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# The diversity of angiogenesis in diabetic vascular complications

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## ABSTRACT

Diabetes is one of the major healthcare problems and it is considered a global epidemic of the 21st century. Long lasting hyperglycaemia contributes to the development of vascular diabetic complications such as retinopathy, nephropathy or diabetic foot syndrome (DFS). The pathogenesis of each diabetic complication is multifactorial. Nevertheless, impaired angiogenesis is one potential component that might be common for many diabetic complications. Angiogenesis is a multi-stage process involving the endothelium, growth factors and their inhibitors, cytokines, endothelial progenitor cells (EPCs) and enzymes. As far as angiogenesis is concerned, diabetes is a paradoxical disease. An excessive angiogenesis is noted in retinopathy or nephropathy, while in diabetic foot syndrome the angiogenic response is insufficient.

**Key words:** angiogenesis, diabetic foot syndrome, retinopathy, nephropathy

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## Introduction

Modern medicine deals with neurodegenerative and cardiovascular diseases increasingly well. After years of high mortality because of heart diseases, now it is time to focus on metabolic disorders, among which diabetes, especially type 2 diabetes mellitus (T2DM), has been playing the key role for centuries.

Since the beginning of this century, an epidemic, or even a pandemic, of diabetes has been observed. Type 2 diabetes has become one of the most life-threatening metabolic diseases for two reasons. Firstly, increasing hyperglycaemia and insulin resistance remain asymptomatic for a long time. The dysfunction of as much as 50% of pancreatic beta cells induces a clinical manifestation. Secondly, untreated or wrongly controlled diabetes contributes to the development of micro- and macroangiopathies that significantly reduce the quality of a patient's life [1]. The vascular complications of diabetes may include retinopathy, nephropathy and diabetic foot syndrome (DFS).

Retinopathy is the leading cause of blindness in the population in developed countries [2]. Nephropathy often leads to renal failure [3]. Amputations, which involve a high risk of mortality, are performed more often in patients with DFS than non-diabetic patients [4]. Thus, the development of diabetic vascular complications is a huge social and economic, and above all

medical, challenge. Understanding the molecular basis of microangiopathy may be the key to the selection of appropriate therapy, because vascular complications are rarely found selectively.

## Vascular complications of diabetes

### Retinopathy

Diabetic retinopathy (DR) is one of the complications of long-lasting and poorly controlled diabetes. According to the World Health Organisation (WHO), 150 million people around the world suffer from retinopathy, and by 2025 this number is expected to have doubled [5]. The epidemiological data indicates that DR develops in 60% of patients with type 2 diabetes that has lasted for more than 20 years, and in the majority of patients with type 1 diabetes. Taking the presence/absence of abnormal new blood vessels as a criterion, there are three DR characters: nonproliferative retinopathy, proliferative retinopathy and macular oedema [2, 5]. Pathologic changes occur in the retina, but at more advanced stages of the disease they may include other parts of the eye (iris, angle of the eye) [2, 5]. Retinopathy is characterised by excessive growth of blood vessels with increased permeability [5]. Hyperglycaemia plays an important role in the pathogenesis of diabetic ret-

inopathy. It activates the polyol metabolic pathway, generates oxidative stress, activates protein kinase C and leads to the production of advanced glycation end products (AGEs) [6–8]. All changes taking place under the influence of hyperglycaemia lead to abnormal blood vessel formation. Excessive formation of immature blood vessels induced by vascular endothelial growth factor (VEGF) is the major disorder in the pathogenesis of diabetic retinopathy.

### Nephropathy

Kidneys are also under the toxic effect of hyperglycaemia. Diabetic nephropathy is the most common cause of end stage renal disease in diabetic patients [9]. The risk of chronic kidney disease is higher in patients with type 1 diabetes, but paradoxically more nephropathy patients are encountered in the population of type 2 diabetes.

Similarly, as in retinopathy, the mechanisms leading to the development of nephropathy are complex. Kidney damage in patients with diabetes is caused by haemodynamic and metabolic changes. Ischaemia, hypoxia, hypertension, oxidative stress, hyperlipidaemia, chronic inflammation, and hyperglycaemia lead to extracellular and intracellular changes in the function and structure of the kidney [10, 11].

Diabetic nephropathy is characterised by glomerular hyperfiltration, increased vascular permeability, alteration within the basement membrane of the capillaries, and mesangial proliferation. The growth factors VEGF, TGF beta (transforming growth factor beta), PDGF (platelet derived growth factor) and enzymes (heparanase) are involved in the pathogenesis of diabetic nephropathy [12, 13,]. Adaptive changes at the early stages of nephropathy involving the secretion of angiogenic factors (mainly VEGF) only initiate the process of angiogenesis, and they are the first step towards the degeneration of kidneys [11], which once started cannot be stopped. Risk factors such as smoking, obesity, and genetics may alter the rate of progression of nephropathy [9], so it is extremely important to select the correct treatment, as treatment may influence disease progression. Strict control of blood pressure, good diabetes control and adequate pharmacotherapy (medicines of the renin-angiotensin system (RAS), angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs)) may slow the changes in the kidney [3].

