Interleukin 6 polymorphism corresponds to the number of severely stenosed coronary arteries

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ABSTRACT. IL6 gene promoter polymorphisms may influence the outcome of cardiovascular diseases. The aim of our study was to find out whether the –174G>C polymorphism, as well as the IL6 secretory profile, may be linked to the number of severely (≥ 75%) occluded coronary arteries in patients with advanced coronary heart disease (CHD). Three hundred and twenty patients awaiting elective coronary artery bypass grafting were enrolled into the study. Blood was taken the day before surgery. The PCR-RFLP method was used for IL6 gene polymorphism analysis. Spontaneous IL6 release was measured by bioassay in supernatants of whole blood cell cultures (WBCC) incubated for 24 h and 48 h. We found that significantly more patients with triple vessel disease were found within the -174GG group as compared to the -174GC and CC genotype carriers. The highest IL6 serum levels were found in the -174GG and the lowest in the -174CC genotype patients. Spontaneous *in vitro* IL6 secretion appeared to be significantly higher at all time points in the -174GG as compared to the CC and GC genotype carriers. The serum concentration of IL6 and the spontaneous IL6 secretion were directly related to the number of obstructed coronary vessels. Our results emphasize the role of IL6 as an important, non-classical risk factor predicting the number of severely affected coronary vessels.

Keywords: multivessel coronary heart disease, IL6

Recent studies indicate that the common G>C promoter polymorphism at position -174 in the 5' region of the IL6 gene may have a predictive strength, not only for the risk of cardiovascular diseases, but also for outcome. The -174GG genotype may be seen as a portent of future atherosclerosis as it been seen to be associated with poorer endothelial function, a phenomenon predating clinical evidence of atherosclerosis by many years, in apparently healthy young men [1]. In two independent studies, the -174GG genotype was prognostic for the grade of carotid intima-media wall thickness in middle-aged and elderly asymptomatic patients [2, 3]. In addition, among the patients who experienced ischemic cardiovascular events, the -174GG genotype conferred the risk of a more severe disease pattern, longer hospitalisation after surgical coronary revascularization, and higher rates of post-operative complications, including death [4-6].

The results of genetic studies go some way to explain these phenomena on the assumption that the -174G allele confers a pro-inflammatory profile. Such an hypothesis has been proposed by authors who found a higher basal serum concentration of IL6 in patients with the GG genotype as compared to those carrying the -174C allele [4, 7-9]. The fact that an elevated interleukin 6 serum concentration has been recognized as a risk factor of future myocardial infarction among apparently healthy men [10] and as a predictor of coronary death among coronary heart disease (CHD) patients [11], may shed light on an association between the -174G>C IL6 promoter polymorphism and clinical findings. It is important however, to realize that only few experimental data on IL6 gene promoter regulation in immune cells have been published so far [7, 12, 13], and its role in regulation of the IL6 gene requires further studies. Moreover, the data obtained on an Italian population seem to be inconsistent with the hypothesis ascribing a higher risk of CHD to the -174G allele. From an analysis of this group of 60-year-old Italian men, it emerged that it was the -174C allele that, either separately [14] or in combination with the apolipoprotein E polymorphism [15], increased the risk of acute myocardial infarction.

The degree of severity of CHD depends on the number, size and extent of stenosis of coronary arteries [16, 17]. It is possible to envisage the scale of CHD progression on the basis of IL6 gene polymorphisms and IL6 secretion patterns. To our knowledge, such a relationship has not been described so far. Recently, different inflammatory markers have been considered as diagnostic in CHD. The fact that inflammatory markers may reflect the functional status of

