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VASCULAR-1 and VASCULAR-2 as a New Potential Angiogenesis and Endothelial Dysfunction Markers in Peripheral Arterial Disease

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Abstract

The quotient of concentrations concerning the key proangiogenic factor, that is, the vascular endothelial growth factor (VEGF-A) and the angiogenesis inhibitor, namely, its soluble receptors (sVEGFR-1 or sVEGFR-2), seems to reflect increased hypoxia and intensity of compensation angiogenesis. Therefore, it can be an ischemic and endothelial dysfunction marker reflected in intermittent claudication (IC) or critical limb ischemia (CLI) in patients with symptomatic peripheral arterial disease (PAD). The main objective of this study was to evaluate the levels of VEGF-A/sVEGFR-1 and VEGF-A/sVEGFR-2—presented using a novelty acronym VASCULAR-1 and VASCULAR-2—in patients with IC and CLI, as well as displayed in 4 classes of severity of PAD. VASCULAR-1 and VASCULAR-2 were calculated using the plasma of venous blood sampled from 80 patients with IC (n = 65) and CLI (n = 15) and the control group (n = 30). Patients with CLI were reported to have a slightly higher index of VASCULAR-1 and double VASCULAR-2 levels as compared to patients with IC (P = nonsignificant), and these markers were significantly higher than controls ($P < .01$ and $P < .01$, respectively). VASCULAR-2 levels were observed to have an increasing tendency in the subsequent degrees of PAD severity according to the Fontaine classification ($P = .02$). In view of the need to consider the role of the proangiogenic and antiangiogenic factor in the assessment of the so-called "angiogenic potential," VASCULAR-1 ratio and VASCULAR-2 ratio may be a new useful biomarker of limb ischemia in patients with IC and CLI. However, this requires further studies and evidence on a very large group of patients with PAD.

Keywords

endothelial dysfunction, angiogenesis, VASCULAR-1 ratio, VASCULAR-2 ratio, peripheral arterial disease

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Introduction

According to the data provided in the references, symptomatic peripheral arterial disease (PAD) affects about one-third of all patients suffering from PAD. The most characteristic symptom of chronic PAD is intermittent claudication (IC) which consists of a pain occurring with walking a certain distance and going away with rest. Nevertheless, rest pains which last longer than 2 weeks comprise a feature of critical limb ischemia (CLI) which is often accompanied with difficult to heal sores or necrosis. Peripheral arterial disease significantly increases the risk of death, whereas advanced ischemic changes very often contribute to amputation of the lower limb.[1,2]

Hypoxia and ischemia stimulate the production of growth factors which facilitate angiogenesis and one of the key angiogenic factors—the process of forming new blood vessels based on the already existing ones—is the vascular endothelial growth factor (VEGF-A). Its proangiogenic effects largely depend on the stimulation of peculiar endothelial

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receptors on many cells, mainly endothelium (Fms-like tyrosine kinase 1 [FLT-1] and Kinase insert domain receptor 1 [KDR-1]).[3,4] Yet, soluble endothelial forms, sVEGFR-1 and sVEGFR-2, may show antagonistic and inhibition activity.

The severity of angiogenesis can be assessed by measuring VEGF-A levels, for example, in venous blood plasma. It is known that age and gender do not have a significant impact on VEGF-A levels, while factors leading to hypoxia (eg, tumor necrosis caused by hypoxia), damaging endothelium (eg, smoking), and physiological processes (eg, healing of wounds) increase the concentration of growth factors for blood vessels.[5,6]

Angiogenesis research was commenced in patients with cancer in whom the formation of new vessels was observed during the growth of cancerous tumor and correlations between angiogenesis and metastases were evidenced.[6] Initially, the so-called "therapeutic angiogenesis" accompanied cancer chemotherapy and consisted of the application of angiogenesis inhibitors in order to slow down tumor growth and reduce metastatic ability. Besides, the study focused on finding a biochemical indicator correlating with cancer severity and prognosis.[7-10]

