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Prognostic usefulness of IL-6 and VEGF for the occurrence of changes in coronary arteries of patients with stable angina and implanted stents

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Abstract. – AIM: The aim of this study was to determine the prognostic significance of interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) in patients with chronic coronary artery disease treated who underwent percutaneous coronary intervention with stent implantation, for assessing the risk of restenosis and the occurrence of *de novo* lesions.

PATIENTS AND METHODS: 498 patients with stable angina were examined during 18 months. 50 patients with significant (> 70%) stenosis of one coronary artery, eligible for the implantation of one stent, were enrolled to the study. Il-6 and VEGF level was measured using ELISA immunoassays during the initial coronary angiography with simultaneous angioplasty and stent implantation and 4 weeks after stent implantation. Coronary angiography was carried out 8-12 months after stent implantation.

RESULTS: Statistically significant increase in IL-6 (from 4.02 ± 4.40 to 10.90 ± 8.23) and VEGF (from 310.13 ± 50.90 to 392.32 ± 106.84) level was observed 4 weeks after stent implantation in the group with restenosis.

CONCLUSIONS: Increased levels of IL-6 and VEGF in the peripheral blood of patients with chronic stable angina pectoris, measured 4 weeks after coronary angioplasty with stent implantation, may indicate an increased risk of angiographic restenosis and *de novo* coronary artery lesions.

Key Words:
Stable angina, Cytokines, Growth factors, Cardiac surgery.

Abbreviations

ACEI = angiotensin-converting-enzyme inhibitor; ASA = acetylsalicylic acid; b-FGF = basic fibroblast growth factor; BMS = bare-metal stent; CABG = coronary artery bypass graft; DES = drug-eluting stent; hs-CRP = high-sensitivity C-reactive protein; IL-1 = interleukin 1; IL-18 = interleukin 18; IL-6 = interleukin 6; LDL = low-density lipoprotein; NSAIDs = non-steroidal anti-inflammatory drugs; PCI = percutaneous coronary intervention; PDGF = platelet-derived growth factor; PTCA = percutaneous transluminal coronary angioplasty; QCA = quantitative coronary angiography; ROC = receiver operating characteristic; SES = sirolimus-eluting stent; TNF-alpha = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.

Introduction

Atherosclerosis is a non-specific inflammatory disease with a multifactorial pathogenesis. Inflammatory mechanisms are involved not only in the formation of, but also in the rupture of atherosclerotic plaque. An important role in the pathogenesis of atherosclerosis is played by endothelial dysfunction, which results in the appearance of adhesion molecules on the surface of leukocytes[1]. Active macrophages, located in the vascular wall, are the source of proinflammatory cytokines such as IL-1, IL-6, IL-18, and TNF-alpha[2]. Another aspect of the progression of atherosclerosis is angiogenesis[3]. VEGF, PDGF, and b-FGF are among the most important growth factors for angiogenesis[4,5]. The role of proinflammatory cytokines and growth factors has been extensively studied in acute coronary syndromes and in patients with unstable angina[6,7]. The results of previous studies are inconclusive.

In stable coronary artery disease, the chronicity of the process makes it difficult to capture significant changes in concentrations of proinflam-

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matory cytokines. Monitoring of serum cytokines after stent implantation allows assessing the level of inflammation in these patients. Therefore, the aim of this study was to determine the prognostic significance of IL-6 and VEGF serum concentration in patients with chronic coronary artery disease who underwent percutaneous coronary intervention with stent implantation (PCI), for assessing the risk of restenosis and the occurrence of *de novo* lesions.

Patients and Methods

The research protocol was approved by the local Bioethical Committee, Poznan University of Medical Sciences (No 203/2009; 5 March 2009).

Patients

498 patients who had been admitted to the First Clinic of Cardiology at Poznan University of Medical Sciences (Poland) with a diagnosis of stable angina entered the study in the period from October 2008 to March 2010. Patients who had undergone surgery on the coronary arteries (PTCA, CABG), as well as patients with multivessel disease, coexisting inflammation (verified by clinical examination), diabetes mellitus, cancer, renal impairment, active peptic or duodenal ulcers, hyperuricemia, and those being treated with NSAIDs or steroids (with the exception of ASA) were excluded from the study.

50 patients with significant (> 70%) stenosis of one coronary artery, eligible for the implantation of one stent, were enrolled to a further study.

Measurement of Il-6 and VEGF Level

Blood to clot was collected to determine the Il-6 and VEGF levels. The blood was centrifuged and stored at –80°C until the analysis. The following 3 studies were carried out in all 50 patients.