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coronary arteries in patients with CHD has been shown in studies on the vasoreactivity of coronary vessels under stress conditions [18]. A study on patients with effort angina of at least one year duration and with luminal diameter stenosis of > 50%, revealed the macrophage colony stimulating factor (M-CSF) as a reliable inflammatory marker of CHD progression. The plasma level of this cytokine was related to the number of occluded coronary vessels [16]. In this paper however, IL6 did not correlate with the number of occluded vessels. A similar relationship has been sought in patients with > 50% occlusion of at least one coronary vessel [17]. None of the inflammatory markers, including CRP, IL6, SAA and sICAM-1, correlated with disease severity, although a non-significant tendency towards higher values accompanied advanced grades of CHD. Summarizing, the expected straightforward relationship between the status of coronary arteries in CHD patients and circulating IL6 levels has not been documented. In addition, it is not known whether IL6 gene promoter polymorphisms could affect the number of stenosed coronary vessels in CHD patients.

Therefore, we asked whether the –174G>C polymorphism and the IL6 secretory profile, *in vivo* and *in vitro*, may be linked to the number of occluded coronary arteries in patients with advanced coronary heart disease.

PATIENTS AND METHODS

Patients

Three hundred and twenty patients, admitted between October 2002 and November 2003 to the Clinic of Cardiosurgery of The Academic Clinical Center in Gdańsk and scheduled for first time elective coronary artery bypass grafting (CABG), were enrolled into the study. Coronary angiography was performed in all patients. Qualification for surgery required that at least one of the major coronary vessels was characterized with ≥ 75% stenosis. The following coronary arteries were affected: left anterior descending coronary artery (LAD) or/and right coronary artery (RCA) or/and circumflex artery (CX). Patients with one, two and triple vessel disease constituted: 14.4%, 40.5% and 45% respectively. Those patients who in addition to coronary heart disease, had been diagnosed with or were suspected of having chronic inflammatory, autoimmune or neoplastic diseases were excluded from the studies. Moreover, all patients who had suffered from any acute infection within the last three months were also excluded from the study. The clinical data were entered into a computerized database. A hundred healthy, age-matched (63.2 ± 6.7 years old; 70% of men and 30% women) people were enrolled as the control group. They had never presented clinical symptoms of CHD and had a normal resting and exercise-related electrocardiogram. They did not have any history of arterial hypertension or diabetes mellitus. They were recruited for comparison with CHD patients for distribution of the –174G>C genotype. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Medical University of Gdańsk. The investigation also conforms to the principles outlined in the Declaration of Helsinki (*Cardiovascular Research* 1997; 35: 2-4). Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or the self-reported use of anti-hypertensive medication. Diabetes was defined according to the American Diabetes Association (*Diabetes Care* 2005; 28: 37-42). Information on the initial diagnosis, smoking history, medication and infections was obtained by interview. Angina pectoris was graded according to the Canadian Cardiovascular Society Classification for angina pectoris (CCS). The New York Heart Association (NYHA) functional classification of patients with heart disease was applied to describe the patient's state. The European System for Cardiac Operative Risk Evaluation (Euroscore) was used for evaluation of the operative risk for the patients.

Methods

Plasma measurements

Blood was taken the day before surgery. Blood was drawn from the ante-cubital vein between 7 and 8 o'clock, frozen in aliquots (-70°C) and used not later than 3 months thereafter. Fasting serum lipids were measured with the enzymatic kits: "Comray Chol", "Comray HDL-Direct" and "Comray-TG" (P.Z. Comray, Poland). High-sensitivity CRP was measured with a particle-enhanced, immunonephelometric assay (N Latex CRP mono, Behring Diagnostics). This assay detects values as low as 0.175 mg/L.

Genotyping protocols

Genomic DNA was isolated with DNA Prep Plus Kit (DNA Gdańsk, Poland) according to the protocol.

IL-6 gene polymorphism

IL-6 gene polymorphism in position -174 was analysed using the PCR-RFLP method, as described Fishman *et al.* [7]. Briefly, PCR was performed with primers 5' AGAAGAACTCAGATGACTGG 3' and 5' GCTGGGCTCCTGGAGGGG 3'. PCR conditions: 40ng genomic DNA, 10pmol of each primer, 200 µM, dNTP, 2 mM $MgCl_2$, 0.5 U DyNAzyme II DNA Polymerase (Finnzymes, Finland), water to the volume of 50 µl. Temperature profile: 95°C 1 mn, 63°C 1 mn, 72°C 2 mn 30 cycles, final elongation 72°C 10 mn. The PCR products were cut by restriction enzyme SfaNI (NEB) at 37°C for 12h, after which agarose electrophoresis was performed in 2% gel stained with ethidium bromide.