Angiogenesis research concerned about cardiovascular diseases related to atherosclerosis and diabetes. Angiogenesis in atherosclerosis is considered by numerous researchers to be a defense mechanism which is the body's response to tissue ischemia such as development of collateral circulation in case of coronary disease and PAD. When it comes to "local" angiogenesis in an atherosclerotic plaque (the so-called "neovasculogenesis *vasa vasorum*"), it affects the destabilization of the plaque, whereas neoangiogenesis in the retina of the eye leads to blindness in diabetic retinopathy. "Therapeutic angiogenesis" applied in cardiovascular disease is focused on the development of collateral circulation.[11]

The studies conducted so far have shown that angiogenetic inhibitors (sVEGFR-1 and sVGFR-2) prevail overgrowth factors (VEGF-A) in healthy organisms.[12] The analysis of the relevant literature shows that angiogenesis was assessed in patients with PAD based on VEGF-A, sVEGFR-1, and sVEGFR-2 levels, obtaining sometimes contradictory results.[13,14] Findley et al observed significant differences in VEGF-A levels between the groups of patients with IC and CLI,[15] while Stehr et al reported significantly high VEGF-A concentrations in class IV based on the Fontaine classification.[16]

Additional information can be provided by the study of the angiogenic potential in the blood of patients with PAD based on the assessment of the quotient of VEGF-A/sVEGFR1 and VEGF-A/sVEGFR2 levels. Our previous studies have shown a tendency of increasing VEGF-A/sVEGFR-1 in 46 patients in subsequent classes based on the Fontaine classification (IIa, IIb, III, and IV).[17] Angiogenesis severity measured with the VEGF-A/sVEGFR-1 or VEGF-A/sVEGFR-2 factors will also appear to differentiate patients with various degrees of tissue ischemia taking into account the inhibitory strength of sVEGFR-1 and sVEGFR-2.

The aim of this study was to assess the ratios of VEGF-A/sVEGFR-1 and VEGF-A/sVEGFR-2 in patients with IC, CLI, and 4 classes of PAD severity according to the commonly used Fontaine classification (in patients classified as IIa, IIb, III, and IV).

Methods

Study Protocol

The study group included 80 patients (27 women and 53 men, average age of 63.5 $\pm$ 9 years) with symptomatic PAD hospitalized in Dr Jan Biziel University Hospital No. 2 in Bydgoszcz (Poland). Among the patients, individuals with IC (subgroup IC, n = 65) and critical ischemia of lower limbs (subgroup CLI, n = 15) were identified. In addition, the sample group was divided with regard to PAD severity according to the Fontaine classification (IIa, n = 11; IIb, n = 54; III, n = 4; and IV, n = 11). The diagnosis of IC may reflect class IIa and IIb, while CLI reflects class III and IV according to the Fontaine classification. In addition to the lack of consent to participate in the study, the exclusion criteria were cancer history and diabetic retinopathy. Peripheral arterial disease was diagnosed based on the history and physical examination and ankle–brachial index (ABI) and for IC on the walking test on the treadmill. Duplex ultrasound of peripheral arteries was performed in all the patients involved in the study. The control group consisted of 30 healthy volunteers (10 women and 20 men, average age of 56 $\pm$ 6 years), who were nonsmokers, without diabetes or prediabetes, without clinically apparent atherosclerosis, with no medicines, and with normal body mass index.

Blood samples were taken from the study group and the controls using the enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, USA) to test VEGF-A, sVEGFR-1, and sVEGFR-2 levels in the plasma of venous blood sampled at early hours after night rest. Results obtained in homogenous units (pg/mL) were converted into proper concentration quotients introducing new terms—acronyms: respectively: "VASCULAR-1" ratio for quotient VEGF-A/sVEGFR-1 and "VASCULAR-2" ratio standing for the VEGF-A/sVEGFR-2 ratio. The statistical analysis used the program Statistica ver. 10.0 (StatSoft, Cracow, Poland), the Kruskal-Wallis test, the Mann-Whitney U test, and Spearman rank correlation coefficient. The significance level of $P < .05$ was assumed.

