High serum interleukin-18 concentrations in patients with coronary artery disease and type 2 diabetes mellitus

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ABSTRACT. ***Aims.*** **The aim of our study was to analyse the serum level of interleukin 18 (IL-18) in coronary artery disease (CAD) patients with type 2 diabetes mellitus (DM), and to relate this to clinical findings.** ***Methods.*** **The IL-18 level was measured by ELISA in serum samples from 130 CAD patients prior to their first, elective, coronary artery bypass surgery. Forty-three of them had been diabetic for several years. A control group consisted of 31 healthy people matched according to age, BMI, lipid and smoking status.** ***Results.*** **The CAD patients with DM were similar to the non-diabetic CAD patients with respect to age, BMI, grade of heart failure, ejection fraction. There were no differences in the duration of CAD, history of myocardial infarction and PTCA or instability of angina. The serum level of IL-18 was higher in the CAD patients than in the control group. The CAD patients with DM had a higher concentration of IL-18 compared to the non-diabetic CAD group. The diabetic patients with triple-vessel disease were characterized by a higher concentration of IL-18 than the non-diabetic patients with the same grade of CAD. Smoking affected the IL-18 concentration, particularly in the diabetic patients.** ***Conclusion.*** **Type 2 DM predisposes patients, especially those with multi-vessel CAD who were smokers, to a higher serum level of IL-18, which may help explain their vulnerability to fatal, secondary cardiovascular events. These patients should be in the first line for stringent, secondary cardiovascular prevention.**

Keywords: interleukin 18, coronary artery disease, type 2 diabetes mellitus

Patients with coronary artery disease and type 2 diabetes are different from those without diabetes. CAD among patients with diabetes attracts more attention because of the high mortality rates. Although in the United States, there has been a decline of 13% in CAD mortality in diabetic men and an increase of 23% for diabetic women [1], this is in the contrast with trends observed in the general CAD population. In all patients with CAD, and in non-diabetic CAD individuals, mortality rates have decreased by 36%-62% in men and by 27%-45% in women respectively [1, 2]. This decrease has been attributed to reductions in population risk factors [2]. Diabetic patients tend to have similar CAD risk factors, but at higher rates [3], an increased risk of recurrent disease, an increased likelihood of angiographic restenosis [4], an increased short-term (7 days) and post-CABG adverse outcome [5], in addition to a poorer overall prognosis. A long-term response to CABG in diabetics was found to be poorer as well [6].

At present, the prevailing hypothesis suggests that the inflammation reflecting responses of innate and acquired immunity plays a crucial role in the development and progression of atherosclerosis and type 2 diabetes [7-12]. Circulating markers of inflammation such as C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, sVCAM-1, sICAM-1 have been shown to predict cardiovascular risk in initially healthy people across different populations [7-9, 13]. The elevated levels of CRP and IL-6 predict the development of type 2 diabetes in healthy women and men [10, 11, 13]. Their application to clinical practice has recently been discussed [14], but only hs-CRP was recommended as a useful predictor of coronary events in the healthy population, however not for secondary prevention clinical practice.

Interleukin-18 (IL-18), as a pro-inflammatory cytokine, is involved in the development and progression of both atherosclerosis and type 2 diabetes. IL-18 induces atherogenesis by stimulating the IL-18 receptors on endothelial and smooth muscle cells, releasing IL-6 and IL-8 from these cells, as well as by up-regulating an expression of adhesion molecules and matrix metalloproteinases [15]. Moreover, the inhibition of IL-18 signalling has been shown to reduce lesion progression and to change the plaque composition towards stable forms [16]. IL-18-deficient, apolipoprotein E-knockout mice exhibited substantially reduced atherosclerotic lesion size, in spite of increased serum cholesterol

[17]. Increased inflammatory activity predisposes atherosclerotic plaques to destabilization [18, 19]. IL-18 concentrations tend to be higher in the patients with unstable angina [20] and acute coronary syndrome [21]. Moreover, patients with documented coronary disease and a high serum level of IL-18 had a 3.3–fold increase in subsequent fatal cardiovascular events, compared to those with a low level of IL-18 [22].

