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VEGF-A and PDGF-BB — angiogenic factors and the stage of diabetic foot syndrome advancement

VEGF-A i PDGF-BB— czynniki angiogenne a stopień zaawansowania zespołu stopy cukrzycowej

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Abstract

Introduction: In patients with diabetic foot syndrome (DFS), an inadequate angiogenic response is observed. The aim of this study was to evaluate the concentrations of VEGF-A, PDGF-BB, sVEGF-R2 and sVEGF-R1 in patients with diabetes-complicated diabetic foot syndrome and analyse them using selected clinical data.

Material and methods: Forty seven diabetic patients, 25 women mean age 63 and 20 men mean age 60.5, with diabetic foot syndrome (DFS) were enrolled in the experimental group. To evaluate angiogenesis factors depending on Wagner grade, the subjects were divided into three subgroups: I — patients with 0 Wagner grade (n = 14); II — patients with 1,2,3 Wagner grades (n = 15); and III — patients with 4,5 Wagner grades (n = 18). The control group consisted of 20 healthy volunteers. The material for research was blood.

Results: Significantly higher levels of VEGF-A and PDGF-BB in the DFS cases compared to controls were observed (VEGF-A p = 0.000001; PDGF-BB p = 0.000051). Analysis of angiogenic parameters according to the stage of diabetic foot syndrome advancement showed higher VEGF-A level (I: p = 0.000867; II: p = 0.001827; III: p = 0.000024) and PDGF-BB (respectively p = 0.004113, p = 0.004224, p = 0.002480) in all the subgroups. Decreased sVEGF-R2 concentrations were observed in the I (p = 0.054) subgroup and the III (p = 0.03524) subgroup. In this study, a strong positive correlation between VEGF-A and PDGF-BB was observed (R = 0.66; p = 0.000001).

Conclusions: Our study revealed that proangiogenic factor levels were increased in DFS. This is associated with lower limb ischaemia and hypoxic conditions. The stage of diabetic foot syndrome advancement influenced VEGF-A and PDGF-BB concentrations. **(Endokrynol Pol 2014; 65 (4):306–312)**

Key words: *angiogenesis; diabetic foot syndrome; vascular endothelial growth factor; platelet-derived growth factor*

Streszczenie

Wstęp: U pacjentów z zespołem stopy cukrzycowej (DFS) obserwuje się nieprawidłową odpowiedź angiogenną. Celem naszej pracy była ocena stężenia VEGF-A,PDGF-BB, sVEGF-R2 i sVEGF-R1u pacjentów z cukrzycą powikłaną ZSC oraz ich analiza pod względem wybranych parametrów klinicznych.

Materiał i metody: Do badania zakwalifikowano 47 pacjentów z ZSC: 25 kobiet (średnia wieku 63 lata), 22 mężczyzn (średnia wieku 60,5 lata). Aby uwzględnić stopień zaawansowania zmian, grupa badana została podzielona na 3 podgrupy: I — 0 stopień Wagnera (n = 14), II — 1, 2, 3 stopień Wagnera (n = 15) oraz III — 4, 5 stopień Wagnera (n = 18). Grupę kontrolną stanowiło 20 zdrowych ochotników. Materiałem do badań była krew żylna.

Wyniki: Zaobserwowano znacząco wyższe stężenia VEGF-A I PDGF-BB u pacjentów w porównaniu do grupy kontrolnej (VEGF-A p = 0,000001; PDGF-BB p = 0,000051). Analizując badane parametry z uwzględnieniem stopnia zaawansowania zmian odnotowano wysokie stężenia VEGF-A (I: p = 0,000867; II: p = 0,001827; III: p = 0,000024) i PDGF-BB (odpowiednio p = 0,004113, p = 0,004224, p = 0,002480) we wszystkich podgrupach. Obniżone stężenie sVEGF-R2 zaobserwowano w I (p = 0,054) i w III (p = 0,03524) podgrupie. W badaniu wykazano pozytywną zależność między stężeniem VEGF-A I PDGF-BB (R = 0,66; p = 0,000001).