Study A: The measurement of IL-6 and VEGF levels during the initial coronary angiography with simultaneous angioplasty and stent implantation (9 x bare-metal stent (BMS), 41 x drug-eluting stents (DES).

Study B: The measurement of IL-6 and VEGF levels 4 weeks after stent implantation,

Study C: Based on the study protocol repeated coronary angiography was performed in all patients 8-12 months after initial PCI for assessing the occurrence of restenosis, or the appearance of *de novo* coronary artery lesions. Coronary stenosis was quantified visually and with the use of Siemens QCA. Restenosis was defined as >50% luminal narrowing at the site of previous dilatation. Coronary angiograms were independently reviewed by 3 observers (experienced invasive cardiologists). When the findings of the three observers differed, the fractional flow reserve (FFR) or intravascular ultrasound (IVUS) was performed, based on inavsive cardiologist preference to resolve the difference

IL-6 and VEGF concentrations were determined in the peripheral blood serum by ELISA, using DRG enzyme immunoassays (DRG International, Inc., Springfield Township, NJ, USA). The angioplasty was performed using the standard technique of radial (80%) or femoral (20%) artery access. The selection of the stent to be implanted in the patient (DES vs. BMS) was left to the decision of the operator. High-pressure plain balloon postdilatation was performed on all patients. Coronary stenoses were quantified visually and with the use of Siemens quantitative coronary angiography system (QCA). A luminal narrowing of > 70% of the cross-sectional area were considered to represent a hemodynamically significant coronary artery lesion. Coronary angiograms were independently reviewed by two observers. When the findings of the two observers differed, the opinion of a third physician was used to resolve a difference.

Statistical Analysis

Statistical analysis was carried out using StatSoft's CSS STATISTICA version 7.0. Arithmetic means and standard deviations of the results were computed using the Descriptive Statistics procedure. Compliance of the variables with the theoretical normal distribution was assessed by the Shapiro-Wilk test. The evaluation of the statistical significance of differences of means in case of abnormal distribution was carried out using the Mann-Whitney test. All hypotheses were verified at the significance level $\alpha = 0.05$. To assess the usefulness of selected features in prediction of coronary lesions, the comparison of areas under the ROC curves was carried out.

Results

The study was completed in all patients. The control angiography examination, which took place 8-12 months following stent implantation, resulted in no restenosis at the previously im-

planted stent and no significant *de novo* coronary artery lesions in 39 patients (group I). In 11 patients (group II), the control coronary angiography revealed restenosis at the previously implanted stent in 7 patients (63.6%; 1 × BMS, 6 × DES) and significant *de novo* coronary artery lesions in 4 patients (36.4%; 1 × BMS, 3 × DES). The two groups did not differ in the diameter of the widened vessel, the minimum diameter of the vessel, the percentage of stenosis before and after surgery, or the length of implanted stent. The angiographic characteristics of studied groups are presented in Table I.

No statistically significant differences between groups I and II were found on the basis of demographic data, risk factors, selected biochemical tests, or treatment (Table I). Hypercholesterolemia was recognized with LDL cholesterol levels above 100 mg/dl.

The results of laboratory tests and the treatments used at the beginning (A) and end (C) of the study in both groups of patients are summarized in Table II.

No statistically significant differences were found in the concentrations of hs-CRP, leukocytes, or platelets between study A and study C, either in patients lacking coronary lesions, or in patients with *de novo* lesions or restenosis ($p > 0.05$).

No significant difference was found in study C between the number of patients receiving β-blockers, ACEI, or ASA in either group ($p > 0.05$).

A statistically significant reduction in the number of patients with elevated LDL cholesterol was observed after 8-12 months of statins therapy.

The mean value, standard deviation, and range of the IL-6 concentration, shown for groups I and II of studies A and study B are presented in Table III. No statistically significant differences in IL-6 serum concentration between study A and study B were found in group I. However, statistically significant increase in Il-6 concentration was observed in group II 4 weeks after stent implantation (study B).

There were no significant differences between the initial concentrations of IL-6 in group I and group II. A statistically significant difference was

Table I. The angiographic and clinical characteristic of studied groups.