The Prospective Epidemiological Study of Myocardial Infarction (PRIME Study) has claimed a strong association between inflammatory biomarkers and classical risk factors (body mass index, smoking status, diabetes, hypertension, lipid status) [7, 23]. Since the concentration of IL-18 was independent of either classical or inflammatory factors, this cytokine was identified as a predictor of coronary events in healthy, middle-aged, European men [23]. In patients with established CAD, IL-18 has been recognised as a strong predictor of cardiovascular death in the AtheroGene Study [22]. IL-18 is also implicated in promoting a low-grade, systemic inflammation in patients with type 2 diabetes. Newly diagnosed, type 2 diabetic patients without micro and macrovascular complications [24, 25], and those with long-lasting diabetes [26, 27] presented higher circulating concentrations of IL-18 compared with non-diabetic subjects. In patients with diabetes and a high level of IL-18, the early atherosclerosis changes were more pronounced [26].

We have not encountered a study analysing serum IL-18 level in patients with established, multi-vessel coronary artery disease and long-lasting, type 2 diabetes. Therefore, it was interesting to look at the concentration of IL-18 in such patients and to find out whether IL-18 may be a discriminating marker for secondary prevention in CAD patients.

PATIENTS AND METHODS

One hundred and thirty consecutive patients admitted between October 2002 and November 2003, to The Clinic of Cardiac Surgery of Medical University in Gdańsk and scheduled for initial, elective, coronary artery bypass surgery, were enrolled in the study. Coronary angiography was performed in all patients. The patients with two or three vessel disease qualified for surgery. They had at least 75% stenosis in two [left anterior descending coronary artery (LAD) + circumflex coronary artery (CX) or LAD+ right coronary artery (RCA)] or three of the major (LAD + CX + RCA) coronary arteries. The clinical data were entered into a computerized database. Written informed consent was obtained from all participants. The present study was approved by the Ethics Committee of the Medical University of Gdańsk. The investigation conforms to the principles outlined in the Declaration of Helsinki [27]. All patients with CAD were divided in two groups: with type 2 diabetes and without diabetes. Diagnosis of type 2 diabetes was based on the criteria of the American Diabetes Association [28]. Diabetic patients were tested for diabetic nephropathy. None of them had an albumin excretion rate above 30 mg/24h.

Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or the self-reported use of anti-hypertensive medication. Information concerning the first diagnosis, smoking history and medication use was obtained by interview. Subjects who reported smoking at least 1 cigarette per day during the year prior to the examination were classified as current smokers. The angina pectoris was graded according to the Canadian Cardiovascular Society Classification for Angina Pectoris (CCS). Unstable angina was defined according to the Braunwald criteria [29]. The New York Heart Association (NYHA) functional classification of patients with heart disease was applied for description of the patient's state. The control group consisted of 31 individuals as presented in *table 1*. They were non-diabetic (fasting plasma glucose < 110mg/dL and negative 75 g oral glucose tolerance test), and without CAD (absence of chest pain at rest and on exertion, no ECG changes at rest, negative exercise stress test). They were recruited from the staff of the Medical University of Gdańsk. They had not suffered from any acute or chronic disease during the 3 months prior to examination.

Table 1
Characteristics of the control group and the group with coronary artery disease