Compliance With Ethical Standards

The local Bioethics Commission gave consent (no. KB 509/2011) to conduct the research. The study was carried out in accordance with the Declaration of Helsinki. All the people involved in the study gave their informed consent for participation in the test confirmed in writing on a dedicated form.

Results

Clinical data concerning the sample group (PAD, n = 80) are shown in Table 1. Table 2 displays mean values of the

Table 1. Clinical Characteristics of the Study Group.

Parameter	Value
Sex (female/male)	27 (34%)/53 (66%)
Age (years ± SD)	63.5 ± 9
IIa class (Fontaine)	11 (14%)
IIb class (Fontaine)	54 (67%)
III class (Fontaine)	4 (5%)
IV class (Fontaine)	11 (14%)
IC subgroup	65 (81%)
CLI subgroup	15 (19%)
IC distance (m ±SD)	100 ± 87
ABI (±SD)	0.5 ± 0.25
BMI (kg/m^2 ±SD)	26.4 ± 4.4
LDL (mg/dL ±SD)	119.7 ± 39.3
TG (mg/dL ±SD)	143.9 ± 73.3
Smokers	74 (92.5%)
Type 2 diabetes	28 (35%)
Ischemic heart disease	36 (45%)
Hypertension	71 (89%)

Abbreviations: ABI, ankle–brachial index; BMI, body mass index; CLI, critical limb ischemia; IC, intermittent claudication; LDL, low-density lipoprotein; SD, standard deviation; TG, triglyceride.

investigated factors, that is, plasma levels of VEGF-A, sVEGFR-1 and sVEGFR-2—in the sample group (PAD, n = 80) divided with regard to the severity class according to the Fontaine classification and to subgroups IC and CLI and in the controls (n = 30). The figures were then used to calculate the quotients of the concentrations.

In PAD groups, significantly higher concentration of VEGF-A and significantly lower levels of sVEGFR-2 were observed as compared with controls ($P < .01$). The analysis of the subgroups revealed 1.7 times higher VEGF-A concentration in patients with CLI in comparison with IC and slightly higher levels of sVEGFR-1 and sVEGFR-2 in the subgroup of CLI versus IC—yet, the differences were statistically insignificant. In the subgroups of patients with PAD, according to the Fontaine classification, the observations showed increasing mean levels of VEGF-A in subsequent severity classes (IIa vs IIb vs III vs IV, $P = .03$) and a statistically insignificant tendency to growing sVEGFR-1 concentrations. No significant sVEGFR-1 differences were reported between the study group and controls. Likewise, the differences in sVEGFR-2 levels between the subgroups of patients were not significant.

A significant negative correlation was observed between VEGF-A and sVEGFR-1 ($R = -0.42$, $P = .02$; Figure 1) in the control group, which suggested that the higher the VEGF-A concentration was the level of sVEGFR-1 decreased. In the study group, a statistically significant positive correlation was achieved between mean plasma levels of VEGF-A and sVEGFR-1 ($R = 0.27$, $P = .02$; Figure 2), that is, the growing concentrations of VEGF-A responded to higher sVEGFR-1 levels.

Table 3 displays mean values of VASCULAR-1, that is, the quotient of VEGF-A/sVEGFR1. The ratio value of 0.16 in the control group, displayed in Table 3, results from the quotient of VEGF-A and sVEGFR-1 and suggests an almost 8-time advantage of VEGF-A inhibition by the sVEGFR-1 receptor. An increase in this ratio toward 1.0 shows an even lesser inhibition. The level of VASCULAR-1 (VEGF-A/sVEGFR-1) in the study group (0.75) was nearly 4 times higher than in the controls (0.16) and the difference turned out to be statistically significant ($P < .01$), which suggests an almost 4 times lower inhibition in the patients. This ratio was reported to be higher in the CLI subgroup as compared with IC (0.90 vs 0.72); however, these differences were statistically insignificant. The VASCULAR-1 quotient was the highest in the subgroup of class IV patients according to the Fontaine classification and amounted to 1.00. An increase of the ratio was observed in the subsequent classes IIa, IIb, and IV—yet, without a statistically significant basis.