	Group I (n = 39)	Group II (n =11)	p
Angiographic Parameters			
Reference diameter (mm)	3.30 ± 0.30	3.28±0.30	NS
Minimal lumen diameter (mm)	0.55 ± 0.25	0.65±0.30	NS
Percentage of stenosis (before PCI)	79.0 ± 9.0	78.0±7.0	NS
Percentage of stenosis (after PCI)	5.0 ± 2.0	4.8±2.2	NS
Length of implanted stent	20.0 ± 4.4	19.0±4.6	NS
Location of change:			
Left anterior descending artery	11 (28%)	5 (45%)	
Circumflex artery	14 (36%)	1 (10%)	NS
Right coronary artery	14 (36%)	5 (45%)	
Demographic data			
Age (years)	61 ± 11	62±12	NS
Number of males	34 (87.1%)	8 (72.7%)	NS
BMI (kg/m²)	29.1 ± 3.9	29.3±4.2	NS
Selected risk factors			
Hypertension	28 (71.7%)	7 (63.6%)	NS
Smoking	18 (46.1%)	7 (63.6%)	NS
Family history	23 (61%)	7 (63.6%)	NS
Selected results of laboratory tests			
hs-CRP (mg/l)	2.9 ± 5.0	3.4±5.5	NS
Leukocytes ($\times 10^9$)	7.3 ± 4.0	8.1±3.2	NS
Platelets (10^9/l)	254 ± 95	281±101	NS
Treatment			
Beta blockers	31 (79.4%)	10 (90.9%)	NS
ACEI	32 (82.0%)	10 (90.9%)	NS
Statins	38 (97.4%)	11 (100%)	NS
ASA	39 (100%)	11(100%)	NS
Clopidogrel	39 (100%)	11 (100%)	NS

Table II. The comparison of selected clinical parameters of patients at the beginning and at the end of the study.

	Study A (initial)		Study C (after 8-12 months)	
	Group I	Group II	Group I	Group II
Selected results of laboratory tests				
hs-CRP (mg/l)	2.9 ± 5.0	3.4 ± 5.5	3.1 ± 4.2	3.2 ± 5.0
Leukocytes (10^9/l)	7.3 ± 4.0	8.1 ± 3.2	7.6 ± 5.1	7.9 ± 6.1
Platelets (10^9/l)	254 ± 95	281 ± 101	239 ± 96	261 ± 98
Hypercholesterolemia	31 (79%)	9 (81%)	10 (24%)	3 (27%)
Treatment				
Beta blockers	31 (79.4%)	10 (90.9%)	30 (76.1%)	10 (90.9%)
ACEI	32 (82.0%)	10 (90.9%)	31 (79.4%)	10 (90.9%)
Statins	38 (97.4%)	11 (100%)	38 (97.4%)	11 (100%)
ASA	39 (100%)	11 (100%)	39 (100%)	11 (100%)
Clopidogrel	39 (100%)	11 (100%)	39 9100%)	11 (100%)

found in study B between groups I and II. Figure 1a shows the values and significance of changes in individual groups and studies.

The mean value, standard deviation, and range of VEGF concentration for groups I and II of studies A and B, are shown in Table IV. There were no significant differences between study A and B in VEGF concentration in group I. A statistically significant increase in VEGF level was observed in group II, 4 weeks after stent implantation. There were no significant differences between the initial levels of VEGF in group I and II; however, a statistically significant difference between group I and II was found in study B. Figure 1b shows the values and significance of changes in individual groups and studies.

The usefulness of increased IL-6 and VEGF serum concentration in predicting coronary lesions in patients with angioplasty was assessed using the ROC curve (Figure 1c and d). The positive predictive value was 90.0%, and the negative prediction value was 71.8% for the cut-off point of increase in IL-6 concentration above 3.0 pg/ml. The resulting area under the ROC curve was 0.88 with $p < 0.0001$. In case of VEGF, the positive predictive value was 72.7% and the negative was 76.92% for the cut-off point of increase in VEGF concentration above 354.8 pg/ml. The resulting area under the ROC curve was 0.75 with $p = 0.0003$.

Discussion

Stent implantation is associated with endothelial damage, and restenosis is an inflammatory response of vessels and is, in some sense, a process of recovery[8]. The role of factors which affect the rate of progression of atherosclerosis and restenosis is poorly understood. Moreover, patients with restenosis after angioplasty also show the progression of atherosclerosis in other vascular segments. Although several studies have shown the important role of inflammatory-immune factors in processes associated with restenosis (re-endothelization),

Table III. IL-6 concentration in group I and II patients, divided into A and B study.

