal vessels at the level of iris and iridocorneal angle led to neovascular glaucoma, with poor clinical outcomes. Ophthalmological periodical screening is extremely important in early identifying and referral to laser therapy. In advanced stages, serious complications appear and the vision loss is irreversible.

Diabetic maculopathy is the most frequent cause of decreased vision encountered in patients with T2DM.

2.6 Diagnosis of DR

Early detection of DR depends on educating DM subjects, as well as their families, friends, and health care providers about the importance of regular eye examination. This holds true for asymptomatic subjects as well.

Initial ophthalmological examination in a patient with suspected/confirmed DR should include the following:

- Visual acuity
- Measurement of intraocular pressure (IOP), due to the possible risk of developing neovascular glaucoma
- Slit-lamp exam +/- gonioscopy if iris neovascularization is observed or IOP is elevated
- Fundus examination with dilated pupil

A variety of imaging techniques are useful to detect, classify and monitor DR, as well as efficacy of treatment: fundus photography, fluorescein angiography, optic coherence tomography (OCT) and OCT angiography.

2.6.1 Ophthalmoscopy and Fundus Photography

Currently, the two most sensitive methods are retinal photography and slit-lamp examination through dilated pupils. Direct ophthalmoscopy by ophthalmologists or trained technicians yields 80% sensitivity and >90% specificity [64]. It is cheap and is considered the method of choice. Fundus photography has the advantage of creating a permanent record, and for that reason, it is the preferred method for retinopathy assessment (**Figures 2–4**).

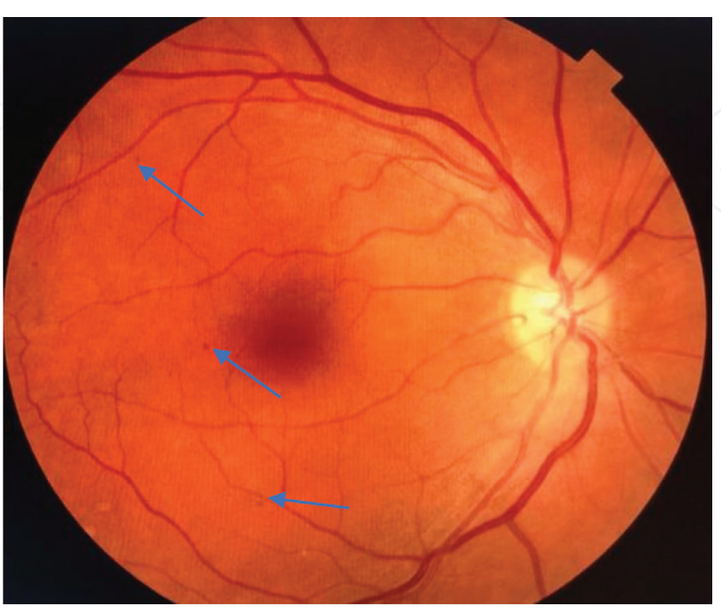

Figure 2.
"Background" diabetic retinopathy: few dot hemorrhages (blue arrows) (Dr. Ana Dascalu's private collection, Emergency University Hospital Bucharest, Ophthalmology Department).

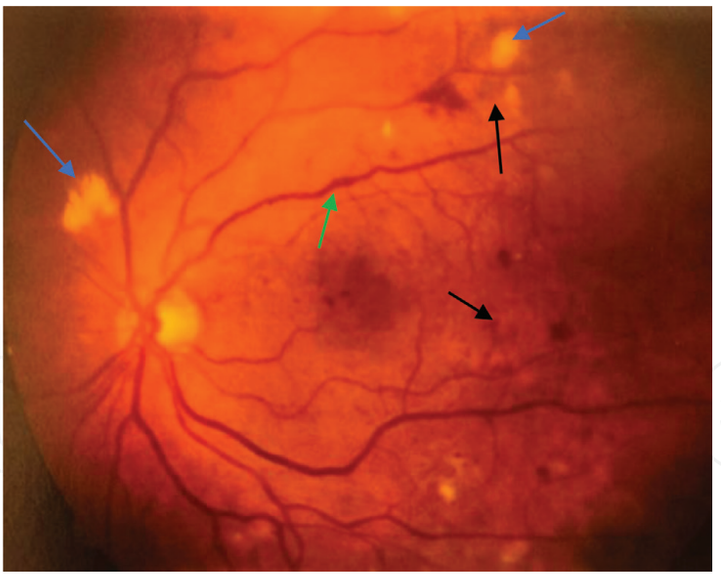

Figure 3.
Retinophotography: Severe NPRD: multiple dot and blot hemorrhages, hard exudates, cotton wool spots (blue arrows), macular edema, VB (green arrow) and IRMA (black arrows) (Dr. Daniela Stana's private collection, Emergency University Hospital Bucharest, Ophthalmology Department, PhD thesis).

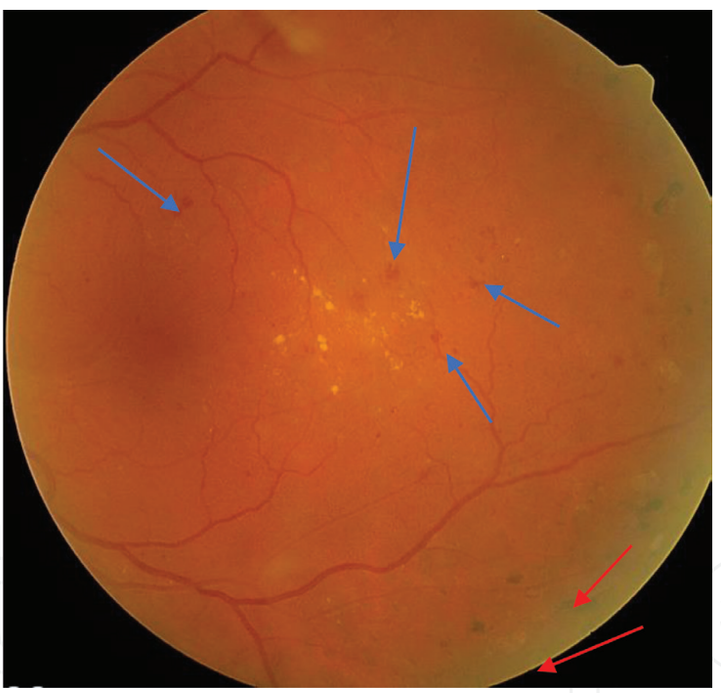

Figure 4.
Incipient PDR: large ischemic area situated temporally to the macular region, with hard exudates, dots hemorrhages, venous loops, IRMA and intraretinal neovessels; in the mid periphery, pigmented lesions post laser photocoagulation (Dr Ana Dascalu's private collection, Emergency University Hospital Bucharest, Ophthalmology Department).

2.6.2 Fluorescein angiography

Fluorescein angiography is an invasive, costly, and time-consuming technique but is a sensitive method to detect vascular changes due to rupture of the inner and outer blood retinal barrier in the course of DR [63, 65, 66]. The retinal vasculature is visualized with great accuracy: the examiner may identify tiny microaneurysms and differentiate between microaneurysms (hyperfluorescent) and punctiform hemorrhage (hypofluorescence by masking effect). It is an indispensable

exploration before planning different laser treatment, for example to distinguish retinal edema by leakage (which appears white due to dye accumulation) from ischemic retinal edema (which appears as hypofluorescent). In the latter case, the application of laser impacts is not recommended because it leads to exacerbation of retinal ischemia (**Figure 5**).

2.6.3 Optical coherence tomography (OCT) and OCT-angiography (OCT-A)

OCT is a completely non-invasive, reproducible and quantifiable. It provides high-resolution images of the retinal layers, choroid, vitreous gel, and the vitreo-retinal interface and has become the gold standard for diagnosis, assessment of treatment response, and follow-up up of patients with diabetic macular edema.