Table 4 displays mean values of VASCULAR-2 (ie, the quotient of VEGF-A/VEGFR-2 levels) in respective PAD subgroups and controls. As the VEGF-A and sVEGFR-2 quotient indicates, the sVEGFR-2-dependent inhibition prevalence over the activity influenced by VEGF-A in controls is 800 times. In the entire group of patients with PAD, the sVEGFR-2-dependent inhibition reduced to 116 times. The analysis of VASCULAR-2, depending on the severity according to the Fontaine classification, suggests the decreasing inhibition of angiogenesis in the blood of patients with PAD: in period II—210 times, IIb—121 times, III—129 times, and IV—69 times (Table 4, Figure 3). The study also revealed a difference in the degree of angiogenic inhibition between the subgroup with CLI and IC. The result in the IC subgroup was 130 times and in CLI—only 79 times (Figure 3).

Discussion

The conducted studies showed that the VEGF-A level was significantly higher in the blood of patients with PAD as compared to healthy controls and the level increased with the progressive limb ischemia. This was also manifested by a significant decrease in the VEGFR-2 level. The comparison of the subgroup of IC patients with CLI patients indicated that the concentration of VEGF-A was borderline significant with $P = 0.07$ and no differences of sVEGFR-1 and sVEGFR-2 were noted between these groups of patients. In the reference literature, there are sometimes inconsistent research results regarding particular parameters of pro- and antiangiogenic factors. We have already analyzed these discrepancies.[17] The observations we have made so far revealed increased VEGF-A levels in the blood of patients with PAD as compared with healthy controls,[16-18] higher levels in CLI patients,[15,19] similar VEGF-A concentrations in PAD individuals versus controls,[20] or even reduced VEGF-A levels in patients with PAD.[21] Similar ambiguities were connected with the invasive determination of the VEGF-A expression in ischemic amputation tissues[22] as well as biopsy.[23,24] Brandão et al obtained higher VEGF-A levels in bioptates from ischemic distal tissues as compared to proximal samples.[23] Similar results were achieved by Palmer-Kazen et al in muscle biopsy of CLI patients,

Table 2. VEGF-A, sVEGFR-1, and sVEGFR-2 Plasma Levels in the Study Group (PAD, n = 80) According to the Fontaine Classification, IC, CLI Subgroup, and Controls (n = 30; pg/mL).

	PAD (n = 80)							Control	
	IIa (n = 11)	IIb (n = 54)	III (n = 4)	IV (n = 11)	IC (n = 65)	CLI (n = 15)	All (n = 80)	(C; n = 30)	*P*
VEGF-A, mean ± SD	49.1 ± 31.1	78.9 ± 50.2	82.1 ± 68.5	145.6 ± 117.6	73.9 ± 48.6	128.7 ± 108.3	84.2 ± 66.7	18.0 ± 7.7	IIa vs C $P = .06$ (NS) IIb vs C $P < .01$ III vs C $P = .05$ IV vs C $P < .01$ IC vs C $P < .01$ CLI vs C $P < .01$ All vs C $P < .01$ IC vs CLI $P = .07$ (NS) IIa vs IIb vs III vs IV $P = .03$
sVEGFR-1, mean ± SD	112.1 ± 28.6	117.4 ± 45.6	135.2 ± 31.3	140.2 ± 37.5	116.5 ± 43.0	138.8 ± 34.9	120.7 ± 42.4	140.5 ± 62.3	IIa vs C $P =$ NS IIb vs C $P =$ NS III vs C $P =$ NS IV vs C $P =$ NS IC vs C $P = .26$ (NS) CLI vs C $P =$ NS All vs C $P = .21$ (NS) IC vs CLI $P = .12$ (NS) IIa vs IIb vs III vs IV $P =$ NS
sVEGFR-2, mean ± SD	10315.6 ± 2084.5	9535.6 ± 2707.9	10570.0 ± 1667.7	10052.4 ± 3547.3	9667.7 ± 2615.0	10190.4 ± 3104.9	9765.6 ± 2700.0	14481.5 ± 3669.9	IIa vs C $P < .05$ IIb vs C $P < .01$ III vs C $P =$ NS IV vs C $P = .01$ IC vs C $P < .01$ CLI vs C $P < .01$ All vs C $P < .01$ IC vs CLI $P = .53$ (NS) IIa vs IIb vs III vs IV $P =$ NS