Type of parameter	Values* according to the division into groups		Differences between Group I and II
	Group I	Group II	
Il- 6 A (pg/ml) (initial)	4.59 ± 5.95 (0.00-25.26)	4.02 ± 4.40 (0.00-14.12)	NS
Il- 6 B (pg/ml) (after 4 weeks)	2.19 ± 3.09 (0.00-12.50)	10.90 ± 8.23 (2.33-26.00)	$p = 0.0001$
Differences between A and B study	NS	$p = 0.01$	

* mean, SD, variance.

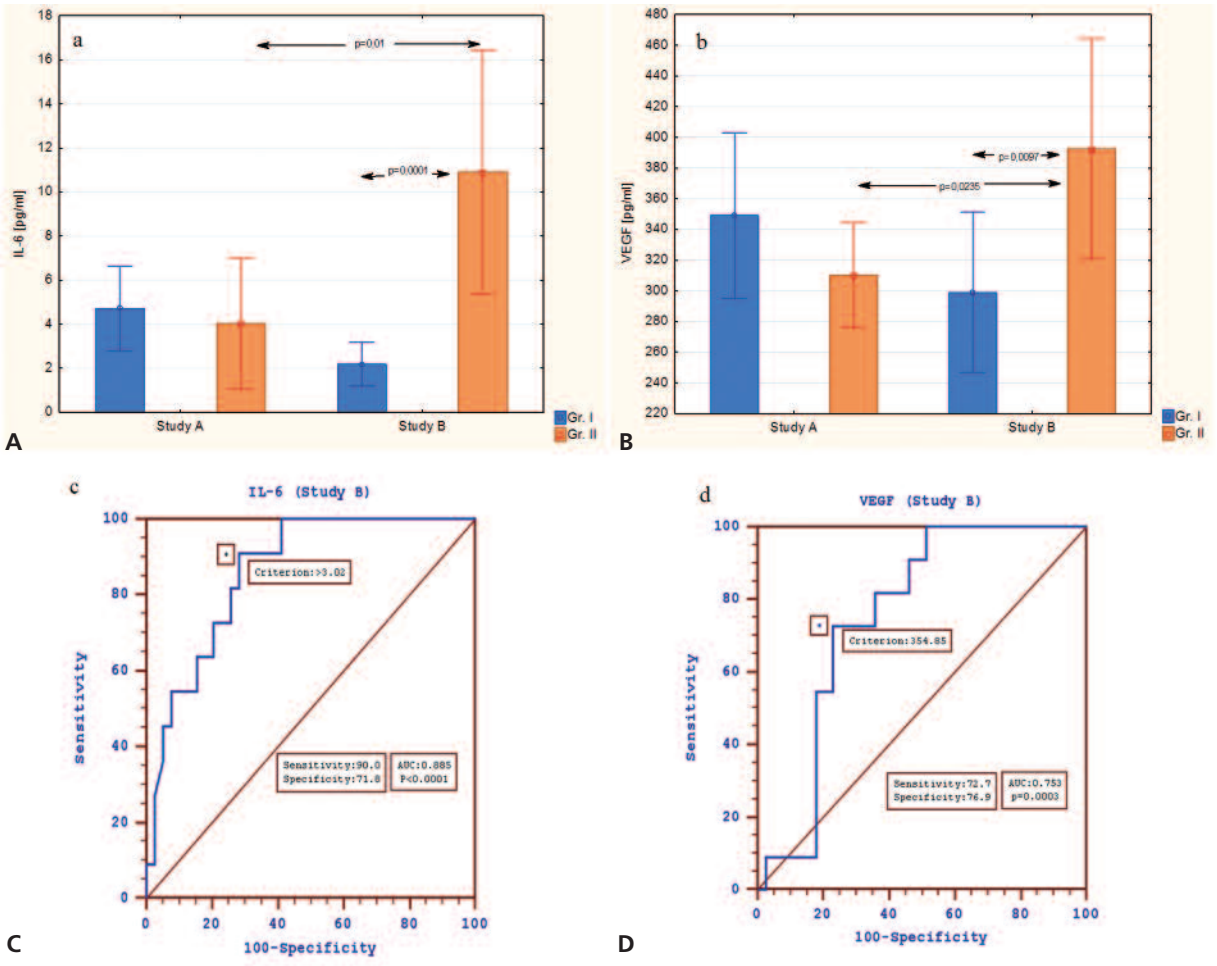

Figure 1. Concentrations of IL-6 in group I and group II (study A and study B) ***(A)***. Concentrations of VEGF in group I and group II (study A and study B) ***(B)***. ROC (receiver operating characteristic) curve to determine the increase in IL-6 after surgery in predicting coronary lesions ***(C)***. ROC curve to determine the increase in VEGF after surgery in predicting coronary lesions ***(D)***.