OCT angiography (OCTA) is a new non-invasive imaging technique that employs motion contrast imaging to high-resolution volumetric blood flow information, rapidly generating images similar to angiographic images [63, 65–67]. It provides a highly detailed view of the retinal vasculature, which allows for accurate delineation of the foveal avascular zone (FAZ) and detection of subtle microvascular abnormalities, including FAZ enlargement, areas of capillary non-perfusion, and intraretinal cystic spaces [66]. The possibility of detecting microvascular changes in diabetic eyes before the presence of visible microaneurysms may have important implications in the future. In this sense, OCTA could be able to quickly identify subjects at risk of DM (**Figures 6** and **7**).

2.7 Treatment

2.7.1 Primary prevention

Follow-up of patients with DR involves the ophthalmologist and the diabetologist. Extensive studies in large groups of diabetic patients have shown the beneficial role of strict control of blood glucose, hypertension and dyslipidemia in both

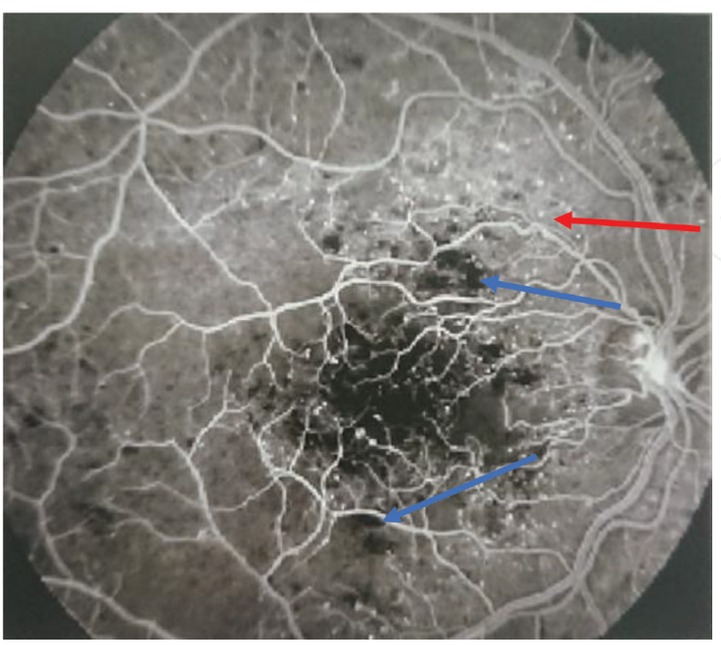

Figure 5.
Fluorescein Angiography: Severe NPRD: numerous microaneurysms (hyperfluorescent dots), areas of non-perfusion (hypofluorescent, blue arrows), venous loops and IRMA, with diffuse leakage (hyperfluorescent, red arrow) (Dr Daniela Stana's private collection, PhD Thesis, Emergency University Hospital Bucharest, Ophthalmology Department).

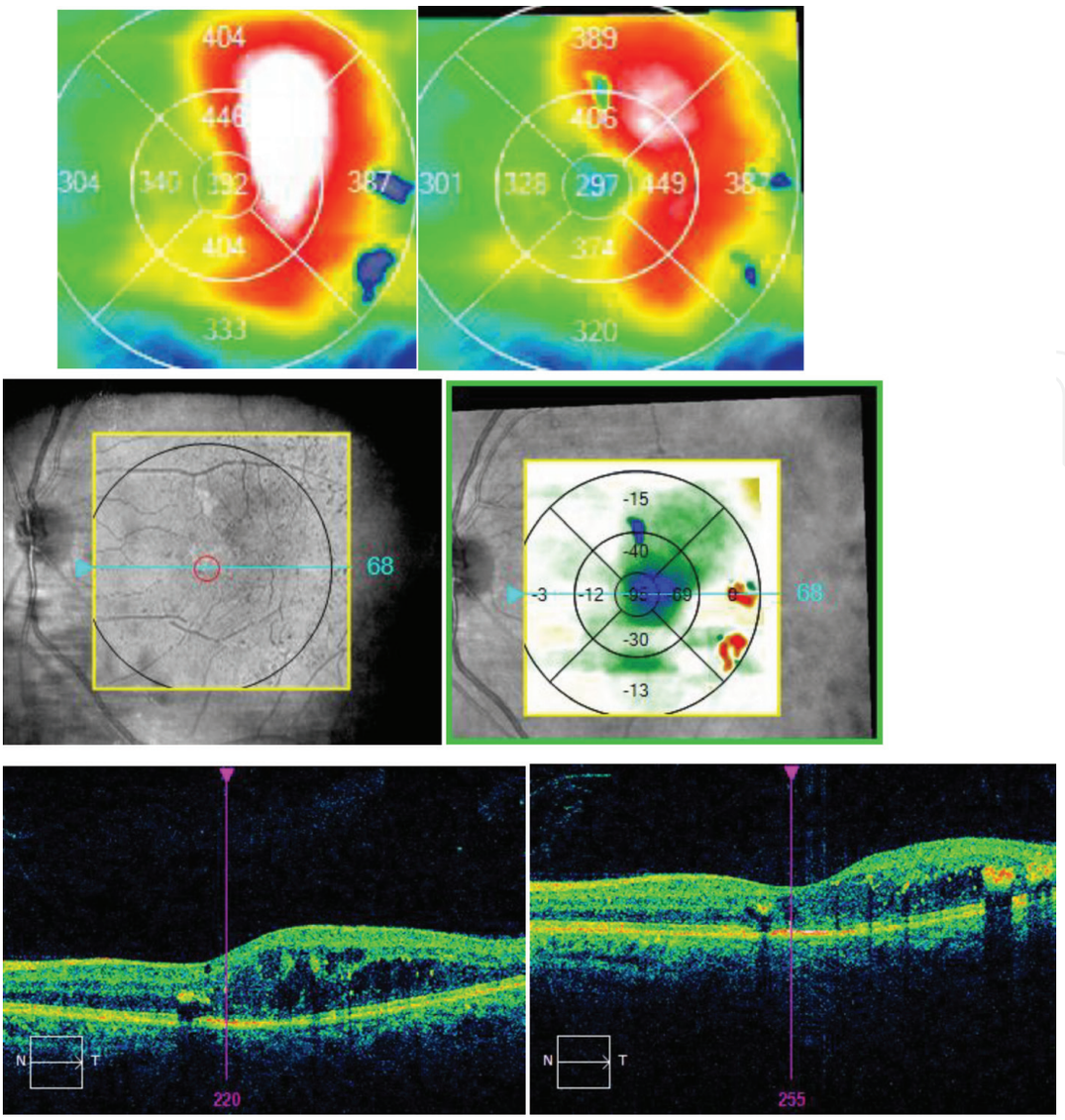

Figure 6.
Optical coherence tomography (OCT) macular change analysis (before and 1 month after intravitreal anti = VEGF): hard exudates intraretinal edema with disorganization of the normal foveal architecture; macular and paramacular temporal edema decreases in area and height (Dr. Ana Dascalu's private collection, Emergency University Hospital Bucharest, Ophthalmology Department).