Characteristics	Control group (n = 31)	CAD patients (n = 130)
Men/women	16/15	96/36
Age (years)	61.2 ± 7.6	62.5 ± 9.0
BMI, kg/m^2	28.7 ± 4.7	28.4 ± 4.3
Total cholesterol (mg/dL)	221.4 ± 46.3	217.0 ± 51.0
LDL cholesterol (mg/dL)	143.2 ± 40.1	133.3 ± 40.7
HDL cholesterol (mg/dL)	48.5 ± 15.7	43.4 ± 11.8
Triglycerides (mg/dL)	168.1 ± 105.3	172.0 ± 118,.0
FPG (mg/dL)	93.6 ± 12.6	116.1 ± 47.4
Smoking status (n (%))		
current smoking	7 (25.6)	31 (23.8)
ex-smokers	16 (48.4)	60 (46.2)
never smoked	8 (25.8)	39 (30.0)
Duration of smoking (years)	26 (2.0-51.0)	27 (1.0-54.0)
Serum level of IL-18 (pg/mL)	248.99 ± 103.69 276.6 (102.5-540.3)	463.48 ± 111.7* 445.0 (262.0-688.0)

Data are presented as: number (percentage) of patients, means ± SD or median (min-max). *p = 0.0001. BMI: body mass index. FPG: fasting plasma glucose.

Blood collection

Blood was drawn from the ante cubical vein the day before surgery, between 7 and 8 o'clock, was frozen in aliquots (-70°C) and used no later than 3 months thereafter.

Laboratory examinations

Fasting plasma glucose (FPG) and lipid were measured by enzymatic tests (Roche Diagnostics GmbH, Germany) and Comray-Chol, Comray-HDL Direct and Comray-TG (P.Z. Comray, Poland). Urinary albumin excretion had been measured in triplicate, timed and overnight, at least twice a year, by immuno-turbidometric assay using Tina-quant® (Boehringer Mannheim GmbH, Germany). Urinary albumin excretion was expressed as the mean of all 24h collections obtained during the 6 months prior to our examination.

Determination of IL-18

Serum IL-18 was measured by ELISA using commercial antibodies purchased from R&D System (Minneapolis, Minn., USA). The sensitivity limit of the assay was 12.5 pg/mL. The intra-assay and inter-assay coefficients of variation were 6.25% and 9.92%, respectively.

Statistical analysis

The results were analysed using the Statistica, version 6 program (StatSoft, Pl). Continuous variables were tested for normality by the Kolmogorov-Smirnov test. Normally distributed variables were analyzed with the ANOVA test. The results of the ANOVA test were presented as arithmetic means ± SD. For comparison of the skew-distributed variables, the non-parametric Mann Whitney U test was employed. The results of the Mann Whitney U test were presented as median (min-max). Nominal variables were analyzed by the χ^2 Pearson test. Correlation was determined by linear and multivariate regression analyses. The non-normally distributed values were log-transformed before performing multivariate regression. In all analyses, a two-tailed significance level < 0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics

Characteristics of the control group in relation to all 130 patients with CAD are presented in *table 1*. The control group was matched with CAD patients with respect to: age, BMI, total cholesterol, LDL-cholesterol, triglycerides, fasting plasma glucose and smoking status.

Baseline characteristics of the CAD diabetics and CAD non-diabetics are reported in *table 2*. As expected, there was a higher percentage of men than women only in the non-diabetic group ($p = 0.03$). Duration of DM was eight years (0.25-30.0). The diabetic and non-diabetic patients were similar with respect to age, BMI, duration of CAD, history of myocardial infarction and PTCA, instability of angina, numbers of affected vessels, grade of heart failure and ejection fraction. Clinical examinations showed that the diabetic patients had more advanced symptoms of CAD according to CSS classification ($p = 0.023$), and a higher prevalence of hypertension ($p = 0.012$) with longer duration ($p = 0.008$). The diabetic patients had a lower total cholesterol ($p = 0.008$) and LDL cholesterol levels ($p < 0.001$) than the non-diabetics. Medications taken by both groups were similar, except for calcium antagonists ($p = 0.048$), which were more common in diabetic patients. A similar proportion of patients in the diabetic and non-diabetic groups were current smokers at the time, with the same smoking habit duration. There were no differences in the number of ex-smokers and patients who had never smoked in those groups.

IL-18 in patient serum

As shown in *table 1*, IL-18 serum concentration was significantly higher in CAD patients compared to healthy, control individuals ($p = 0.0001$).