Abbreviations: CLI, critical limb ischemia; IC, intermittent claudication; NS, nonsignificant; PAD, peripheral arterial disease; SD, standard deviation; VEGF, vascular endothelial growth factor.

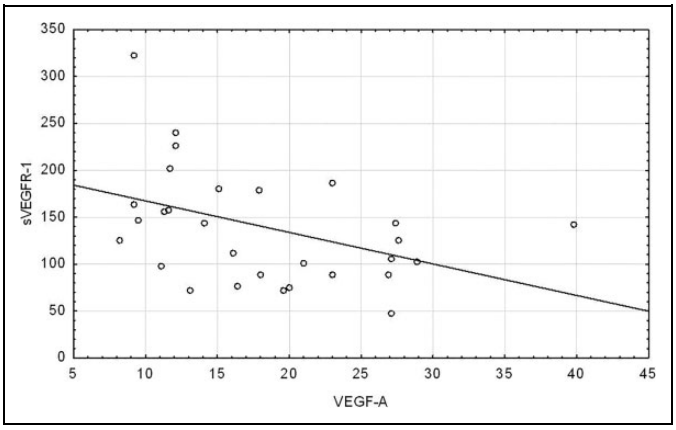

Figure 1. Correlation between VEGF-A and sVEGFR-1 plasma concentrations in controls (n = 30), $R = -0.42$, $P = .02$. VEGF indicates vascular endothelial growth factor.

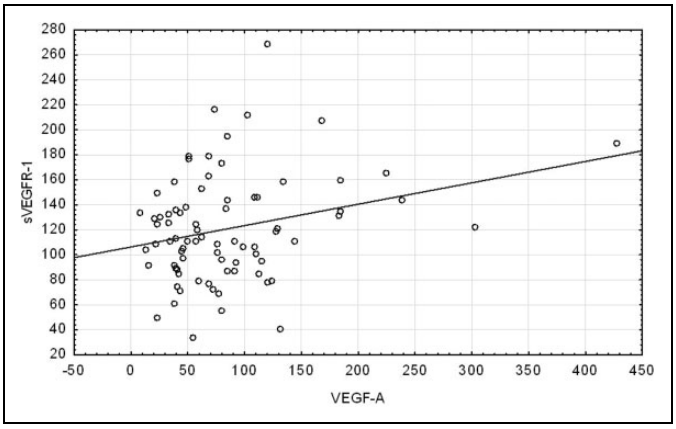

Figure 2. Correlation between VEGF-A and sVEGFR-1 plasma concentrations in the study group (PAD, n = 80), $R = 0.27$, $P = .02$. PAD indicates peripheral arterial disease; VEGF, vascular endothelial growth factor.

whereas lower levels of VEGF-A were observed in skin biopsy.[24] The confusions concerning the diversified role of VEGF-A and sVEGFR-1 were challenged with silico models.[25] Also Kikuchi et al suggest that inflammation-driven expression of the antiangiogenic VEGF-A isoform can contribute to impaired collateralization in ischemic cardiovascular disease.[26]