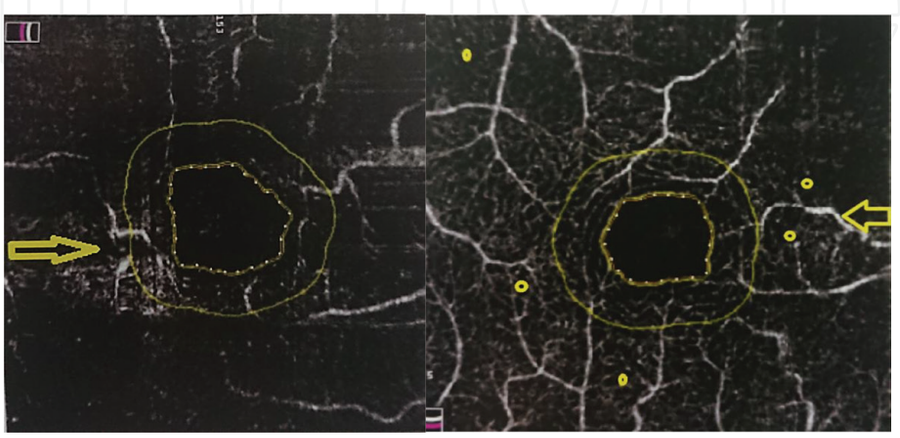

Figure 7.
OCT-A: (a) enlargement of FAZ and perifoveolar area of microvascular abnormalities; (b) mild FAZ enlargement, multiple microaneurysms (Dr. Daniela Stana's private collection, PhD Thesis, Emergency University Hospital Bucharest, Ophthalmological Department).

preventing and slowing the progression of DR. DCCT and UKPDS showed the importance of a good glycemic control in preventing microvascular damage in diabetes [68, 69]. Furthermore, every decrease with 10 mm Hg of systolic blood pressure is associated with a reduction of 35% in the risk of DR progression and of 50% in the risk of blindness [69]. Maintaining HbA_{1c} below 7.0% (53 mmol/mol) and a systolic blood pressure below 140 mmHg is considered a realistic therapeutic target in clinical practice. Currently, the recommended serum lipid levels in DM are an optimal LDL cholesterol concentration of <100 mg/dl and desirable triglycerides levels of <150 mg/dl [69–72].

2.7.2 Retinopathy screening

DR remains clinically silent, a long period of time until damages become irreversible. Ophthalmologic monitoring of DM subjects is essential. Frequency of screening depends on the severity of DR and the co-existence of risk factors. The follow-up schedule recommended by the ICO (International Council of Ophthalmology) Guidelines for Diabetic Eye Care is presented in **Table 2** [61].

2.7.3 Laser photocoagulation

2.7.3.1 Classical laser

Laser therapy has been used in DR for over 60 years and remains the mainstay of treating the ischemic retina. Applied early in severe NPDR and PDR, laser therapy leads to the prevention/regression of neovascularization and to the remission of retinal edema. Clinical studies confirm the effectiveness of laser photocoagulation by reducing vision loss by approximately 50% in patients with PDR. It is based on application of 1000-2000 laser shots, lasting 100 milliseconds, 200-250 mW of power with a size of 200-500 micrometers at the level of the middle and extreme periphery of the retina, spaced at a distance by a spot diameter, in order to destroy

DR staging	**Follow-up Schedule for ophthalmologists**	**Therapy**
No apparent DR	1-2 years	Observation
Mild NPDR	6-12 months	Observation
Moderate NPDR	3-6 month	Observation
Severe NPDR	<3 months;	Pan-retinal photocoagulation should be considered
Proliferative DR	<1 month;	Pan-retinal photocoagulation
Stable (Treated) PDR	6-12 months	Observation
Diabetic Macular Edema severity	**Follow-up Schedule for management by ophthalmologists**	
Noncentral-involved DME	3-6 month;	Focal laser photocoagulation should be considered
Central-involved DME	1-3 month;	Focal laser photocoagulation/ anti-VEGF therapy should be considered
Stable DME	3-6 month	Observation

Table 2.
ICO Guidelines for Diabetic Eye Care: screening and follow-up schedule for diabetic retinopathy.

the VEGF-secreting ischemic retina. Immediate complications are related to eye discomfort (tingling sensation/low-intensity pain) and mild ocular inflammation (caused by retinal burns). For this reason, it is recommended to space the laser photocoagulation in 3-4 sessions. In the long run, potential complications include hemeralopia, "fan shaped" visual field changes, or even concentric narrowing of the visual field through widening of scars and subretinal fibrosis. Other less frequent side effects are membrane injury, with secondary choroidal neovascularization, damage of ciliary nerves with permanently mydriasis and loss of accommodation, uveal effusion, angle closure glaucoma, serous retinal detachment, and vitreous hemorrhage [73–75].

2.7.3.2 Multispot laser

This technique allows the delivery of laser shots in a much shorter time and in a semi-automatic manner. These are much finer and at a lower intensity, threshold or subthreshold, causing lesser heat and consequently inflammation in the pigmented retinal epithelium. Clinical studies have shown that the effectiveness of the method is similar to classical laser, but fewer complications are encountered regarding retinal scarring and the impact on the visual field.

2.7.3.3 Laser photocoagulation in diabetic macular edema (DME)

In the case of exudative non-central DME, laser treatment may be considered as an alternative or in combination with intravitreal injections with anti-VEGF. In this case, the laser spots are finer, with a size of 50-100 μm, and lower energy. Laser treatment is inefficient in the case of ischemic macular edema, and so fluorangiography is necessary before therapeutic planning. In the early treatment diabetic retinopathy study (ETDRS) study, when comparing laser photocoagulation with no treatment, there was a decrease in DME from 24% to 12% after 3 years follow-up, while visual acuity improved in only 3% of patients [76].

2.7.4 Intravitreal anti-VEGF

Clinical and experimental studies have revealed increased VEGF concentration in ocular samples early in the evolution of DR, documenting its role both in increased vascular permeability and in vascular proliferation. Hence, anti-VEGF agents were used first in the treatment of DME and later in PDR management [77] (**Table 3**).

Clinical studies showed that Pegaptanib is the least effective in preventing neovascularization. Comparative studies between bevacizumab, ranibizumab and aflibercept found that they are all effective to decrease DME. However, aflibercept is most powerful in subjects with worse visual acuity [77, 78]. Still, the effect of intravitreal anti- VEGF is temporary and intravitreal therapy should be repeated according to clinical outcome.

2.7.5 Intravitreal steroids

In cases of DME resistant to anti-VEGF therapy after 3 monthly injections, intravitreal triamcinolone injection or fluocinolone are a therapeutic alternative to reduce DME and improve vision [79]. Their main untoward effects are cataract and transient increase of intraocular pressure.

	Description of the molecule	FDA approval	Dose
Pegaptanib (Macugen; Eyetech Inc, Cedar Knolls, NJ, USA)	RNA aptamer that binds to the heparin binding site of the VEGF-A165 isomer	For wet age related macular degeneration only	0.3 mg /0.09ml
Bevacizumab (Avastin; Genentech, San Francisco, CA, USA)	Full-length recombinant humanized anti-VEGF monoclonal antibody	No; "off-label" use	1.25 to 2.5 mg (0.05-0.1ml)
Ranibizumab (Lucentis; Genentech, San Francisco, CA, USA/ Novartis Ophthalmics, Basel, Switzerland)	Recombinant fragment of the humanized anti-VEGF monoclonal antibody; increased binding affinity for all VEGF isoforms	Yes	0.3 or 0.5 mg in 0.05 mL
Aflibercept (Eylea; Regeneron, Tarrytown, NY, USA)	Recombinant fusion protein of the binding domains of human VEGF-R1 and VEGF-R2, fused with the Fc domain of human IgG1 bind VEGF with greater affinity compared to other anti VEGF and prevent activation of VEGF-R	Yes	2mg/0.05mL

Table 3.
Anti VEGF agents used in DME treatment.

2.7.6 Surgical management of DR

Vitreoretinal surgery is crucial in managing advanced DR, in order to mitigate visual loss. Its main indications include vitreous hemorrhage interfering with photocoagulation, tractional and combined tractional and rhegmatogenous retinal detachment, dense premacular hemorrhage and DME with with vitreo-macular traction [80]. The objectives of surgical removal of the vitreous (vitrectomy) include removal of vitreous opacity (usually blood) and/or fibrovascular proliferation, relieving retinal traction, achieving retinal reattachment, and allowing completion of scatter laser photocoagulation. A large case series showed that sight threatening complications are rare and in approximately 90% of cases, vision is improved or stabilized [81]. Vitrectomy may also be beneficial for maculopathy when traction from the vitreous gel contributes to fluid accumulation.