As can be seen from *table 3*, IL-18 levels were found to be significantly higher when diabetic CAD patients were compared to non-diabetic CAD patients($p = 0.04$), and when a subgroup of the triple-vessel disease CAD diabetics was compared to the triple-vessel disease CAD non-diabetics ($p = 0.02$). Diabetic men showed significantly higher concentrations of IL-18 than non-diabetic men ($p = 0.05$). Hypertension and unstable angina did not affect IL-18 concentrations in diabetic and non-diabetic patients. The diabetic patients who had received nitrates, β-blockers, calcium antagonists, statins, ACE-inhibitors, and aspirin had still higher concentration of IL-18 compared to the non-diabetic individuals receiving these drugs. The differences were significant for nitrates ($p = 0.05$), statins ($p = 0.03$), ACE-inhibitors ($p = 0.015$), aspirin ($p = 0.05$). IL-18 levels were found to be significantly higher in the group of diabetics who received ACE inhibitors ($p = 0.05$) compared to diabetics who did not receive them. Smoking status affected only the diabetic patients. Those who had never smoked had the lowest levels of IL-18 compared with ex-smokers ($p = 0.05$) and current smokers ($p = 0.04$). There were no differences in the concentrations of IL-18 between ex-smokers and current smokers.

IL-18 levels in CAD patients with and without DM, together with healthy, controls are presented in *figure 1*. The IL-18 levels found in CAD patients were in a higher range as compared to the controls. IL-18 concentrations were significantly higher in CAD DM+ patients than in the CAD DM^- ones ($p = 0.04$).

Relationship between IL-18 and clinical parameters

Linear regression analysis revealed, as presented in *table 4*, that the concentration of IL-18 in the CAD DM+ group did not to correlate with: age, BMI, fasting plasma glucose, HDL cholesterol, LDL cholesterol and total cholesterol, triglycerides, EF, duration of CAD and DM, hypertension, or smoking.

A low but significant inverse correlation was only found between IL-18 and LDL cholesterol values in the non-diabetic, CAD group ($r = -0.28$, $p = 0.05$).

To identify independent factors that might have affected serum concentrations of IL-18, a multivariate regression

Table 2
Demographic and clinical data of patients with coronary heart disease with or without type 2 diabetes

Characteristics	CAD DM+ (n = 43)	CAD DM - (n = 87)	p
Men/women (%)	26/17 (60.5/39.5)	68/19 (78.2/21.8)	0.03*
Age (years)	65 (48-77)	63 (45-82)	0.9
Duration of DM (years)	8 (0.25 –30.0)	-	
Duration of CAD (years)	5 (0.125-30.0)	5 (0.25-30.0)	0.9
History of MI (n (%))	27 (62.8)	55 (63.2)	0.9
Previous PTCA (n (%))	4 (9.3)	4 (4.6)	0.3
CSS I-II/III-IV (n (%))	9/34 (20.9/79.1)	35/52 (40.2/59.8)	0.023*
Unstable angina (n (%))	7 (19)	19 (21,8)	0,5
Triple-vessel disease (n (%))	30 (69.8)	49 (57.0)	0.2
NYHA 1/2/3/4 (n (%))	19/5/12/7 (44.2/11.6/27.9/16.3)	50/8/17/12 (57.5/9.2/19.5/13.8)	0,5
EF (%)	51.1±11.1	52.4±11.0	0,5
Hypertension (n (%))	36 (83.7)	54 (62.1)	0.012*
Duration of hypertension (years)	15 (0.25-40)	5 (0.25-40)	0.008*
BMI (kg/m^2)	29.0 ± 4.8	28.2 ± 4.2	0.3
Total cholesterol (mg/dL)	196.5 (116.0-357.0)	221.5 (129.0-355.0)	0.008*
LDL cholesterol (mg/dL)	106.0 (55.0-194.0)	140.5 (74.0-255.0)	0.001*
HDL cholesterol (mg/dL)	38.0 (28.0-70.0)	44.5 (12.0-94.0)	0.1
Triglycerides (mg/dL)	155.0 (64.0-982.0)	140.0 (57.0-660.0)	0.6
FPG (mg/dL)	141.0 (45.0-329.0)	95.0 (71.0-158.0)	0.001*
Medication			
Nitrates (n (%))	37 (86.1)	75 (86.2)	0.98
ß-blockers (n (%))	35 (81.4)	70 (81.4)	1.00
Calcium antagonists (n (%))	14 (32.6)	15 (17.2)	0.048*
Statins (n (%))	31 (73.8)	66 (75.9)	0.6
ACE inhibitors (n (%))	31 (73.8)	52 (60.5)	0.1
Aspirin (n (%))	39 (90.7)	77 (88.5)	0.7
Diuretics (n (%))	11 (29.7)	21 (25.9)	0.7
Diabetes treatment			
Oral hypoglycemic agents (n (%))	29 (67.4)		
Insulin	14 (32.6)		
Smoking status (n (%))			
current smoking	10 (23.3)	21 (25.3)	0.8
ex-smokers	21(48.8)	39 (47.0)	0.8
never smoked	12 (27.9)	27(31.7)	0.7
Duration of smoking (years)	30.0 (1.0-54.00)	25.0 (2.0-50.0)	0.8