The index used in this paper and calculated based on the ratio of the concentration levels of the circulating natural angiogenic inhibitors (sVEGFR-1 and sVEGFR-2) interacting with (proangiogenic) VEGF-A illustrates the so-called "angiogenic potential." The analysis of the blood of healthy controls and obese patients suggests that these indices show the increasing PAD depending on the growing ischemic severity according to the Fontaine classification. VASCULAR-1 calculated based on the proportion of the blood sVEGFR-1 and VEGF-A level aiming at the level of 1.0 suggests even weaker inhibition in patients with class IV ischemia according to the Fontaine classification. Due to the considerable level of sVEGFR-2 in the blood of patients versus controls as compared

Table 3. VASCULAR-1 Ratio in the Study Group (PAD, n = 80) According to the Fontaine Classification, IC, CLI, and Controls (n = 30; pg/mL:pg/mL).

	PAD (n = 80)							
	IIa (n = 11)	IIb (n = 54)	III (n = 4)	IV (n = 11)	IC (n = 65)	CLI (n = 15)	All (n = 80)	Control (C; n = 30)
Mean ± SD	0.45 ± 0.26	0.77 ± 0.58	0.62 ± 0.52	1.00 ± 0.64	0.72 ± 0.55	0.90 ± 0.62	0.75 ± 0.57	0.16 ± 0.12

P
IIa vs C $P = .04$
IIb vs C $P < .01$
III vs C $P = .22$ (NS)
IV vs C $P < .01$
IC vs C $P < .01$
CLI vs C $P < .01$
All vs C $P < .01$
IC vs CLI $P =$ NS
IIa vs IIb vs III vs IV $P = .16$ (NS)

Abbreviations: CLI, critical limb ischemia; IC, intermittent claudication; NS, nonsignificant; PAD, peripheral arterial disease; SD, standard deviation.

Table 4. VASCULAR-2 Ratio in the Study Group (PAD, n = 80) According to the Fontaine Classification, IC, CLI, and Controls (n = 30; pg/mL:pg/mL).

	PAD (n = 80)								
	IIa (n = 11)	IIb (n = 54)	III (n = 4)	IV (n = 11)	IC (n = 65)	CLI (n = 15)	All (n = 80)	Control (C; n = 30)	*P*
Mean ± SD	0.005 ± 0.003	0.009 ± 0.005	0.008 ± 0.006	0.018 ± 0.024	0.008 ± 0.005	0.015 ± 0.021	0.009 ± 0.01	0.001 ± 0.0006	IIa vs C $P = .05$
									IIb vs C $P < .01$
									III vs C $P < .05$
									IV vs C $P < .01$
									IC vs C $P < .01$
									CLI vs C $P < .01$
									All vs C $P < .01$
									IC vs CLI $P = .14$ (NS)
									IIa vs IIb vs III vs IV $P = .02$

Abbreviations: CLI, critical limb ischemia; IC, intermittent claudication; NS, nonsignificant; PAD, peripheral arterial disease; SD, standard deviation.

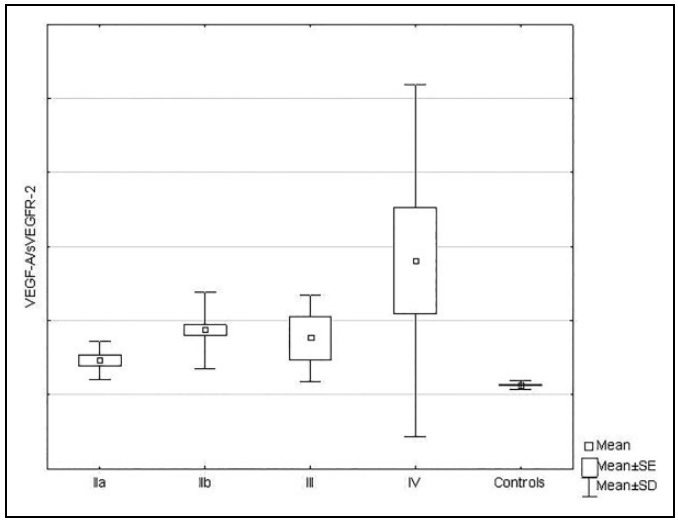

Figure 3. VASCULAR-2 ratio in the study group (PAD, n = 80) according to the Fontaine classification and controls (n = 30; pg/mL:pg/mL). PAD indicates peripheral arterial disease.