Whole blood cell cultures (WBCC)

Whole blood was collected into EDTA Vacutainer tubes. Five-hundred µl of whole blood was diluted (1:1) with RPMI (Gibco, Life Technologies Inc., USA) containing 5% fetal calf serum (Gibco, Life Technologies Inc., USA) and incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h and 48 h on 24-well, plastic plates (Corning, Science Products, New York, USA) without any additional stimulants. Before plating, blood was adjusted for an equal WBC number in a unit volume so that equal numbers of WBC were put into each well of the plate. The samples were prepared in triplicate. After incubation, the supernatants were removed and kept at -70°C. Spontaneous IL6 secretion was evaluated in culture supernatants.

Bioassay for interleukin 6

Bioassay for IL6 was performed as previously described [28]. The IL6-dependent murine hybridoma cell line B9 was cultured for 48 hours on 96-well plastic plates (Corning, Science Products, Rochester, NY, USA) at a concentration of $20x10^3$cells/well and $10x10^3$cells/well respectively in RPMI medium (Gibco, BRL Life Technologies, Gaithersburg, USA) containing 5% FCS (Gibco, BRL Life Technologies, Gaithersburg, USA), 2 mmol L-glutamate and penicillin-streptomycin (Sigma Chemical Co., St. Louis, USA). Ten µl of the sera were added to each well of the plate, in triplicate. Cell viability was measured using the colorimetric MTT assay. The optical densities obtained from experimental wells were fitted with titration standards of rIL6. Neutralizing rabbit anti-IL6 antibodies (Genzyme, Cambridge, MA, USA) were added (1:10, 1:20, 1:50) in order to confirm the specificity of the test. The parallel control samples received normal rabbit serum (1:50). All neutralizing antibodies completely blocked the three biological tests. The IL6 assay had a detection limit of 1.0 pg/mL. The intra-assay coefficients of variation ranged between 8.5 and 12.8%. The inter-assay coefficient of variation ranged between 16 and 25%. The results were read at 570 nm on the automated plate reader (Multiscan MCC/340, Labsystems, and Helsinki, Finland).

Statistical analysis

The results were analysed using the Statistica, Version 6 program (StatSoft, Pl). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Normally distributed variables were analysed with the ANOVA test. For comparison of the skew-distributed variables the non- parametric Kruskal-Wallis ANOVA and Mann-Whitney U tests were applied. All results were presented as an arithmetic mean ± SD. Nominal variables were analysed using the χ^2 Pearson test. Conformation of the allele frequencies to Hardy-Weinberg equilibrium proportions was tested by the χ^2 test. The multivariate linear stepwise regression was applied to assess correlates of multivessel CHD. The level of significance was set at $p < 0.05$ and two-sided tests were performed as the standard.

RESULTS

General characteristics of patients

The preoperative characteristics of the patients are presented in *table 1*. There were no significant differences between the traditional cardiovascular risk factors, metabolic parameters, medications used and cardiovascular status of patients with different -174G>C IL6 genotypes. The distribution of the –174G>C genotypes was similar ($p = 0.5$) in the CHD patients (GG = 28.9%; GC = 47.5%; CC = 23.6%) and the healthy controls (GG = 32%; GC = 47%; CC = 21%), and was compatible with the Hardy-Weinberg equilibrium.