Wnioski: U pacjentów zaobserwowano podwyższone stężenia czynników proangiogennych, co związane jest z silnym niedokrwieniem kończyny dolnej oraz istniejącym stanem hipoksji. Stopień zaawansowania zmian znacząco wpływa na stężenia VEGF-A i PDGF-BB. **(Endokrynol Pol 2014; 65 (4): 306–312)**

Słowa kluczowe: *angiogeneza; zespół stopy cukrzycowej; naczyniowo śródbłonkowy czynnik wzrostu; płytkopochodny czynnik wzrostu*

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Introduction

The complexity of angiogenesis, the variety of growth factors and cytokines taking part in this process, the function of the endothelial progenitor cells, and an increasing role for this process in the pathogenesis of many diseases has meant that angiogenesis has been arousing increasing interest in the world of science. In this remarkable process of blood vessel formation, a lot of cytokines and growth factors are involved, of which vascular endothelial growth factors are the most important.

Angiogenesis plays an important role in wound healing. Healing begins at the moment of tissue injury, and the whole process can take weeks, months or even years. The process of wound healing includes four stages: haemostasis, inflammation, proliferation and tissue remodelling [1, 2]. Vasoconstriction, platelet adhesion, aggregation and their degranulation and the activation of plasma cascade coagulation occur at the site of the injured vessel. In the inflammatory phase, neutrophils, monocytes/macrophages and lymphocytes are involved. These cells migrate from the circulation in response to chemotactic factors released from injured tissue and by other inflammatory cells [1]. Proliferation includes epithelialisation, collagen synthesis, matrix formation and angiogenesis [1]. The final phase of wound healing consists of collagen remodelling, fibroblast transformation into myofibroblasts, and extracellular matrix reorganisation [1–3]. The reconstruction of vascular structures in damaged tissue is the most important stage of healing due to the restoration of normal blood circulation [3].

Physiologically, angiogenesis is a dynamic process regulated by numerous factors, and a strong balance is preserved between stimulators and inhibitors. Fully matured, correctly functioning blood vessel formation requires the co-operation of all cytokines, integrins, and receptor proteins because a deficiency, excess or malfunction of one component of the cascade process may contribute to pathological angiogenesis development. Abnormal blood vessel creation and associated impaired wound healing are observed in patients with diabetic foot syndrome (DFS).

In 2011, according to the International Diabetes Federation, 366 million people worldwide suffered from diabetes [4]. Diabetic foot syndrome is characterised by a triad: neuropathy, ischaemia and infection is a most unfortunate late diabetic complication due to reduced quality of life associated with amputation. Infections are the most frequent consequence of DFS and led to non-healing wounds resulting from impaired angiogenesis. 15%-20% of patients with DFS require amputation [5]. Improving the process of blood vessel formation is associated with the acceleration of wound healing.

In 1997, the US Food and Drug Administration approved only one growth factor for the treatment of chronic lower extremity diabetic neuropathic ulcers, which was rhPDGF-BB [6]. Platelet-derived growth factor (PDGF) is responsible for angiogenesis induction, and in co-operation with basic fibroblast growth factor stabilises the newly formed vessels by pericytes recruitment [7, 8]. Thus PDGF plays an essential role in all stages of blood vessel creation: in the early days of angiogenesis, it is released from platelets and endothelial cells, and after a few weeks it takes part in remodelling involving collagen turnover and cross-linking. PDGF freed from platelets acts chemotactically for neutrophils, macrophages and fibroblasts that migrate into the site of injured tissue. After 2–3 weeks, collagenase expression, active collagen turnover and cross-linking are the effect of PDGF [6]. PDGF induces vascular structure formation through priming vascular smooth muscle cells/pericytes (VSMCs/pericytes) to release angiogenic factors, including vascular endothelial growth factor (VEGF) [6]. PDGF-AB (heterodimer) is freed by platelets, and PDGF-BB (homodimer) is synthesised by endothelial cells (ECs), megakaryocytes and muscle cells. Both heterodimer and homodimer initiate pericytes migration and proliferation, although the more effective action in maintaining vessel stability is shown by PDGF-BB [7].