3. Diabetic foot ulcers (DFUs)

3.1 Risk factors

The main risk factors of DFUs include DM duration and high HbA_{1c} [82–88]. The EURO-Diab group has identified hypertension, smoking and lipid disorders (hypertriglyceridemia, hypercholesterolemia) as additional risk factors [82, 83]. In Western countries, the male sex appears to be more commonly affected, with a risk ratio of 1.6. The co-existence of other microvascular complications (DR, nephropathy) increases the risk of DFUs.

Precipitating trauma is important. However, history of trauma is only identified in 48% of patients with DFUs. By contrast, foot injury without an apparent cause usually results from repeated minor injuries by inappropriate footwear [88–90].

Amin and Doupis have estimated that 45-60% of DFUs are mainly due to neuropathy, while 45% of DFUs are due to both diabetic neuropathy and peripheral arterial disease [87]. Like DR, diabetic neuropathy is also a very frequent DM complication and its prevalence increases with DM duration [90]. The other important driver of DFUs is ischemia from peripheral arterial disease (PAD). Visual impairment, foot deformities and past history of DFU also increase the risk of DFUs [85, 86].

3.2 Pathophysiology

The underlying mechanisms of DFUs include diabetic neuropathy, PAD and infection.

Diabetic neuropathy may affect the motor, sensory and autonomic nerves. Thickening of the basement membranes, endothelial hyperplasia in the vasa nervorum lead to thinning of the vascular lumen and secondary ischemia [90, 91]. On the other hand, metabolic disorders caused by chronic hyperglycemia, with the formation of AGEs, polyol pathways, increased oxidative stress levels and enzymatic activation of PKC also have direct toxic effects on nerve fibers.

Autonomic neuropathy causes arteriolo-venular shunts with secondary decreased blood flow in capillaries, but also anhidrosis, resulting in dry skin, thin, prone to cracking and ulceration. Sensory neuropathy leads to sensory (inability to feel warm/cold, pain, pressure), rendering the foot prone to undetected acute or chronic traumas [90, 91]. Motor neuropathy leads to imbalance between plantar flexors and extensors, with characteristic deformities, such as hammer toe, claw toe etc, and leading to high planter pressures in some small foot areas [92–94].

PAD leads to lower-extremity ischemia. In diabetes, it is usually located in the infra-popliteal arteries, less so at the iliofemoral level [89, 95]. Ischemia portends even more ominous outcomes [91, 96].

Pesently, micro and macrocirculatory disorders are considered not as separate entities, but more as a continuum in DM [97], neovascularization of vasa vasorum, with secondary hemorrhages and platelet aggregation facilitating the progression of atherosclerosis and intraluminal obstruction.

DFUs are frequently infected. The most common germs involved are staphylococci and streptococci, but deep infections are usually polymicrobial including gram positive, gram negative and even anaerobic germs [98, 99]. Chronic hyperglycemia and chronic hypoxia predispose to severe infections [99].

3.2.1 Clinical signs-staging systems

DFU represents any full-thickness ulcer below the ankle in DM. The initial signs and symptoms depend on the pathophysiological mechanism involved (neuropathy and/or PAD). Subjects with diabetic neuropathy are usually initially asymptomatic, but a minority of them may later develop neuropathic symptoms (numbness, paresthesia, lancinating or burning pain) with nocturnal exacerbation. In the event of PAD, intermittent claudication or even ischemic rest pain and gangrene may develop (**Figure 8**).

Usually, DFUs develop in an area exposed to increased pressure, with a non-healing tendency, often neglected in early stages due to diminished pain sensation. In the vent of infection, signs of local inflammation may be added (redness, swelling, pain, pus secretion etc).

Several staging systems were developed in order to characterize the pathogenic pathway and the severity and extension of ulcer. The International Working Group on the Diabetic Foot Risk Classification System (IWGDF) refers mainly at the severity of neuropathy and coexistence or not of the peripheral ischemia [100], while the Wagner classification describes the extension and depth [101] (**Tables 4** and **5**).

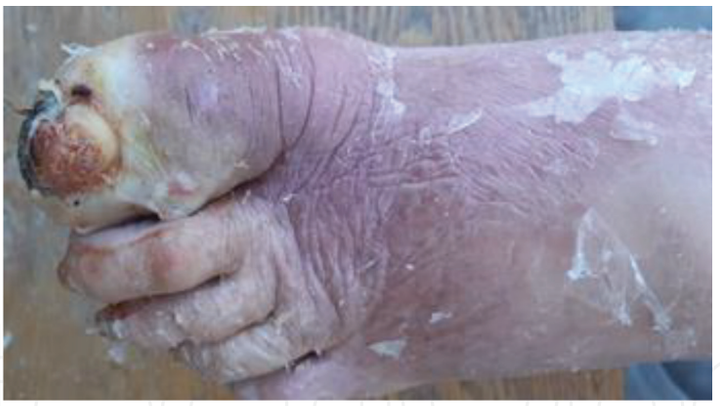

Figure 8.
Distal toe gangrene and extensive infection and inflammation at the level of the forefoot and mid foot (Dr. Dragos Serban's private collection).

Grade 0	Intact sensation
Grade 1	Diminished sensation
Grade 2	Diminished sensation+ foot deformities (hammertoes, claw toes) +/-peripheral arterial disease
Grade 3	Previous/present ulcer or amputation

Table 4.
IWGDF risk classification [96].

Grade 0	No ulcer in high-risk patients
Grade 1	Superficial ulcer
Grade 2	Ulceration involving tendons, ligaments, muscles, joints, not exposed to bone, without cellulitis or abscess
Grade 3	Deeper ulcers, with frequent bone complications of osteomyelitis, abscesses or cellulitis.
Grade 4	Forefoot gangrene.
Grade 5	Gangrene extended to midfoot/hindfoot

Table 5.
Wagner Classification of DFU [101].

3.3 Diagnosis

3.3.1 Clinical examination

Patient history is necessary to provide information on DM duration, glycemic control, associated risk factors and any prior lesions/amputations.

Clinical examination should look for skin disorders, foot deformities, nail lesions, blisters etc. also be documented. It must also include an evaluation of neuropathic deficits, PAD and infection. Signs of limb threatening infection include bullae, ecchymoses, soft tissue crepitus and rapid spread of infection [102, 103].

Evaluation of sensory neuropathy is very important to establish whether the patient has lost the protective sensation, making him prone to accidental trauma. Hot/cold discrimination, pain perception, light touch and vibration perception, as well as protective sensation must be tested [95–99]. The latter is best assessed by the 10 g Semmes Weinstein monofilament or the measurement of the vibration perception

threshold (VPT) with a neurothesiometer [93–95, 102, 103]. Tendon reflexes and muscular strength are also a part of the examination [95–99]. Finally, sudomotor dysfunction (reduced sweat production) is best examined by the Neuropad indicator test, which is based on a colour change from blue to pink [96, 97]. Indeed, this test has recently been identified as an independent risk factor of DFUs at 5 years [104].

3.3.1.1 Peripheral neuropathy screening

Evaluation of bilateral sensorial neuropathy in clinical practice requires neurological trained specialist and electrophysiological tests, which an increased burden on the national healthcare systems. In order to better select the patients who are more probably affected by neuropathy, a simpler tool was developed in 1994, namely Michigan Neuropathy Screening Instrument (MNSI) [105, 106]. It comprises a 2-step evaluation: first, a 15-item self-administered questionnaire that is scored by summing abnormal responses, followed by lower extremity examination (deformities, non-healing ulcers), assessment of ankle reflexes and of vibratory sensation. According to Herman and col., a score of more than 4 should raise the suspicion of peripheral sensorial neuropathy [106].