### Diabetic foot syndrome

It is believed that diabetic foot syndrome (DFS) is the most medically and socio-economically devastating complication of diabetes. DFS significantly increases mortality in diabetic patients [10]. The high mortality

is associated with the development of non-healing wounds, which are caused by neuropathy, progressive ischaemia and impaired angiogenesis [14]. Wound contamination by bacteria, which leads to non-healing ulcers, is the factor increasing the mortality rate in patients with DFS. According to the International Diabetes Federation (IDF), each year nearly 4 million ulcers are diagnosed [15–17]. Diabetic foot ulcers represent 84% of all lower limb amputations [10]. Interestingly, DFS develops also in patients with newly diagnosed diabetes [10]. Abnormal angiogenesis plays the main role in the pathogenesis of DFS. It impairs the phase of migration and proliferation of cells. Wound healing consists of four phases: coagulation, inflammation, migration with proliferation, and remodelling. Angiogenesis occurs during the migration and proliferation phase of the wound healing process [14]. The formation of normal blood vessels is a critical point during the healing process, because of supplying the surrounding tissue with oxygen. Abnormal vascular structures are not able to provide optimal blood supply, resulting in an increase of hypoxia. Progressive ischaemia and hypoxia lead to tissue necrosis.

The aetiology of microangiopathy is different and multifactorial, but a common component of vascular diabetic complications might be the abnormal angiogenesis.

### Angiogenesis in the diabetic vascular complications

Physiological angiogenesis is a process of interaction between specific factors, cytokines, enzymes and cells, whose appearance at the appropriate concentration, quantity and time results in new blood vessels formation. Despite the complexity of this process, some steps can be identified: increase of the permeability of existing vessels, stimulation of endothelial cells (ECs morphological change), the degradation of the extracellular matrix, the migration and proliferation of endothelial cells (tubule vessel formation), the maturation of new blood vessels (stabilisation by pericytes and establishment of intercellular connections) [11, 15]. A balance between angiogenesis stimulators and inhibitors ensures that new, properly functioning blood vessels form. The deterioration of this balance leads to an excessive vascularisation or to insufficient blood vessel formation. Abnormal angiogenesis occurs in many pathological conditions such as cancer, impaired wound healing, and cardiovascular diseases [10]. Diabetes, which is a metabolic disorder, is extremely fascinating from the angiogenic point of view. This is because of the development of diabetic vascular complications, where abnormal formation of blood vessels plays a key role in the pathogenesis. Hypoxia, which is caused by hyperglycaemia, may develop. The effects of angiogenesis, as a result of hypoxia, are different in

various organs. Thus, impaired angiogenesis may lead to excessive formation of blood vessels with increased permeability in the eye (retinopathy), increased permeability of blood vessels in the kidneys (nephropathy), and insufficient vessel formation in hypoxic tissues during the healing process (diabetic foot syndrome).

Vascular endothelial growth factor (VEGF) is the best known regulator of angiogenesis. The increased concentration of this factor has been observed in the vitreous in patients with proliferative diabetic retinopathy (PDR) [7, 17]. Studies have shown that serum VEGF levels vary with changes in the progression of retinopathy. Ozturk et al. observed a higher concentration of VEGF in the serum of patients with nonproliferative retinopathy compared to a group with PDR. In this study, significant correlations between serum VEGF and the duration of diabetes and the cytokines IL-1 $\alpha$ , IL-6 were noted [18].

Raised VEGF expression is also observed in diabetic nephropathy, but only at the early stages of the disease. Studies in animal models [19, 20] suggest that the level of VEGF decreases with progressive renal injury. Similar results were reported by Bortoloso et al. in patients with type 2 diabetes [21]. This study showed a negative correlation between glomerular mRNA VEGF and AER (albumin excretion rate) and the relationship of VEGF and mRNA VEGF-R2 (vascular endothelial growth factor receptor 2). However, there was no relationship between VEGF and VEGF-R1 (vascular endothelial growth factor receptor 1). [21]. The increased expression of VEGF at the initial stages of nephropathy may be the result of toxic effects of hyperglycaemia and glomerular hypertension. It seems that at this stage the increased VEGF secretion has a protective function. However, initiated angiogenesis contributes to the destruction of renal structures. Damaged podocytes and endothelial cells (the major source of VEGF in the kidney) are not able to produce VEGF, and therefore its expression decreases with the progress of nephropathy [11, 22].

Studies of VEGF in patients with diabetic foot syndrome shows that the concentration of this factor is reduced [14]. In studies performed in the 1990s, decreased levels of basic fibroblast growth factor (bFGF), VEGF and PDGF in experimental diabetic wounds were observed [23–26]. Later studies have confirmed the reduced levels of VEGF and PDGF during wound healing in diabetic animals [27, 28].