VEGF also stabilises newly formed vascular tubules. VEGF-A directly stimulates ECs proliferation and migration [9]. Hypoxia inducible factor-1 (HIF-1) is a transcription factor regulating VEGF expression. VEGF-A is produced by many cells: endothelial cells, smooth muscle cells, keratinocytes, and macrophages, and acts via its specific receptors, mainly VEGF-R1 and VEGF-R2 [10, 11].

Patients with type 2 diabetes are a special group of patients because of the development of late diabetic vascular complications. Diabetic retinopathy is characterised by abnormal excessive angiogenesis with elevated VEGF levels. On the other hand, impaired wound healing is observed in patients with DFS. This pathological process results from impaired angiogenesis. There have been many studies into the role of PDGF in DFS treatment and VEGF in retinopathy or nephropathy. Unfortunately, very little information is available regarding VEGF-A and PDGF-BB concentrations and their relationship in patients with DFS. VEGF-A is predominantly involved in the early stages of angiogenesis (activating vascular structure formation), while the role of PDGF-BB is focused on the final stage of this process (stabilising already formed vessels).

Therefore, the aim of this study was to evaluate the concentrations of VEGF-A, sVEGF-R1, sVEGF-R2 and PDGF-BB in patients with diabetes-complicated

Table I. *Clinical characteristics of studied subjects*
Tabela I. *Charakterystyka kliniczna pacjentów*

Parameter	Women	Men
Age (mean) (years)	63	60.5
DM duration(mean) (years)	20	16.5
Type DM 1/2	1/24	1/21
DFS duration (mean) (years)	4	2
Wagner grade 0	7	7
Wagner grades 1–3	10	5
Wagner grades 4–5	8	10
Smoking	14	18
BMI (mean) [kg/m^2]	28	30.7
Hypertension	19	15
Heart infarction	1	1
Ischaemic heart disease	5	4

diabetic foot syndrome and analyse them using selected clinical data.

Material and methods

Forty seven diabetic patients with diabetic foot syndrome were enrolled in the experimental group. Patients were treated in the Diabetic Foot Clinic, University Hospital and the Department of Vascular Surgery and Angiology, University Hospital of A. Jurasz, in Bydgoszcz, Poland. The criteria for inclusion in the study were: glycated haemoglobin value above 7.0% (mean 8.3%) providing poor diabetes control and the presence of vascular complication with diagnosed diabetic foot syndrome and classified grade of lesions according to the Wagner classification. Patients were qualified for this study on the basis of history and physical examination including vascular research. The patients were treated with insulin, appropriate diet and physical effort. The comparative analysis of the examined group included the influence of factors such as: body mass index (BMI), Wagner grade (Grade 0: no ulcer in a high risk foot, Grade 1: superficial ulcer involving the full skin thickness but not underlying tissues, Grade 2: deep ulcer, penetrating down to ligaments and muscle, but no bone involvement or abscess formation, Grade 3: deep ulcer with cellulitis or abscess formation, often with osteomyelitis, Grade 4: localised gangrene, Grade 5: extensive gangrene involving the whole foot)[Wagner 1983], diabetes duration, and DFS duration on measured parameters.

To evaluate angiogenesis factors depending on the Wagner grade, the subjects were divided into three subgroups: I — patients with 0 Wagner grade (without oxygen deficiency and normal wound healing; n = 14); II — patients with 1,2,3 Wagner grades (ischaemia, impaired wound healing, inflammation; n = 15); and III - patients with 4,5 Wagner grades (critical ischaemia, necrosis; n = 18). Any patient who was being treated with medications affecting haemostasis, or had had an operation within the last month, or who had end stage renal failure, chronic liver disease or any other severe medical condition requiring active treatment was excluded from this study. The characteristics of the subjects in terms of gender are set out in Table I. The diabetes duration and DFS duration were longer in women than in men. The women enrolled into this study were older compared to the men. However, the men had the most advanced lesions classified as Wagner grades 4 or 5. The following accompanying diseases were reported: arterial hypertension (n = 33), myocardial infarction (n = 2), ischaemic heart disease (n = 9), and lipid disorders (n = 17). In addition, 11 subjects had nephropathy.