Table IV. VEGF concentration in group I and II patients, divided into A and B study.

Type of parameter	Values* according to the division into groups		Differences between Group I and II
	Group I	Group II	
VEGF A (pg/ml) (initial)	350.03 ± 167.94 (55.40 - 689.88)	310.13 ± 50.90 (210.34 - 401.03)	NS
VEGF B (pg/ml) (after 4 weeks)	295.21 ± 162.29 (25.22 - 793.63)	392.32 ± 106.84 (265.02 - 670.45)	$p = 0.009$
Differences between A and B study	NS	$p = 0.02$	

* mean, SD, variance.

their ultimate significance is not clear[9-11]. The levels of serum cytokines are highly variable, conditioned by as yet not fully identified factors[12]. The discrepancies in the results not only reflect the diverse pathogenesis of ischemic heart disease, but also result from methodological differences. The authors believe that careful selection of the study group is a significant restriction on the effect of various factors on cytokines levels. In this study, the patient and control group did not differ in terms of demographic data or the prevalence of risk factors. There were no statistically significant differ-

ences in the level of platelets, or studied inflammatory markers, i.e., hs-CRP, leukocyte levels, between the initial and the control study (i.e. after 8-12 months). The groups also did not differ in the angiographic parameters. The treatment was identical, which allows the impact of therapy on the results to be excluded. A similar decrease in LDL-cholesterol levels was observed in both groups over 12 months. In Speidl et al report[13], concerning the prediction of restenosis in 85 patients, VEGF was measured at baseline and 24 hours after the implantation of DES (a total of 159 implantations). Control coronary angiography, performed 6–8 months later, revealed the occurrence of restenosis in 14% of respondents. It was shown that high concentrations of VEGF before coronary angioplasty may have a protective role, whereas the increase in VEGF 24 hours after implantation of DES was associated with an increased risk of in-stent restenosis. The Authors, therefore, stress the ambiguous role of VEGF in the process of vascular diameter loss following coronary angioplasty.

Similarly, we observed a statistically significant increase in VEGF levels 4 weeks after stent implantation in the group with restenosis. For VEGF values above 354.85 pg/ml, the positive predictive value was 72.7% and negative predictive value was 76.92%.

The ambiguous role of VEGF was shown by Kochiadakis et al[14], who evaluated VEGF levels and VEGF gene expression in peripheral blood leukocytes in 44 patients with stable coronary artery disease who were implanted with a stent in one coronary artery; the study was randomized between the SES (sirolimus-eluting stents) and BMS groups. VEGF in the BMS group showed a growth trend, while in the SES group a statistically significant decrease in VEGF levels was observed, as compared with the initial value. There was no correlation between the occurrence of restenosis and the concentration of VEGF, but a correlation was found with the gene expression of VEGF in patients with restenosis, both in the SES and BMS group.

The data on the role of IL-6 in the process of restenosis is also divergent. We observed a statistically significant increase in IL-6, 4 weeks after stent implantation in the group with restenosis. ROC analysis revealed that IL-6 > 3.0 pg/ml has a positive predictive value of 90.9% and negative predictive value of 71.8%. Similar findings were obtained by Zurakowski et al[15] in 73 patients with stable coronary artery disease who underwent stent implantation, and had the level of IL-6 measured initially and after 6 months. The group with significant in-stent restenosis was characterized by elevated levels of IL-6.

On the other hand, in the work of Hudzik et al[16], IL-6 was measured in 37 patients divided into three groups: early restenosis, late restenosis, and history of restenosis. There were no significant differences between these groups, and no correlation between the diameter of the vessel and the concentration of IL-6, was observed.

The increased levels of VEGF and IL-6 observed in our research 4 weeks following invasive treatment in patients who developed restenosis or *de novo* coronary artery lesions, may serve as an early marker of changes (restenosis or *de novo* lesion). Our report and review of the literature suggest that, in patients with stable coronary artery disease and implanted stents, different consequences of inflammation are observed in different periods of time. It cannot be excluded that the inflammation induced by stent implantation may contribute to restenosis and *de novo* coronary artery lesions. For example, the results of the PROSPECT study, published by Stone et al[17], do not exclude such a possibility. However, further researches are required to confirm or deny this hypothesis.

Conclusions

The increased levels of IL-6 and VEGF in the peripheral blood of patients with chronic stable angina pectoris, measured 4 weeks after coronary angioplasty with stent implantation, may indicate an increased risk of angiographic restenosis and *de novo* coronary artery lesions.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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