3.3.1.2 Peripheral arterial disease

Documenting the presence and the severity of ischemia is extremely important. Examination includes: a) palpation of peripheral pulses at the dorsalis pedis and the posterior tibial arteries; b) measurement of the ankle-brachial index (ABI) by a Doppler device [99, 100]. ABI evaluates the ratio of systolic arterial pressure at the brachial over the ankle level [107, 108]. Normal values range between 0.9-1.3, while values exceeding 1.3 point to calcified, uncompressible arteries, in which case the test cannot be used [99]. Similarly, one may measure the toe-brachial index (TBI), given that small digital arteries are rarely calcified: TBI<0.7 confirms the diagnosis of PAD [108]. More sophisticated evaluation (ultrasound, angiography) are used when necessary, especially to guide interventional treatment [95–99].

3.3.1.3 Assessment of the severity of the infection

If infection is suspected, it is best to use a tissue culture to identifying pathogens [109, 110]. X-rays, computed tomography and magnetic resonance imaging are used to evaluate bone infection or abscess formation, as well as to guide surgical treatment [86, 96, 97].

3.4 DFU management

3.4.1 Prophylaxis of DFU

Patients at risk of DFU should be managed by an interdisciplinary approach, including a diabetologist, a vascular surgeon, a podiatrist, a general surgeon, an orthopedic surgeon, a plastic surgeon and other specialists [82, 94, 102]. Stringent glycemic control is essential both in primary prevention of DFU and in ensuring wound healing. Management of high blood pressure and dyslipidemia is also important [86, 96, 97].

High-risk patients need education about the importance of wearing comfortable footwear, rigorous local hygiene, keeping feet dry and avoiding possible causes of local trauma (including barefoot walking) and frequent self-examinations [86, 96, 97]. Callus debridement, off-loading, and correct treatment of nail pathology are simple but

extremely efficient measures for the prevention of foot ulcers [86, 96, 97]. LEADER trial suggests that treatment with liraglutide in patients with type 2 diabetes and at high risk of CV events did not increase the risk of DFU events and was associated with a significantly lower risk of DFU-related amputations compared with placebo [109].

3.4.2 Therapeutic management of DFUs: main principles

Management of DFUs is aimed at correcting the pathogenic triad of neuropathy, PAD and infection. Off-loading with appropriate footwear and/or casts, debridement of callus and/or necrotic tissue, revascularization (by-pass grafting or intraluminal angioplasty) and infection control are the top priorities [86, 96, 97]. These may be aided by special dressings, skin substitutes, growth factors and other modalities [111–116]. Special care must be taken to recognize and promptly deal with emergencies requiring surgery and other urgent interventions [117].

4. Conclusions

Diabetic retinopathy and diabetic foot ulcer are both disabling complications, with a significant impact on the patient's quality of life and healthcare systems [118]. Microvascular impairment and local inflammation play a significant role in the both pathological mechanisms. Prevention and early detection, along with optimal control of blood sugar, hyperlipemia and arterial hypertension are the most efficacious measures against these fearful complications.

Conflict of interest

Peter Kempler has received honoraria from and/or is an advisory member of the following companies: Ely Lilly, Novo Nordisk, Novartis, Miro, Boehringer-Ingelheim, Woerwag-Pharma, Pfizer, Sanofi, Di-Care Zrt., 77 Elektronika Kft., Teva, Astra-Zeneca.

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References

[1] Ciulla TA, Amador AG, Zinman B. Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. Diabetes Care. 2003 Sep;26(9):2653-2664. doi: 10.2337/diacare.26.9.2653.

[2] Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BE. The Wisconsin Epidemiologic Study of Diabetic Retinopathy XXII: the twenty-five-year progression of retinopathy in persons with type 1 diabetes. Ophthalmology 2008;115(11):1859-1868.

[3] Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL 3rd, Klein R; American Diabetes Association. Retinopathy in diabetes. Diabetes Care. 2004;27 Suppl 1:S84-S87.

[4] Nathan DM, DER Group: The diabetes control and complications trial/epidemiology of diabetes interventions and complicationsstudy at 30 years: overview. Diabetes Care 2014;37(1): 9-16.

[5] Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med. 2017 Jun 15;376(24):2367-2375.

[6] Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. Ann N Y Acad Sci. 2018 Jan;1411(1):153-165.

[7] Moss SE, Klein R, Klein BE. Long-term incidence of lower-extremity amputations in a diabetic population. Arch Fam Med 1996;5:391-398

[8] Yau, J. W., Rogers, S. L., Kawasaki, R., Lamoureux, E. L., Kowalski, J. W., Bek, T., ... & Haffner, S. (2012). Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*, *35*(3), 556-564.

[9] King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. Br J Clin Pharmacol. 1999 Nov;48(5):643-648. doi: 10.1046/j.1365-2125.1999.00092.x.

[10] Barsegian A, Kotlyar B, Lee J, Salifu MO, McFarlane SI. Diabetic Retinopathy: Focus on Minority Populations. Int J Clin Endocrinol Metab. 2017;3(1):034-045. doi: 10.17352/ijcem.000027.

[11] UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes (UKPDS 38). BMJ 1998;317:703-713.

[12] Liu L, Quang ND, Banu R, Kumar H, Tham YC, Cheng CY, Wong TY, Sabanayagam C. Hypertension, blood pressure control and diabetic retinopathy in a large population-based study. PLoS One. 2020 Mar 5;15(3):e0229 665.

[13] Lurbe, E., Redon, J., Kesani, A., Pascual, J. M., Tacons, J., Alvarez, V., & Batlle, D. (2002). Increase in nocturnal blood pressure and progression to microalbuminuria in type 1 diabetes. *New England Journal of Medicine*, *347*(11), 797-805.

[14] **Klein R**, Klein BEK, Moss SE, *et al*. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XVII. The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. Ophthalmology 1998; 105:1801-1815.

[15] **Klein R**, Klein BEK, Moss SE, *et al*. Is blood pressure a predictor of the incidence or progression of diabetic retinopathy? Arch Intern Med 1989;149:2427-2432.

[16] Donaldson, M., Dodson, P. Medical treatment of diabetic retinopathy. *Eye* **17,** 550-562 (2003).

[17] Hadjadj S, Duly-Bouhanick B, Bekherraz A, BrIdoux F, Gallois Y, Mauco G, Ebran J, Marre M. Serum triglycerides are a predictive factor for the development and the progression of renal and retinal complications in patients with type 1 diabetes. Diabetes Metab. 2004;30(1):43-51.

[18] Klein BE, Moss SE, Klein R, Surawicz TS. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XIII. Relationship of serum cholesterol to retinopathy and hard exudate. Ophthalmology. 1991;98(8):1261-1265.

[19] Yu JY, Lyons TJ. Modified Lipoproteins in Diabetic Retinopathy: A Local Action in the Retina. J Clin Exp Ophthalmol. 2013 Dec 18;4(6):314. doi: 10.4172/2155-9570.1000314..

[20] Ankit BS, Mathur G, Agrawal RP, Mathur KC. Stronger relationship of serum apolipoprotein A-1 and B with diabetic retinopathy than traditional lipids. Indian J Endocrinol Metab. 2017 Jan-Feb;21(1):102-105.

[21] Chew EY, Klein ML, Ferris FL 3rd, Remaley NA, Murphy RP, Chantry K, Hoogwerf BJ, Miller D. Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy. Early Treatment Diabetic Retinopathy Study (ETDRS) Report 22. Arch Ophthalmol. 1996;114(9):1079-1084.

[22] Buraczynska M, Ksiazek P, Baranowicz-Gaszczyk I, Jozwiak L. Association of the VEGF gene polymorphism with diabetic retinopathy in type 2 diabetes patients, *Nephrology Dialysis Transplantation*, 22 (3):827-832, 2007.

[23] Jalal D, Kalia K: Impact of *VEGF* Gene Polymorphisms on Progression of Diabetic Retinopathy in an Indian Population**,** Biochemistry and Molecular Biology, 31(S1) 780.10-780.10, 2017.