The control group consisted of 20 healthy volunteers selected with regard to age and gender. This study was conducted according to the tenets of the Declaration of Helsinki. The Bioethics Commission of the Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Toruń approved the experimental protocol (no. KB/367/2009). All patients were informed about the aim of the study and they signed their agreement to participate.

The material for research was blood plasma. The blood samples were drawn from the antecubital vein after 12 hours of fasting. The plasma samples were obtained from the whole blood collected into tubes containing EDTA and centrifuged at 3,000 rpm for 15 minutes. All the samples were stored at –80°C until analysis, for no longer than six months. The concentrations of soluble VEGF receptors were determined in the plasma samples. PDGF and VEGF concentrations were determined in the plasma samples considering the known release of them by platelets. The growth factors and receptors were measured using ELISA kit: Quantikine Human Immunoassay (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

The statistical analysis was performed using Statistica 8.0 for Windows (StatSoft). Shapiro-Wilk test was used for the evaluation of variables distribution. The parameters with parametrical distribution were analysed in the groups using Student-t test and were described with mean (X) and standard deviation (SD). U-Mann-Whitney rank-sum test was used for variables with non-parametrical distribution. These parameters were presented as median (Me), upper quartile (Q1)

Table II. *VEGF-A, sVEGF-R1, sVEGF-R2, and PDGF-BB levels in the study group compared to the control group*
Tabela II. *Stężenia VEGF-A, sVEGF-R1, sVEGF-R2, PDGF-BB w grupie badanej i w grupie kontrolnej*

Parameter [pg/mL]	Study group n = 47		Control group n = 20		p value
	X/Me	SD/Q1;Q3	X/Me	SD/Q1;Q3	
VEGF-A	70.84	40.11;198.57	26.87	25.22;27.51	0.000001
sVEGF-R1	137.16	49.48	119.90	71.12	0.27
sVEGF-R2	9,182.32	3,742.56	10,693.13	1,716.52	0.09
PDGF-BB	375.55	198.40;868.26	118.78	49.18;209.63	0.00005

X — mean; Me — median; SD — standard deviation; Q1 — lower quartile; Q3 — upper quartile; p-value; p < 0.05 statistical significant

and lower quartile (Q3), as well as a range of values minimum and maximum. The comparison of a larger number of groups was performed by ANOVA. For nonparametric data, the Spearman correlation coefficient was used. The analysis of correlation between parameters with normal distribution was done through Pearson correlation coefficient. A P-value of < 0.05 was considered statistically significant.

Results

Table II shows angiogenesis parameters in patients with DFS and healthy volunteers. sVEGF-R1 and sVEGF-R2 concentrations are presented as average and standard deviation (parametric distribution), while VEGF-A and PDGF-BB concentrations are described with median and quartiles. Significantly higher levels of VEGF-A and PDGF-BB in the study group compared to the control group were observed. The differences were statistically significant (VEGF-A p = 0.000001; PDGF-BB p = 0.000051). There were no significant differences in VEGF receptors between patients and controls.

Our analysis of the angiogenesis parameters including Wagner grades demonstrated significantly higher VEGF-A level in all the subgroups compared to the control group (I: p = 0.000867; II: p = 0.001827; III: p = 0.000024) (Fig. 1). The highest VEGF-A concentration was noted in the III subgroup. PDGF-BB concentration was statistically significantly higher in all the subgroups than in the controls (respectively p = 0.004113, p = 0.004224, p = 0.002480) (Fig. 2). The patients with grade 0 had the highest PDGF-BB concentration. Statistically significant decreased sVEGF-R2 concentrations were observed in the I subgroup (p = 0.054) and the III subgroup (p = 0.03524) compared to the control group. No statistically significant difference was observed in sVEGF-R1 concentration. For further research, the angiogenic variables were tested among the subgroups. No statistically significant differences were noted while analysing I *vs.* II, I *vs.* III and II *vs.* III. Based on the