to the VEGF-A level, VASCULAR-2 was 0.001. Patients with PAD were reported to have higher level of VASCULAR-2, which grew from 0.005 in class IIa to 0.018 in class IV based on the Fontaine classification. Yet, a considerable difference was observed in the concentration of VASCULAR-2 and, to a lesser extent, in VASCULAR-1 levels between CLI and IC patients.

Failure to obtain the statistical significance for the VASCULAR-1 ratio between IC and CLI as well as between class IIa, IIb, III, and IV appears to have resulted from the small sample size. However, the relatively varied level of sVEGFR-2 in patients with the different severity of PAD with the increasing concentration of VEGF-A in subsequent Fontaine's classes may explain almost twice as high VASCULAR-2 level (ie, the quotient of VEGF-A/sVEGFR-2) in the subgroup of CLI versus IC. It should also be noted that VASCULAR-2 clearly distinguished patients with CLI (0.015) from the ones with chronic ischemia (IC: 0.008).

So far, oncology has studied the quotient of plasma VEGF-A and sVEGFR-1 levels in patients with pancreatic cancer and acute myeloid leukemia (AML). The study conducted by Chang et al, which involved 92 patients with pancreatic cancer, showed that VEGF-A/sVEGFR-1 ratio was an independent prognostic factor in this neoplasm disease. The higher VEGF-A/sVEGFR-1 ratio measured before the treatment was connected with the clinically shorter life span, and from the viewpoint of pathomorphology, it was associated with the higher severity of pancreatic cancer.[7] Similar observations were made by Aref et al who studied 43 patients with AML, in which the higher level of this factor also meant a shorter life expectancy.[8] The study by Toi et al involving 110 patients with breast cancer assessed the ratio of VEGF-A and sVEGFR-1 levels in the sampled neoplastic tissue. A worse prognosis was observed in patients with VEGF-A levels outweighing the sVEGFR-1 levels (the so-called S/V ratio, ie, sVEGFR-1/

VEGF-A was investigated).[9] The tissue expression of VEGF-A and sVEGFR-1 (sVEGFR-1/VEGF) was tested in patients with colorectal cancer in terms of clinical parameters and prognosis —the publication by Yamaguchi et al found that sVEGFR-1 prevalence was connected with a better prognosis.[10]

The VEGF-A/sVEGFR-1 ratio and the VEGF-A/sVEGFR-2 ratio have not been analyzed in patients with PAD so far. The importance of VASCULAR-1 and VASCULAR-2 used in this study results from the possibility of considering not only the contribution of VEGF-A, which has the strongest proangiogenic properties, but also the role of natural inhibitors, comprising the kind of a compensation mechanism, in the assessment of the proangiogenic impact in patients' blood. The analysis of VASCULAR-1 and VASCULAR-2 levels in patients with PAD of different severity suggests that oxygen deficit not only stimulates synthesis and the secretion of VEGF-A to blood. It appears that insufficient and progressive failure of the organism within the synthesis and secretion of natural angiogenic inhibitors, that is, sVEGFR-1 and sVEGFR-2, is particularly significant. Osada-Oka et al suggest that hypoxia and hypoxia-induced VEGF-A expression are also involved in the pathogenesis of progressive atherosclerosis.[27]