[24] Awata T, Kurihara S, Takata N, Neda T, Iizuka H, Ohkubo T, Osaki M, Watanabe M, Nakashima Y, Inukai K, Inoue I, Kawasaki I, Mori K, Yoneya S, Katayama S. Functional VEGF C-634G polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes. Biochem Biophys Res Commun. 2005 Aug 5;333(3):679-685. doi: 10.1016/j.bbrc.2005.05.167. PMID: 15963467.

[25] Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P: Association of the VEGF Gene With Proliferative Diabetic Retinopathy But Not Proteinuria in Diabetes, Diabetes Mar 2004, 53 (3) 861-864; **DOI:** 10.2337/diabetes.53.3.861

[26] Braunger BM, Leimbeck SV, Schlecht A, Volz C, Jägle H, Tamm ER. Deletion of ocular transforming growth factor β signaling mimics essential characteristics of diabetic retinopathy. *Am J Pathol*. 2015;185(6):1749-1768.

[27] Beránek M, Kanková K, Benes P et al., "Polymorphism R25P in the gene encoding transforming growth factor-beta (TGF-β1) is a newly identified risk factor for proliferative diabetic retinopathy," *American Journal of Medical Genetics*, vol. 109, no. 4, pp. 278-283, 2002.

[28] Anjum, A., et al. "Diabetic Retinopathy and Sex Hormones." *OSR Journal of Dental and Medical Sciences (IOSR-JDMS)* 16.1 (2017): 110-5.

[29] Akushichi M, et al. "A Case of Progressive Diabetic Retinopathy Related to Pregnancy Followed on Optical Coherence Tomography Angiography." *Ophthalmic Surgery, Lasers and Imaging Retina* 50.6 (2019): 393-397.

[30] Jesia H, Chew EY. "Retinopathy in diabetic pregnancy." *A Practical Manual of Diabetes in Pregnancy* (2017): 269.

[31] Rosenn, B., Miodovnik, M., Kranias, G., Khoury, J., Combs, C. A., Mimouni, F., ... & Lipman, M. J. (1992). Progression of diabetic retinopathy in pregnancy: association with hypertension in pregnancy. *American journal of obstetrics and gynecology*, *166*(4), 1214-1218.

[32] Knol JA, van Kooij B, de Valk HW, Rothova A. Rapid progression of diabetic retinopathy in eyes with posterior uveitis. Am J Ophthalmol. 2006 Feb;141(2):409-412. doi: 10.1016/j.ajo.2005.09.005. PMID: 16458715.

[33] Denniston, Alastair K., et al. "The UK Diabetic Retinopathy Electronic Medical Record (UK DR EMR) Users Group, Report 2: real-world data for the impact of cataract surgery on diabetic macular oedema." *British Journal of Ophthalmology* 101.12 (2017): 1673-1678.

[34] Chung, Jin, et al. "Effect of cataract surgery on the progression of diabetic retinopathy." *Journal of Cataract & Refractive Surgery* 28.4 (2002): 626-630.

[35] Rübsam A, Parikh S, Fort PE. Role of Inflammation in Diabetic Retinopathy. *Int J Mol Sci*. 2018;19(4):942. Published 2018 Mar 22. doi:10.3390/ijms19040942

[36] Oswal KS, Sivaraj RR, Murray PI, Stavrou P. Clinical course and visual outcome in patients with diabetes mellitus and uveitis. *BMC Res Notes*. 2013;6:167. Published 2013 Apr 29. doi:10.1186/1756-0500-6-167

[37] Vagaja NN, Binz N, McLenachan S, et al: Influence of endotoxin-mediated retinal inflammation on phenotype of diabetic retinopathy in Ins2Akita miceBritish Journal of Ophthalmology 2013;97:1343-1350.

[38] Demircan N., Safran B.G., Soylu M., Ozcan A.A., Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (London) 2006;20:1366-1369. doi: 10.1038/sj.eye.6702138.

[39] Yoshida S., Yoshida A., Isнibashi T., Elner S.G., Elner V.M. Role of MCP-1 and MIP-1alpha in retinal neovascularization during postischemic inflammation in a mouse model of retinal neovascularization. J. Leukoc. Biol. 2003;73:137-144. doi: 10.1189/jlb.0302117.

[40] Rusnak S., Vrzalova J., Sobotova M., Hecova L., Ricarova R., Topolcan O. The measurement of intraocular biomarkers in various stages of proliferative diabetic retinopathy using multiplex xmap technology. J. Ophthalmol. 2015;2015:424783. doi: 10.1155/2015/424783

[41] Doganay S., Evereklioglu C., Er H., Turkoz Y., Sevinc A., Mehmet N., Savli H. Comparison of serum no, TNF-alpha, IL-1beta, SIL-2r, IL-6 and il-8 levels with grades of retinopathy in patients with diabetes mellitus. Eye (London) 2002;16:163-170. doi: 10.1038/sj/eye/6700095.

[42] Chalam K.V., Grover S., Sambhav K., Balaiya S., Murthy R.K. Aqueous interleukin-6 levels are superior to vascular endothelial growth factor in predicting therapeutic response to bevacizumab in age-related macular degeneration. J. Ophthalmol. 2014;2014:502174. doi: 10.1155/2014/502174.

[43] Zhu W, Meng Y, Wu Y, Lu J: Anti-diabetic medications and risk of macular edema in patients with type 2 diabetes: a systemic review and meta-analysis, Int J Clin Exp Med 2018;11(12):12889-12901

[44] Poulaki V, Qin W, Joussen AM, Hurlbut P, Wiegand SJ, Rudge J, Yancopoulos GD and Adamis AP. Acute intensive insulin therapy exacerbates

diabetic blood-retinal barrier breakdown via hypoxia-inducible factor-1alpha and VEGF. J Clin Invest 2002; 109: 805-815.

[45] Merante D, Menchini F, Truitt KE and Bandello FM. Diabetic macular edema: correlations with available diabetes therapies--evidence across a qualitative review of published literature from MEDLINE and EMBASE. Drug Saf 2010; 33: 643-652.

[46] Liazos E, Broadbent DM, Beare N and Kumar N. Spontaneous resolution of diabetic macular oedema after discontinuation of thiazolidenediones. Diabet Med 2008; 25: 860-862.

[47] Shin ES, Sorenson CM, Sheibani N. Diabetes and retinal vascular dysfunction. J Ophthalmic Vis Res. 2014 Jul-Sep;9(3):362-73. doi: 10.4103/2008-322X.143378. PMID: 25667739; PMCID: PMC4307665.

[48] Rodríguez ML, Pérez S, Mena-Mollá S, Desco MC, Ortega ÁL. Oxidative Stress and Microvascular Alterations in Diabetic Retinopathy: Future Therapies. Oxid Med Cell Longev. 2019 Nov 11;2019:4940825.

[49] Martins, B.; Amorim, M.; Reis, F.; Ambrósio, A.F.; Fernandes, R. Extracellular Vesicles and MicroRNA: Putative Role in Diagnosis and Treatment of Diabetic Retinopathy. *Antioxidants* **2020**, *9*, 705.

[50] Robinson R, Barathi VA, Chaurasia SS, Wong TY, Kern TS. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. Dis Model Mech. 2012 Jul;5(4):444-456.

[51] Intine RV, Sarras MP Jr. Metabolic memory and chronic diabetes complications: potential role for epigenetic mechanisms. Curr Diab Rep. 2012 Oct;12(5):551-559. doi: 10.1007/s11892-012-0302-7.

[52] Raczyńska D Zorena K, Urban B, Zalewski D, Skorek A, Malukiewicz G, Sikorski BL. "Current Trends in the Monitoring and Treatment of Diabetic Retinopathy in Young Adults", *Mediators of Inflammation*, vol. 2014, Article ID 492926, 13 pages, 2014. https://doi.org/10.1155/2014/492926

[53] Wu H., Hwang D.K., Song X., Tao Y. Association between aqueous cytokines and diabetic retinopathy stage. J. Ophthalmol. 2017;2017:9402198. doi: 10.1155/2017/9402198.