More and more attention is being paid to the contribution of the extracellular matrix (ECM) in the process of blood vessel formation. ECM is not only the environment for the cells. ECM is an environment where there are numerous enzymes, cytokines and proteins that enable signal transduction [15]. Extracellular matrix components are produced by various cells, among others by fibroblasts, in response to stimulating factors. Transforming growth factor beta (TGF- $\beta$ ) is one of the factors

that induce the production of ECM components and increase the expression of PDGF, FGF and VEGF, that shows its proangiogenic properties [29]. The study of endothelial cell cultures and retinal pericytes shows that TGF- $\beta$  may play an important role in the pathogenesis of diabetic retinopathy. Van Geest et al. have observed that TGF- $\beta$  has a greater influence on the pericytes than endothelial cells and may regulate the synthesis of ECM [29]. Despite the fact that the role of TGF- $\beta$  in the pathogenesis of diabetic retinopathy is not fully understood, in the nephropathy TGF- $\beta$  is considered to be a factor causing basal lamina thickening in the glomerulus [29]. Enzymes play no less important a role in angiogenesis than next-angiogenic growth factors. One of them is heparanase, the function of which has been well studied in the pathogenesis of cancers. Heparanase cleaves heparan sulphate (HS), the main polysaccharide of basement membrane (BM). HS ensures the integrity of the basement membrane and controls the activity of cytokines, growth factors, through their ECM binding. The degradation of HS by heparanase leads to the elimination of ECM barriers and the release of bioactive molecules. In this way, heparanase emphasises its participation in tissue remodelling, angiogenesis and inflammation [13]. Very few studies have shown that heparanase activity is increased in the retina neovascularisation. Increased expression of this enzyme was observed in STZ-diabetic rats [streptozotocin-diabetic rats], that may lead to the development of retinopathy [30, 31]. In nephropathy, increased activity of heparanase has also been observed. Shafan et al. reported higher levels of heparanase in the plasma and increased urinary excretion in patients with T2DM. Furthermore, a relationship between heparanase in urine and blood glucose has been observed [32]. Rops et al. reported increased urinary heparanase activity [33]. Unfortunately, so far there is no data on heparanase in DFS. It is well known that abnormal angiogenesis delays diabetic wound healing. In 2007, Kawanabe et al. published a study that shows that sphingosine-1-phosphate [S1P] can accelerate the healing process in mice with induced diabetes [34]. Platelets and haematopoietic cells are a known source of S1P [35]. Sphingosine phosphate is responsible for the migration and proliferation of endothelial cells; it is required for the maturation of blood vessels and it is involved in angiogenesis, inflammation, wound healing, and thrombosis [34, 35]. S1P transmits a signal by specific receptors. It enables the formation of N-cadherin-based connections between endothelial cells and smooth muscle cells [36]. Thus, S1P contributes to generating stable blood vessels. Kawanabe et al. found that administration of S1P as a subcutaneous injection significantly accelerated wound healing in diabetic mice compared to control mice (controls did not receive S1P injections) [34]. A study by Skoura et al.

reported increased sphingosine-1-phosphate receptor (S1P<sub>2</sub>R) expression in the retina of mice under the influence of hypoxia. The researchers suggest that hypoxic conditions may contribute to neovascularisation in the eye [36]. Increased levels of S1P were also observed at the early stages of nephropathy [37]. Among many growth factors, enzymes and angiogenic cytokines, also erythropoietin (Epo) is worth noting. Epo shows a similar activity to VEGF, but their mechanism of action is independent [38]. It has been shown that Epo-induced angiogenesis promotes wound healing, remodelling myocardial ischaemia and reperfusion in the brain [39, 40]. The role of Epo in the pathogenesis of diabetic retinopathy is not fully understood. Studies have shown elevated levels of Epo in the vitreous [39]. However, supplementation with Epo in the early stages of diabetic retinopathy may have a protective effect on neurons and a suppressive effect on hypoxia-induced neovascularisation [39]. We have not found any studies reporting on Epo in angiogenesis during nephropathy and DFS.

The pathogenesis of diabetic vascular complications involves a variety of processes; however, angiogenesis is closely related to inflammation. Basic fibroblast growth factor (FGF-2) is one of the factors that have both pro-inflammatory and proangiogenic properties [16]. Inflammation may initiate FGF-dependent angiogenesis. FGF-2 increases the expression of VEGF, cyclooxygenase-2 (COX-2), IL-6 or osteopontin (OPN, monocyte chemotactic factor), and activates fibroblasts [9, 16]. Fibroblasts also proliferate in response to PDGF and TGF- $\beta$ . They produce erythropoietin and are responsible for maintaining extracellular homeostasis through the synthesis of ECM components [9]. Fibroblasts phenotypic change is observed in patients with non-healing wounds. The authors indicate a decreased migration and proliferation of fibroblasts isolated from ulceration and callus. Moreover, fibroblasts derived from diabetic ulcers are characterised by a reduced response to angiogenic factors [41, 42].

## Conclusions

The process of blood vessels formation is very important for diabetic patients. Insufficient angiogenesis contributes to impaired wound healing, while excessive vessels formation may lead to blindness. There are many well known growth factors, enzymes or cytokines involved in angiogenesis.

Unfortunately, it still remains to be elucidated why in a diabetic person both insufficient and excessive angiogenesis occurs. This may be an effect of prolonged hyperglycaemia on structurally different tissues. Future studies will bring new insights into the field of vascular diabetic complications.

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