Data are presented as: number (percentage) of patients, means ± SD or median (min-max). CAD DM+: diabetic patients. CAD DM-: non-diabetic patients. MI: myocardial infarction. PTCA: percutaneous transluminal coronary angioplasty. CCS: Canadian Cardiovascular Society Classification for Angina Pectoris. NYHA: New York Heart Association classification. EF: ejection fraction. FPG: fasting plasma glucose. * Statistically significant ($p < 0.05$).

analysis controlling for selected variables (age, BMI, lipid status, duration of CAD, angina status, EF, FPG, DM, duration of DM, hypertension, duration of hypertension, number of affected vessels, smoking, medications) was performed. In a model that explained only 16% of variation of serum IL-18 levels *(table 5)*, triglycerides ($\beta = 0.29$, $p = 0.01$), type 2 diabetes ($\beta = 0.19$, $p = 0.03$) and smoking ($\beta = 0.22$, $p = 0.01$) were independent determinants of serum IL-18 concentration in patients with coronary heart disease.

DISCUSSION

In our study and for the first time, IL-18 serum concentrations were measured in patients with advanced coronary heart disease with at least two stenoses, with > 75% diagnosed in the major coronary artery. In all studies conducted so far, a high level of this cytokine has been observed in patients with early atherosclerosis [26] and in patients with at least one stenosis, > 30% [22] or > 75% [21]. We have chosen IL-18 as an inflammatory marker because it seems to be independent of the well-known classical risk factors and other inflammatory markers such as CRP, IL-6 and fibrinogen [22]. We have demonstrated that patients with at least two-vessel CAD had a significantly elevated level of IL-18 compared with the control group. A slightly lower level of IL-18 in a much smaller group of patients with acute coronary syndrome was found in one previous study [21]. In our study, we concentrated on the IL-18 serum levels among patients with advanced CAD and long-lasting type 2 diabetes. The IL-18 concentration appeared to be significantly higher in the diabetic CAD group compared to the non-diabetic CAD individuals.

Table 3
Serum IL-18 concentrations in patients with coronary artery disease with or without type 2 diabetes