Status of coronary arteries

Carriers of the -174G>C genotypes differed in the number of severely (≥ 75% stenosis) obstructed coronary arteries. Significantly ($p = 0.001$) more patients (56.6%) with triple vessel disease [*i.e.* left anterior descending coronary artery (LAD), right coronary artery (RCA) and circumflex artery (CX)] were found within the -174GG group as compared to the -174GC (41%) and CC (38.8%) genotype-carrying patients *(table 2)*.

IL-6 serum level and the -174G>C polymorphism

IL6 serum levels were dependent on the –174 G>C polymorphism ($p = 0.007$). The highest level was found in the -174GG and the lowest in -174CC genotype (*post hoc*: GG *versus* CC $p = 0.03$ and GG *versus* GC $p = 0.001$) patients. Further, patients were subdivided into groups with and without previous acute myocardial infarction (MI). It appeared that the -174GG genotype carriers, who had suffered at least one MI, were characterized by higher serum IL6 levels than those without MI in their history ($p = 0.03$). In patients with other genotypes there was no difference in IL6 serum levels between post-MI patients and those without history of MI *(table 3)*.

Secretion of IL6 in vitro and the 174G>C polymorphism

Whole blood cell cultures of all patients were carried out for 4 h, 24 h and 48 h on 24-well plastic plates without any stimulants. The spontaneous IL6 secretion into culture supernatants appeared to be significantly higher at all time points in the -174GG as compared to the CC and GC genotype carriers (4 h, $p = 0.04$; 24 h, $p = 0.03$; 48 h, $p = 0.04$) *(figure 1)*.

IL6 serum levels and obstructed coronary arteries

The patients were divided into one, two or triple vessel groups if they had the following coronary arteries affected with ≥ 75% stenosis: left anterior descending coronary artery (LAD) or/and right coronary artery (RCA) or/and circumflex artery (CX). Serum IL6 concentrations were clearly increasing with the number of obstructed coronary vessels ($p = 0.0001$) *(figure 2)*.

IL6 in vitro secretion and obstructed coronary arteries

Next, we concentrated on the relationship between *in vitro* IL6 secretion and the number of vessels affected with ≥ 75% stenosis. Spontaneous IL6 secretion by WBCC was measured in non-stimulated 24 h and 48 h cultures. It appeared that spontaneous IL6 secretion after 24 h and 48 h was directly related to the number of severely stenosed arteries 24 h ($p = 0.03$) and 48 h ($p = 0.03$) *(figure 3)*.

Serum CRP level, -174G>C IL6 polymorphism and obstructed vessels

The values for CRP remained in a similar range ($p = 0.7$) for patients with single (1.4 ± 0.8 mg/L), double (1.5 ± 1.2 mg/L) and triple (1.7 ± 1.2 mg/L) vessel disease. There was no correlation between the circulating IL6 and CRP levels ($p = 0.7$). The IL6 gene polymorphism did not correspond to the CRP values ($p = 0.8$).

The multivariate linear regression, adjusted for the classical risk factors such as age, total cholesterol, HDL-

Table 1
Basic clinical parameters in patients with different IL6 -174G>C genotypes