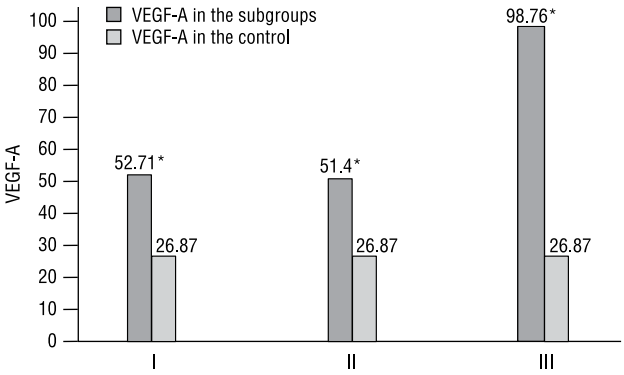

Figure 1. *VEGF-A level in patients divided into the subgroups compared to the control group; Data is presented as the median; *p significant*

Rycina 1. *Stężenie VEGF-A u pacjentów w podgupach oraz w grupie kontrolnej*

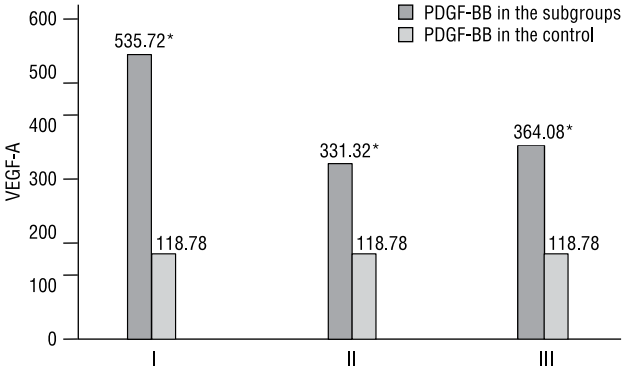

Figure 2. *PDGF-BB level in patients divided into the subgroups compared to the control group ; Data is presented as the median; *p significant*

Rycina 2. *Stężenie PDGF-BB u pacjentów w podgupach oraz w grupie kontrolnej*

Spearman coefficient, a strong positive correlation between VEGF-A and PDGF-BB was observed (R = 0.66; p = 0.000001). The positive value of coefficient correlation shows the coexistence of increased concentrations

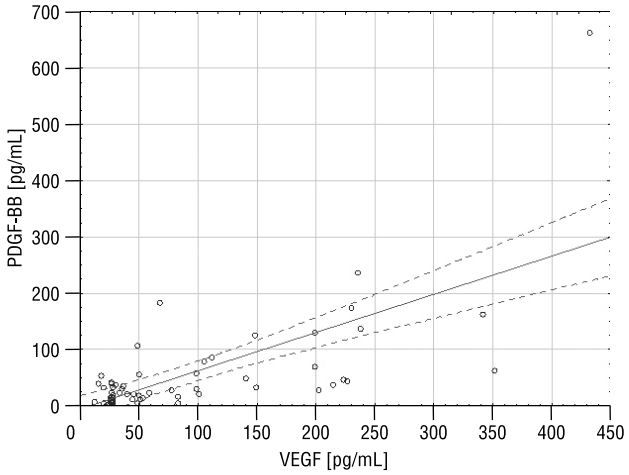

Figure 3. *The correlation between VEGF-A and PDGF-BB concentrations*

Rycina 3. *Zależność pomiędzy stężeniem VEGF-A i PDGF-BB*

of VEGF-A and PDGF-BB. This relation is illustrated in Figure 3. Moreover, a negative correlation of sVEGF-R2 concentration and age was observed ($R = –0.33$; $p = 0.032833$), as well as a negative correlation of PDGF-BB concentration and age ($R = –0.31$; $p = 0.035032$).

Discussion

It is known that in patients with diabetes, the levels of proangiogenic factors are decreased. Our findings are therefore controversial. In this study, we observed increased VEGF-A and PDGF-BB concentrations in patients with diabetic foot syndrome.