[54] Chang YC, Wu WC. Dyslipidemia and diabetic retinopathy. *Rev Diabet Stud*. 2013;10(2-3):121-132. doi:10.1900/RDS.2013.10.121

[55] Loukovaara, S., Piippo, N., Kinnunen, K., Hytti, M., Kaarniranta, K. and Kauppinen, A. (2017), NLRP3 inflammasome activation is associated with proliferative diabetic retinopathy. Acta Ophthalmol, 95: 803-808.

[56] Wheeler SE, Lee NY. Emerging Roles of Transforming Growth Factor β Signaling in Diabetic Retinopathy. J Cell Physiol. 2017 Mar;232(3):486-489. doi: 10.1002/jcp.25506. Epub 2016 Sep 21. PMID: 27472503.

[57] Lopes De Jesus CC. Diabetic Retinopathy, Diabetic Retinopathy, Mohammad Shamsul Ola, IntechOpen, 2012. DOI: 10.5772/28955. Available from: https://www.intechopen.com/books/diabetic-retinopathy/diabetic-retinopathy

[58] De La Cruz JP, Gonzalez-Correa JA, Guerrero A, De la Cuesta FS, et al. Pharmacological approach to diabetic retinopathy. Diabetes/metabolism research and reviews 2004;20:91-113.

[59] Siemianowicz K, Gminski J, Telega A, Wójcik A, Posielezna B, Grabowska-Bochenek R,Francuz T. Blood antioxidant parameters in patients with diabetic retinopathy.

International Journal of Molecular Medicine 2004;14:433-437.

[60] Mamputu JC, Renier G. Advanced glycation end-products increase monocyte adhesion to retinal endothelial cells through vascular endothelial growth factor-induced ICAM-1 expression: inhibitory effects of antioxidants. Journal of Leukocyte Biology 2004;75:1062-1069.

[61] http://www.icoph.org/downloads/Diabetic-Retinopathy-Scale.pdf

[62] Kanski J, Bowling B: Kanski's Clinical Ophthalmology 8th Edition, Saunders Ltd, 2015.

[63] Corcóstegui B, Durán S, González-Albarrán MO, Hernández C, Ruiz-Moreno JM, Salvador J, Udaondo P, Simó R. Update on Diagnosis and Treatment of Diabetic Retinopathy: A Consensus Guideline of the Working Group of Ocular Health (Spanish Society of Diabetes and Spanish Vitreous and Retina Society). J Ophthalmol. 2017;2017:8234186. doi: 10.1155/2017/8234186. Epub 2017 Jun 14.

[64] Jiménez-Báez MV, Márquez-González H, Bárcenas-Contreras R, Morales-Montoya C, Espinosa-García LF. (2015). Early diagnosis of diabetic retinopathy in primary care. *Colombia Médica*, *46*(1), 14-18.

[65] Spaide R. F., Klancnik J. M., Jr., Cooney M. J. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. *JAMA Ophthalmology*. 2015;133:45-50.

[66] Khadamy J, Abri Aghdam K, Falavarjani KG. An Update on Optical Coherence Tomography Angiography in Diabetic Retinopathy. J Ophthalmic Vis Res. 2018 Oct-Dec;13(4):487-497. doi: 10.4103/jovr.jovr_57_18.

[67] de Barros Garcia JM, Isaac DL, Avila M. Diabetic retinopathy and OCT angiography: clinical findings and future prespectives. *International Journal of Retina and Vitreous*. 2017;3:p. 14. doi: 10.1186/s40942-017-0062-2.

[68] Hsu CR, Chen YT, Sheu WH. Glycemic variability and diabetes retinopathy: a missing link. J Diabetes Complications. 2015 Mar;29(2):302-306. doi: 10.1016/j.jdiacomp.2014.11.013. Epub 2014 Dec 3. PMID: 25534877.

[69] Scanlon PH, Aldington SJ, Stratton IM. Epidemiological issues in diabetic retinopathy. Middle East Afr J Ophthalmol 2014;20:293-300. DOI: 10.4103/0974-9233.120007

[70] Berco E, Rappoport D, Pollack A, Kleinmann G, Greenwald Y: Management of Diabetic Retinopathy and Other Ocular Complications in Type 1 Diabetes, Major Topics in Type 1 Diabetes, Kenia Pedrosa Nunes, 2015, IntechOpen, DOI: 10.5772/61276.

[71] American Diabetes Association, author. Management of Dyslipidemia in Adults in Diabetes. Diabetes Care. 2007;25(1):S74–S77.

[72] Patel JI, Jenkins L, Benjamin L, Webber S. Dilated pupils and loss of accommodation following diode panretinal photocoagulation with sub-tenon local anaesthetic in four cases. Eye Lond Engl. 2002;16(5):628-632. doi: 10.1038/sj.eye.6700004.

[73] Moriarty AP, Spalton DJ, Shilling JS, Ffytche TJ, Bulsara M. Breakdown of the blood-aqueous barrier after argon laser panretinal photocoagulation for proliferative diabetic retinopathy. Ophthalmology. 1996;103(5):833-838. doi: 10.1016/S0161-6420(96)30607-6.

[74] Fong DS, Girach A, Boney A. Visual side effects of successful scatter laser photocoagulation surgery for proliferative diabetic retinopathy: a

literature review. Retina. 2007; 27(7):816-824. doi: 10.1097/IAE.0b013e318042d32c.

[75] Mori, Y., Suzuma, K., Uji, A. *et al.* Restoration of foveal photoreceptors after intravitreal ranibizumab injections for diabetic macular edema. *Sci Rep* **6,** 39161 (2016). https://doi.org/10.1038/srep39161

[76] Romero-Aroca P, Reyes-Torres J, Baget-Bernaldiz M, Blasco-Suñe C. Laser treatment for diabetic macular edema in the 21st century. Curr Diabetes Rev. 2014 Mar;10(2):100-12. doi: 10.2174/1573399810666140402123026. PMID: 24852439; PMCID: PMC4051253.

[77] Zhao Y, Singh RP. The role of anti-vascular endothelial growth factor (anti-VEGF) in the management of proliferative diabetic retinopathy. Drugs Context. 2018 Aug 13;7:212532. doi: 10.7573/dic.212532.

[78] Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, Browning DJ, Elman MJ, Ferris FL, Friedman SM, Melia M, Pieramici DJ, Sun JK, Beck RW: Diabetic Retinopathy Clinical Research Network,. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N Engl J Med. 2015 Mar 26;372(13):1193-1203. doi: 10.1056/NEJMoa1414264.

[79] Busch, C., Zur, D., Fraser-Bell, S. *et al.* Shall we stay, or shall we switch? Continued anti-VEGF therapy versus early switch to dexamethasone implant in refractory diabetic macular edema. *Acta Diabetol* **55,** 789-796 (2018).

[80] Ellis D, Burgess PI, Kayange P. Management of diabetic retinopathy. Malawi Medical Journal : the Journal of Medical Association of Malawi. 2013 Dec;25(4):116-120..

[81] Tesfaye S, Chaturvedi N, Eaton SE, *et al.* Vascular risk factors and diabetic neuropathy. N Engl J Med 2005; 352: 341-350.

[82] Vincent AM, Hinder LM, Pop-Busui R, *et al.* Hyperlipidemia: a new therapeutic target for diabetic neuropathy. J Peripher Nerv Syst 2009; 14: 257-267.

[83] Amin N, Doupis J. Diabetic foot disease: From the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. World J Diabetes. 2016 Apr 10;7(7):153-164. doi: 10.4239/wjd.v7.i7.153.