Characteristics	CAD DM+ (n = 43)	CAD DM- (n = 87)	p[1]
Patients	500.0 (274.0-684.0)	430.0 (262.0-688.0)	0.04*
Men	510.0 (310.0-684.0)	433.5 (262.0-688.0)	0.05*
Women	451.0 (274.0-652.0)	430.0 (293.0-680.0)	0.2
p[2]	0.2	0.3	
Hypertension			
yes	487.0 (274.0-684.0)	430.0 (274.0-688.0)	0.07
no	531.0 (274.0-684.0)	424.0 (262.0-680.0)	0.3
p[2]	0.6	0.7	
Unstable angina			
yes	524.0 (420.0-680.0)	472.0 (293.0-680.0)	0.3
no	482.0 (274.0-684.0)	424.0 (262.0-688.0)	0.07
p[2]	0.3	0.3	
Triple-vessel disease			
yes	513.0 (344.0-684.0)	432.0 (274.0-688.0)	0.02*
no	445.0 (274.0-652.0)	424.0 (262.0-680.0)	0.5
p[2]	0.2	0.6	
Smoking status			
1. never smoked	441.0 (274.0-556.0)	429.0 (284.0-680.0)	0.8
2. ex-smokers	551.0 (529.0-577.0)	495.0 (390.0-531.0)	0.2
3. current smoking	510.0 (333.0-684.0)	435.0 (262.0-688.0)	0.07
p[2] *1-2*	0.05*	0.2	
1-3	0.04*	0.4	
2-3	0.7	0.9	
Medication			
Nitrates			
yes	508.0 (274.0-684.0)	430.0 (262.0-688.0)	0.05*
no	452.0 (360.0-570.0)	440.0 (293.0-636.0)	0.7
p[2]	0.6	0.9	
β-blockers			
yes	502.7 ± 90.7	464.9 ± 116.8	0.07
no	437.6 ± 118.5	408.9 ± 95.4	0.5
p[2]	0.09	0.06	
Calcium antagonists			
yes	452.0 (274.0-684.0)	404.0 (293.0-633.0)	0.5
no	524.0 (310.0 –680.0)	433.0 (262.0-688.0)	0.03*
p[2]	0.2	0.6	
Statins			
yes	500.0 (310.0-684.0)	430.0 (262.0-688.0)	0.03*
no	482.0 (274.0-580.0)	429.0 (302.0-680.0)	0.8
p[2]	0.2	0.8	
ACE inhibitors			
yes	524.0 (310.0-684.0)	437.0 (274.0-668.0)	0.015*
no	451.0 (274.0-577.0)	426.5 (262.0-688.0)	0.9
p[2]	0.05*	0.8	
Aspirin			
yes	500.0 (310.0-684.0)	432.0 (262.0-688.0)	0.05*
no	484.0 (274.0-552.0)	395.5 (302.0-680.0)	0.7
p[2]	0.4	0.3	

Data are presented as: median (min-max). p[1]: difference between patients in the group DM+ and DM-. p[2]: difference within the group DM+ or DM-. CAD DM+: diabetic patients. CAD DM-: non-diabetic patients. * Statistically significant ($p < 0.05$).

The differences in the IL-18 concentrations in the diabetic and non-diabetic patients were more pronounced when patients with triple-vessel disease were compared. The mechanisms responsible for this elevation of the IL-18 serum level in diabetic patients have not been fully clarified. However, conditions such as poor glycaemic control, diabetic nephropathy, obesity and inflammation must be taken into consideration as possible reasons for the high serum IL-18 concentrations. Recently, a pivotal role for acute hyperglycaemia in the induction of adhesion molecules and pro-inflammatory cytokines has been identified [30, 31]. An elevated glucose level has been blamed for an

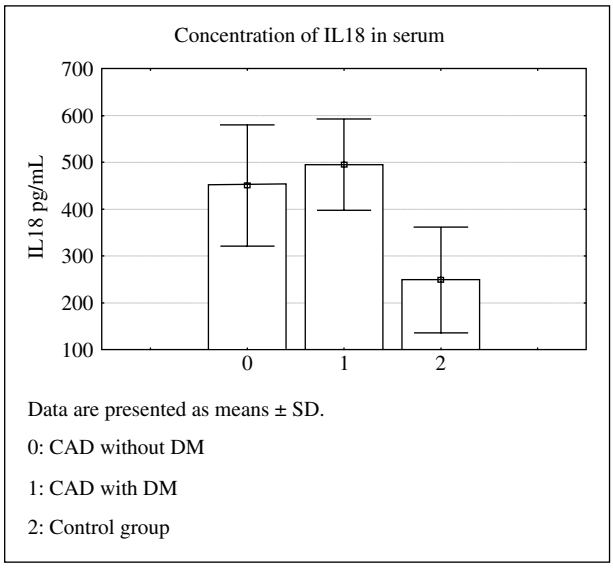

Figure 1
Serum IL-18 concentration in the control group, in patients with coronary heart disease with or without type 2 diabetes.