Angiogenesis plays a pivotal role in wound healing [10]. VEGF is the main factor inducing both physiological and pathological angiogenesis. This specific mitogen for vascular endothelial cells is synthesised by the endothelial cells, myocytes, macrophages, lymphocytes CD4+, and megakaryocytes [11, 12]. It is stored in leukocytes and platelets from where it is released into the circulation in a hypoxic condition [12] and under the influence of IL-6 and IL-8. Hypoxia, acting via hypoxia inducible factor-1α (HIF-1α) is the most important factor that enhances VEGF-A expression. Our data showed an increased VEGF-A concentration in the patients with DFS compared to the control group. The reasons for the increased VEGF-A level are complex. Patients with DFS are characterised by impaired perfusion leading to hypoxia. Hypoxia activates VEGF-A expression. Analysis of the study group based on Wagner classification showed that patients with Wagner grades 4 and 5 (grade 4: localised gangrene, grade 5: extensive gangrene involving the whole foot) [13] had a two-fold higher VEGF-A concentration than patients with Wagner grade 0 (grade 0: no ulcer in a high risk foot) and four-fold higher compared to the control group.

We suggest that this may result from the deep microcirculation disturbance in the ischaemic area and severe tissue hypoxia.

It is postulated that overexpression of VEGF-A in patients with DFS is also associated with chronic inflammation. The inflammatory process presents in patients with grade 2 and above according to the Wagner classification. Increased levels of proinflammatory cytokines such as C-reactive protein, fibrinogen, IL-6, IL-1, and tumour necrosis factor α (TNF-α) have been observed in diabetic patients [14]. The elevated level of IL-6 contributes to the release of VEGF-A stored in platelets and leukocytes. The average value of patients' BMI was 29.4, i.e. most patients were overweight or obese. Adipose tissue is considered to be an endocrine organ and releases hormones, enzymes and cytokines, including VEGF and IL-6 [14, 15]. In 2010, Loebig et al. observed a positive correlation between BMI and serum VEGF level [15]. A similar result was obtained by Doupis et al. Their research showed increased VEGF level in the serum of non-obese patients with diabetes, and in obese patients with diabetes. However, the highest concentration of VEGF was observed in the group of obese non-diabetics [16].

Unfortunately, there is not enough data in the available literature about VEGF-A concentration in diabetic patients complicated by DFS. The concentration of vascular endothelial growth factor varies greatly in other late complications of diabetes such as nephropathy and retinopathy. In patients with proliferative diabetic retinopathy, an increased level of VEGF-A in the vitrous and aqueous body of the eye was noted [17]. The studies conducted by Izuta et al. also showed an elevated level of VEGF-A in the vitrous body [18]. In diabetic nephropathy, VEGF-A expression is dependent on the severity of changes in the glomeruli. At the early stages of diabetic nephropathy, an increasing expression of VEGF-A is observed, then the expression decreases [19, 20]. Moreover, in patients with type 2 diabetes, the urine VEGF-A concentration is elevated [21]. In our study, the influence of other microvascular complications (retinopathy, nephropathy) on concentrations of VEGF and PDGF in patients with DFS was investigated. There were no statistically significant differences in concentrations of angiogenic parameters in patients with and without retinopathy and in patients with and without nephropathy.

On the other hand, VEGF-A levels may be due to differences in the genotype of VEGF in diabetic patients. The research conducted by Bleda et al. shows that VEGF gene polymorphisms predispose to different vascular complications of diabetes. What is more, the –2578 CA

genotype was associated with a statistically significant increase in the concentration of VEGF in serum [Bleda 2011].

The haemostasis process is abnormal in patients with diabetes. The study conducted by Roth et. al. indicated a reduced mitogenic activity of VEGF-A_{165} as a result of plasmin cutting (VEGF-A_{165} faster degradation in non-healing ulcers) [23]. This may suggest that the action of VEGF is not effective due to its premature inactivation.