[84] Edo AE, Edo GO, Ezeani IU. Risk factors, ulcer grade and management outcome of diabetic foot ulcers in a Tropical Tertiary Care Hospital. Niger Med J. 2013 Jan;54(1):59-63. doi: 10.4103/0300-1652.108900

[85] Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med 2017;376: 2367-2375

[86] Boulton AJM, Armstrong DG, Kirsner RS, et al. Diagnosis and management of diabetic foot complications. Arlington (VA): American Diabetes Association; January 2020. PMID: 32105420

[87] Shahi SK, Kumar A, Kumar S, Singh SK, Gupta SK, Singh TB. Prevalence of diabetic foot ulcer and associated risk factors in diabetic patients from North India. J Diabetic Foot Complications. 2012;4:83-91.

[88] Moss SE, Klein R, Klein BE. The prevalence and incidence of lower extremity amputation in a diabetic population. Arch Intern Med. 1992;152: 610-616.

[89] Sharma M, Sharma A, Gothwal SR, Dixit S, Lunia AK, Sharma M. Diabetic foot ulcers: A prospective study of 100 patients based on wound based severity score. IOSR J Dent Med Sci. 2014;13:79-89.

[90] Yagihashi S, Mizukami H, Sugimoto K. Mechanism of diabetic

neuropathy: Where are we now and where to go? J Diabetes Investig. 2011 Jan 24;2(1):18-32. doi: 10.1111/j.2040-1124.2010.00070.x.

[91] Boulton AJ, Vinik AI, Arezzo JC, *et al.* Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care 2005; 28: 956-962

[92] Strotmeyer ES, de Rekeneire N, Schwartz AV, *et al.* Sensory and motor peripheral nerve function and lower-extremity quadriceps strength: the health, aging and body composition study. J Am Geriatr Soc 2009; 57: 2004-2010.

[93] Vinik AI, Liuzze FJ, Holland MT, *et al.* Diabetic neuropathies. Diabetes Care 1992; 15: 1926-1975

[94] Rogers LC, Frykberg RG, Armstrong DG, Boulton AJ, Edmonds M, Van GH, Hartemann A, Game F, Jeffcoate W, Jirkovska A, Jude E, Morbach S, Morrison WB, Pinzur M, Pitocco D, Sanders L, Wukich DK, Uccioli L. The Charcot foot in diabetes. Diabetes Care. 2011 Sep;34(9):2123-2129.

[95] Pendsey SP. Understanding diabetic foot. Int J Diabetes Dev Ctries. 2010; 30(2):75-79.

[96] Boulton AJM. The diabetic foot: grand overview, epidemiology and pathogenesis. Diabetes Metab Res Rev. 2008;24 Suppl 1:S3-S6.

[97] Papanas N, Maltezos E. The diabetic foot: established and emerging treatments. Acta Clin Bleg 2007; 62(4): 230-238.

[98] Ziegler D, Papanas N, Schnell O, Nguyen BDT, Nguyen KT, Kulkantrakorn K et al. Current concepts in the management of diabetic polyneuropathy. J diabetes Investig 2020;Sep 12 [Online ahead of print].

[99] Papanas N. Diabetic neuropathy collection: progress in diagnosis and screening. Diabetes Ther 2020;11: 761-764.

[100] Papanas N, Paschos P, Papazoglou D, Papatheodorou K, Paletas K, Maltezos E, Tsapas A. Accuracy of the Neuropad test for the diagnosis of distal symmetric polyneuropathy in type 2 diabetes. Diabetes Care. 2011;34(6):1378-1382.

[101] Papanas N, Boulton AJ, Malik RA, Manes C, Schnell O, Spallone V et al. A simple new non-invasive sweat indicator test for the diagnosis of diabetic neuropathy. Diabet Med. 2013;30(5):525-534.

[102] Brownrigg JR, Apelqvist J, Bakker K, Schaper NC, Hinchliffe RJ. Evidence-based management of PAD & the diabetic foot. Eur J Vasc Endovasc Surg. 2013;45(6):673-681. doi:10.1016/j.ejvs.2013.02.014

[103] Vas, PRJ, Edmonds, M, Kavarthapu, V, et al. The diabetic foot attack: "tis too late to retreat!" Int J Low Extrem Wounds. 2018;17:7-13.

[104] Panagoulias GS, Eleftheriadou I, Papanas N, Manes C, Kamenov Z, Tesic D et al. Dryness of foot skin assessed by the visual indicator test and risk of diabetic foot ulceration: a prospective observational study. Front Endocrinol (Lausanne). 2020;11:625.

[105] Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care. 1994 Nov;17(11):1281-1289. doi: 10.2337/diacare.17.11.1281. PMID: 7821168.

[106] Herman WH, Pop-Busui R, Braffett BH, et al. Use of the Michigan Neuropathy Screening Instrument

as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabet Med*. 2012;29(7):937-944. doi:10.1111/j.1464-5491.2012.03644.x

[107] Høyer C, Sandermann J, Petersen LJ. The toe-brachial index in the diagnosis of peripheral arterial disease. J Vasc Surg. 2013 Jul;58(1):231-238. doi: 10.1016/j.jvs.2013.03.044.

[108] Potier L, Halbron M, Bouilloud F, Dadon M, Le Doeuff J, Ha Van G, Grimaldi A, Hartemann-Heurtier A. Ankle-to-brachial ratio index underestimates the prevalence of peripheral occlusive disease in diabetic patients at high risk for arterial disease. Diabetes Care. 2009;32(4):e44.

[109] Dhatariya K, Bain SC, Buse JB, Simpson R, Tarnow L, Kaltoft MS, Stellfeld M, Tornøe K, Pratley RE; LEADER Publication Committee on behalf of the LEADER Trial Investigators. The Impact of Liraglutide on Diabetes-Related Foot Ulceration and Associated Complications in Patients With Type 2 Diabetes at High Risk for Cardiovascular Events: Results From the LEADER Trial. Diabetes Care. 2018 Oct;41(10):2229-2235. doi: 10.2337/dc18-1094.

[110] Demetriou M, Papanas N, Panopoulou M, Papatheodorou K, Bounovas A, Maltezos E. Tissue and swab culture in diabetic foot infections: neuropathic versus neuroischemic ulcers. Int J Low Extrem Wounds. 2013;12(2):87-93.

[111] Demetriou M, Papanas N, Panagopoulos P, Panopoulou M, Maltezos E. Antibiotic resistance in diabetic foot soft tissue infections: a series from Greece. Int J Low Extrem Wounds. 2017;16(4):255-259.

[112] Papanas N, Maltezos E. Becaplermin gel in the treatment of diabetic neuropathic foot ulcers. Clin Interv Aging. 2008;3(2):233-240.

[113] Eleftheriadou I, Samakidou G, Tentolouris A, Papanas N, Tentolouris N. Nonpharmacological management of diabetic foot ulcers: an update. Int J Low Extrem Wounds. 2020; Oct 19:1534734620963561. doi: 10.1177/1534734620963561. Online ahead of print.

[114] Papanas N, Demetzos C, Pippa N, Maltezos E, Tentolouris N. Efficacy of a new heparan sulfate mimetic dressing in the healing of foot and lower extremity ulcerations in type 2 diabetes: a case series. Int J Low Extrem Wounds. 2016;15(1):63-67.

[115] Perren S, Gatt A, Papanas N, Formosa C. Hyperbaric oxygen therapy in ischaemic foot ulcers in type 2 diabetes: a clinical trial. Open Cardiovasc Med J. 2018;12:80-85.

[116] Papanas N, Papachristou S, Kefala C, Kyroglou S. Use of the new Vergenix soft tissue repair matrix to heal a chronic neuropathic diabetic foot ulcer. Int J Low Extrem Wounds. 2020;19(2):205-206.

[117] Vas PRJ, Edmonds M, Kavarthapu V, Rashid H, Ahluwalia R, Pankhurst C, Papanas N. The diabetic foot attack: "'tis too late to retreat!" Int J Low Extrem Wounds. 2018;17(1):7-13.

[118] Papanas N, Papi M, Rerkasem K. A wealth of science. Int J Low Extrem Wounds. 2020;19(1):5-6