enhanced expression of vascular cell adhesion molecule (VCAM-1), expressed on human endothelial cells [30]. Through the oxidative mechanism, glycaemia regulates the serum concentrations of IL-6, TNF-α and IL-18 in subjects with normal and impaired glucose tolerance [31]. In non-diabetic patients with a first myocardial infarction, hyperglycaemic stress may contribute to the increased levels of IL-18, CRP and of T-cell activation markers [32]. Some authors have observed that in patients with long-lasting type 2 diabetes, the serum level of IL-18 correlates with glycaemic control [33]. Furthermore, others have found distinctly higher IL-18 serum levels in patients with microalbuminuria or clinical albuminuria than in those without microalbuminuria [33]. An enhanced IL-18 level found in non-diabetic CAD individuals may be regarded as a result of obesity and insulin resistance. It has recently been reported that serum IL-18 concentrations tend to increase in healthy, obese women and decrease after a weight loss [34, 35]. Interestingly, the impact of obesity on the IL-18 concentration was studied only within the female group. Moreover, there was no correlation between the BMI and the IL-18 concentration in the apparently healthy male group [23]. In our study, we did not see any correlation between the IL-18 serum level, fasting plasma glucose (FPG) and BMI in the diabetic patients. However, we could not exclude the role of acute hyperglycaemia in inducing an IL-18 increase in our diabetic patients, because post-prandial hyperglycaemia was thought to be a better marker of plasma glucose level oscillations than FPG [31].

In our studies, the gradation of IL-18 levels was noticeable; the lowest levels were seen in the control group, intermediate levels in the non-diabetic CAD patients, and higher levels in the diabetic CAD ones. The highest levels were found in the diabetic CAD patients with triple-vessel disease. This implies that the type 2 DM patients with CAD, especially with multi-vessel disease, are characterized by the highest score of systemic inflammation. This group is at great risk of further cardiovascular events, and therefore secondary prevention should be taken particularly seriously. The use of statins is highly recommended in this group. The fact that type 2 DM patients are susceptible to statins has been demonstrated in a randomised CARDS trial in the primary prevention model [36]. In 2 838 diabetic patients without high LDL cholesterol, the rates of first cardiovascular events were significantly reduced after four years of treatment with a low dose of atorvastatin. High doses of atorvastatin produced a marked decrease of the high sensitivity to C-reactive protein seen in diabetic patients, after 30 weeks of treatment [37]. Statins have been shown to reduce the circulating, pro-inflammatory marker levels (CRP, IL-6, sICAM-1, sVCAM-1) in hypercholesterolemic and hypertriglyceridemic patients [38-40]. Results of large clinical trials involving patients with coronary artery disease (the PRINCE trial and the MIR-ACL study) have demonstrated that statins strongly decrease the circulating level of CRP [41, 42]. Pleiotropic vascular effects of statins are mediated by inhibition of isoprenoid synthesis and increase in nitric oxide release or its bioavailability [43]. So far, there are only limited data focused on the effects of statins on IL-18 production [44-47]. Although the benefit of statins as anti-inflammatory therapy is well documented, it is noteworthy that other drugs such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs),

Table 4
Correlations between serum IL-18 concentrations and clinical parameters in patients with coronary artery disease with or without type 2 diabetes