Vascular maturation is the final stage of angiogenesis. This process involves pericytes that cover newly formed vessels and it is controlled by PDGF [24]. We observed an elevated level of PDGF-BB in patients with DFS compared to healthy subjects. The analysis of the study group divided into subgroups according to their Wagner grade was particularly interesting. The highest concentration of PDGF-BB was observed in subgroup I (Wagner 0). This result shows that the concentration of PDGF-BB was decreasing with the progress of the changes. We suggest that this may be associated with progressive endothelium damage. Endothelial cells do not function properly, therefore they are not able to synthesise and release PDGF-BB. Armulik et al. showed that the knockout of PDGF and PDGFR leads to abnormalities in the structure of blood vessels. The lack of pericytes has secondary effects on endothelial cells contributing to endothelium hyperplasia, cell connection disorders, and excessive liminal membrane folds. The endothelial dysfunction is compensated for by the increasing VEGF-A secretion [25]. On the other hand, the advance of hypoxia induces HIF-1α, which activates the PDGF gene. Therefore, in spite of the decreasing concentration of PDGF-BB in the subgroups, it is still higher compared to the control group.

The data about PDGF presented in the literature is inconclusive. In 2009, Doupis et al. published a study which showed that PDGF-AA concentration was reduced in patients with painful, and painless, neuropathy compared to a control group. However, the concentration of PDGF in the combination AB/BB was elevated in both study groups [26]. Three years later, Doupis et al. presented decreased levels of PDGF-AA in the serum of non-obese diabetic patients and obese diabetic patients [16]. Animal studies conducted by Tanii et al. demonstrated that in STZ-DM mice (streptozotocin induced diabetes mellitus), PDGF-B gene expression is impaired. The accumulation of AGEs (advanced glycation end products) was associated with reduced PDGF-B expression. The supplementation of PDGF-B gene restored normal level of PDGF-BB in the tissue and protected against ischaemic limb autoamputation [27].

The results of our study indicate a decreased level of sVEGF-R2 in patients with DFS in the subgroup with Wagner grades 4–5. This may reflect the wearing out of the receptor and deficiency in its expression by the damaged endothelial cells. In the available literature, we did not find any information about sVEGF-R2 concentrations in patients with DFS.

The presented results demonstrate a relationship between a high level of VEGF-A and a high level of PDGF-BB. This might prove intensified hypoxia, which regulates the expression of both genes [28–30]. Furthermore, the study conducted by Ball et al. on a cellular model indicated that VEGF-A acts through PDGF receptors (PDGF-Rα and PDGF-Rβ). Furthermore, VEGF-A_{165} competes with PDGF-AA and-BB for binding to mesenchymal stem cells [31].

The results of our study show elevated levels of angiogenic factors in patients with DFS. Nevertheless, angiogenesis and the associated wound healing are impaired. In patients with diabetes, especially complicated by diabetic foot syndrome, ischaemic changes are so strong that they contribute to VEGF-A overexpression. Greenberg et al. noted that VEGF might act as an antiangiogenic factor. The authors postulate that connecting VEGF-A with VEGF-R2 inhibits the binding of PDGF to PDGF-Rβ. Consequently, it leads to unstable blood vessel creation [9]. In our study, we assessed the concentration of the soluble forms of VEGF receptors, which are classified as angiogenesis inhibitors [29]. Although VEGF in patients with DFS is present in the circulation in large amounts, it seems that its angiogenic activity is abolished by the formation of the complex with sVEGF-R2.

We found a positive correlation between VEGF-A and PDGF-BB (in the whole study group, without dividing into subgroups). This might be associated with the regulation of the expression of one factor by another. Hypoxic VEGF transcriptional activity is controlled by a number of cytokines and factors, including by PDGF [29].

Determining the concentration of VEGF-A in patients with DFS may be useful in selecting appropriate therapy. The research carried out by Skóra et al. shows that administration of VEGF gene with simultaneous autotransplantation mononuclear bone marrow cells leads to elevated levels of VEGF. They noted that this therapy improved critical limb ischaemia [32].

Conclusions

Our study has demonstrated increased VEGF-A and PDGF-BB concentrations in diabetic patients with DFS. This is associated with lower limb ischaemia and hypoxic conditions. The highly significant positive correlation between VEGF-A and PDGF-BB shows their proangiogenic co-operation (interaction). Impaired

angiogenesis and wound healing on a limb complicated by DFS indicates the ineffectiveness of these factors, something that may result from sVEGF-R2 inhibition.

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