A different trend of the VEGF-A and sVEGFR-1 correlations in the blood of patients with PAD and healthy controls indicates the distortion of the angiogenic inhibition mechanism. In the controls, the growing VEGF-A concentrations were accompanied with the lowering levels of sVEGFR-1, while in the group of the patients with PAD, higher VEGF-A concentrations correlated with increased sVEGFR-1 levels. This observation is hard to interpret in the light of available studies and due to the lack of tests analyzing the scale of VEGF-A inhibition by circulating natural angiogenic inhibitors. Blood tests allowing to analyze free sVEGF-A and the proangiogenic factor in sVEGF-A/sVEGFR-1 and sVEGF-A/sVEGFR-2 complexes would be incredibly useful in these circumstances and their results could shed new light on the phenomenon of angiogenesis. It can be assumed that in the event of low physiological VEGF-A levels in the blood of healthy controls, inhibitor sVEGFR-1 plays a significant role in maintaining the angiogenesis balance. sVEGFR-1 referred to as a trap receptor prevails over the activator and forms complexes with VEGF-A, reducing VEGF-A access to the stationary receptor VEGFR-2 on the surface of endothelial cells, thus preventing the activation of angiogenesis. The situation is different in patients with PAD when high sVEGF-A blood levels considerably exceed the inhibition capabilities of sVEGFR-1.

Therefore, it seems that calculating the quotients of VEGF-A/sVEGFR-1 or VEGF-A/sVEGFR-2 levels may help to assess the severity of peripheral tissue ischemia in patients with PAD, and thus, it may serve not only for the purpose of preliminary clinical evaluation (ABI and the walking test in addition to the history and physical examination) but also, for example, for the purpose of noninvasive monitoring of treatment efficiency, for example, conservative treatment including marching training. One of the VEGF-A measurement methods applied so far to assess the efficiency of treatment has been an invasive method, namely, tissue biopsy. The study of Jones et al assessed the tissue level of VEGF-A before and after a 3- and 12-week marching training. Original VEGF-A levels were not different in the PAD group and controls, but the walking training resulted in decreased VEGF-A levels in sampled tissues.[28] Therefore, the potential advantage of measuring VASCULAR-1 and/or VASCULAR-2 may be low invasiveness of such a procedure as easily accessible venous blood is used as material and not tissue.

Although IC and CLI have traditionally been thought as early and late variants, respectively, of the same disease, recent studies have suggested that 2 conditions might be caused by distinct pathophysiological mechanisms. The ischemia-induced increases in VEGF-A levels may induce dysregulation of other factors (especially angiopoietin-2 and sTie2 pathway), with unpredictable consequences on tissue ischemia.

Besides, the new potential ischemic marker which is VASCULAR-1 and/or VASCULAR-2 ratio could serve as a parameter to develop innovative therapeutic strategies which concern not only growth factors but also antireceptor medicines. The results suggest it is desirable to pursue further relevant studies on a large number of patients with PAD and they provide a new research tool.

Limitations of the Study

The main limitation of the study was a too small number of the sample group (PAD, n = 80), especially the number of patients with CLI (n = 15). Significant differences were observed between patients and controls, whereas the detailed analysis concerning the subgroups of patients (IC vs CLI, between classes based on the Fontaine classification) provided only observations regarding certain trends, usually statistically insignificant but setting the tendency of limb blood supply changes. Probably, investigation of temporal changes and oscillations in VEGF-A, s-VEGFR-1, and s-VEGFR-2 levels in patients with PAD may be clinically relevant. Other measurements of plasma levels of these markers might be useful to confirm their clinical role.

Conclusions

The quotient of VEGF-A/sVEGFR-2 (the so-called "VASCULAR-2 ratio") can comprise a biochemical indicator allowing to distinguish the severity of tissue ischemia and so angiogenesis in patients with IC and critical PAD, but it needs to be supported in further studies on a larger number of patients with PAD, especially with CLI.

VASCULAR-1 ratio and/or VASCULAR-2 ratio may be a useful no-invasive ischemic marker in the future, but its potential clinical value, for example, to monitor the efficiency of treatment, requires further studies on a large group of patients with PAD.

Declaration of Conflicting Interests

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