Different factors	CAD DM+	CAD DM-
Age (years) ‡	0.12	-0.01
BMI (kg/m^2) †	0.04	-0.08
FPG (mg/dL) †	-0.14	-0.09
Total cholesterol (mg/dL) †	-0.16	-0.13
LDL cholesterol (mg/dL) †	-0.25	-0.28*
HDL cholesterol (mg/dL) †	-0.01	-0.06
Triglycerides (mg/dL) †	0.01	0.21
EF (%) †	0.08	-0.11
Duration of CAD (years) ‡	0.14	-0.02
Duration of DM (years) ‡	-0.01	
Duration of hypertension (years) ‡	0.18	-0.03
Duration of smoking (years) ‡	0.18	0.12

Data are presented as Pearson† or Spearman‡ correlation coefficients. Triglycerides were log-transformed for analysis. Statistically significant *p = 0.05.

Table 5
Multivariate analysis of relationships between serum IL-18 concentrations and selected variables in patients with coronary artery disease

Variables	β	p
Age (years)	0.03	0.75
BMI (kg/m^2)	-0.09	0.35
Total cholesterol (mg/dL)	-0.14	0.38
LDL cholesterol (mg/dL)	-0.23	0.11
HDL cholesterol (mg/dL)	0.03	0.76
Triglycerides (mg/dL)	0.29	0.01*
Duration of CAD (years)	0.01	0.45
Unstable angina (n)	0.17	0.06
Ejection fraction (%)	-0.01	0.94
FPG (mg/dL)	-0.01	0.96
DM (n)	0.19	0.03*
Duration of DM (years)	-0.03	0.78
Hypertension (n)	-0.05	0.60
Duration of hypertension (years)	0.10	0.32
Triple-vessel disease (n)	0.10	0.28
Smoking (n)	0.22	0.01*
Duration of smoking (years)	0.13	0.16
β-blockers (n)	0.01	0.97
Calcium antagonists (n)	-0.19	0.06
Statins (n)	0.01	0.96
ACE inhibitors (n)	0.13	0.17
Aspirin (n)	0.10	0.25

$R^2 = 0.16$. * Statistically significant ($p < 0.05$).

β-blockers, tioglitazones are thought to possess an anti-inflammatory activity as well [48-53].

In our study, we could not show any differences in IL-18 serum concentrations between recipients and non-recipients of statins, β-blockers or aspirin. Also, the multivariate regression analysis did not show any effect of medical treatment on IL-18 serum levels. This probably was due of the unequal distribution of the recipients versus non-recipients (about 80% were recipients).

Interestingly, our results relating smoking to IL-18 concentrations lend support to the notion of the particularly hazardous effects of smoking on diabetic patients. The IL-18 concentrations were still high in the diabetic patients who had been smokers in the past. Smoking, as well diabetes, promote endothelial dysfunction through an excessive generation of reactive oxygen species (ROS), resulting in a decreased nitric oxide (NO) production [54-56]. Endothelium-derived nitric oxide (NO) is not only the major mediator of endothelium-dependent vasodilatation, but it also has important anti-inflammatory, anti-thrombotic and pro-fibrinolytic properties that are relevant at all stages of the disease. However, the differences between the influence of smoking and diabetes on endothelium exist [54, 55]. There are no experimental data focused on both smoking and hyperglycaemia with relation to endothelial injury. However, an association between smoking and type 2 diabetes has recently been reported, which points to a strong and consistent increase in the incidence of type 2 diabetes in heavy smokers in different populations [57, 58]. Smoking status did not affect the serum level of IL-18 in apparently healthy men [23]. Further studies are needed to establish the influence of smoking on diabetic patients.

A limitation of the present study is that the determination of IL-18 was performed only once. It was impossible to measure the IL-18 level twice because the patients were admitted to hospital the day before surgery, and they came from a large area of north Poland.

The current study provides support for an important role of IL-18 in type 2 diabetes mellitus patients. Type 2 diabetes mellitus predisposes patients, especially those with multivessel CAD and with a smoking habit, to high serum levels of IL-18, which may help explain their vulnerability to fatal, secondary cardiovascular events. These patients should be in the first line for stringent secondary cardiovascular prevention.

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