–174 IL-6 genotype	GG	GC	CC	p value
N (%)	93 (29)	151 (47)	76 (24)	
Age (years)	63.5 ± 9.2	61.3 ± 8.8	63.1 ± 8.3	0.12
Duration of disease	9.3 ± 8.0	7.6 ± 6.5	7.0 ± 7.1	0.1
Men #	76.0	69.5	74.3	0.51
Hypertension #	63.0	73.4	66.0	0.25
Diabetes #	33.0	27.0	21.0	0.26
Smokers #				
Current	8	9	14	0.52
Ex-smokers	64	67	56	
Non-smokers	28	24	30	
Body mass index, (kg/m^2)	27.8 ± 4.0	28.4 ± 4.1	28.0 ± 4.0	0.51
Total cholesterol (mmol/L)	5,54 ± 1,21	5,67 ± 1,36	5,49 ± 1,26	0,60
LDL (mmol/L)	3.74 ± 1.16	3.43 ± 1.10	3.45 ± 1.13	0.22
HDL (mmol/L)	1.13 ± 0.30	1.13 ± 0.36	1.16 ± 0.28	0.85
TGL (mmol/L)	1.70 ± 0.73	1.86 ± 1.38	1.85 ± 1.13	0.85
Beta-blockers #	77	88	79	0.07
ACEI #	68	64	70	0.67
Statins #	84	75	78	0.28
ASA #	98	91	92	0.09
CCS #				
1	6.5	4.0	5.5	0.96
2	32.5	36.5	31.5	
3	52.0	50.0	55.0	
4	9.0	9.5	8.0	
Previous MI #	63.3	65.3	60.8	0.80
Number of MI #				
0	36	35	39	0.51
1	45	44	43	
2	14	18	15	
3	4	3	3	
4	1	0	0	
Previous PTCA #	10.0	9	13.0	0.72
LVEF before surgery	51.1 ± 10.4	51.6 ± 7.9	52.4 ± 8.5	0.64
NYHA class #				
1	60.0	56.0	67.0	0.50
2	10.0	15.5	6.5	
3	19.0	20.0	17.0	
4	11.0	8.5	9.5	
EuroSCORE	3.46 ± 2,5	3.8 ± 2,44	3.10 ± 2,2	0.47

ACEI, angiotensin-converting enzyme inhibitors; ASA, acetylsalicylic acid; CCS, Canadian Cardiovascular Society Classification for Angina Pectoris; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional classification of patients with heart diseases; Euroscore, European System for Cardiac Operative Risk Evaluation.
Differences were calculated using the ANOVA, Kruskal-Wallis ANOVA and Pearson χ^2 tests.
Data are presented as arithmetic means ± S.D or percentage (#).

Table 2
IL6 gene polymorphism and number of obstructed coronary vessels

Affected vessels	- 174 G>C		
	GG	GC	CC
1	2 (3.3%)	32 (21.5)	11 (14.6)
2	38 (40.1)	57 (37.5)	36 (46.6)
3	53 (56.6)	62 (41)	29 (38.8)
Statistical significance (Pearson χ^2)		p = 0.001	

= the number of affected vessels with ≥ 75% stenosis. The following arteries were affected: left anterior descending coronary artery (LAD) or/and right coronary artery (RCA) or/and circumflex artery (CX).
Data indicate the number and percentage (%) of patients. Patients with one vessel; n = 45, two vessels; n = 131, three vessels occluded; n = 144.

cholesterol, BMI, smoking, history of hypertension and diabetes mellitus, and family history of CHD revealed that CHD duration ($\beta = 0.176$ $p = 0.0005$) and the -174G>C polymorphism ($\beta = 0.152$ $p = 0.0005$) remained in positive association with multi-vessel CHD. This association was independent of the classical risk factors.

DISCUSSION

We are, to our knowledge, the first to demonstrate that the –174G>C polymorphism may be associated with the number of critically occluded, main coronary arteries. We have shown that carriers of the -174GG genotype were significantly more likely to have triple vessel disease

Table 3
Concentration of IL6 in serum according to IL6 -174G>C genotypes

Genotypes	-174GG		-174GC		-174CC		Statistical significance		
Patients n (%)	90 (29)		150 (48)		70 (23)				
IL6 pg/mL	*44.1 ± 29.5		37.0 ± 24.1		18.4 ± 7,1		p = 0.007		
Myocardial infarction	0	1	0	1	0	1	p		
IL6 pg/mL	**21 ± 15	40 ± 32$^{\#}$	44 ± 18	39 ± 17$^{\#\#}$	18 ± 7	18 ± 4	$^{\#}$0.03	$^{\#\#}$0.5	$^{\#\#\#}$0.9

*Differences were calculated using the Kruskal-Wallis ANOVA test or Mann-Whitney **U test. Data are presented as arithmetic means ± S.D.
Myocardial infarction: 1 = patients who suffered at least 1 myocardial infarction, 0 = patients without myocardial infarction.

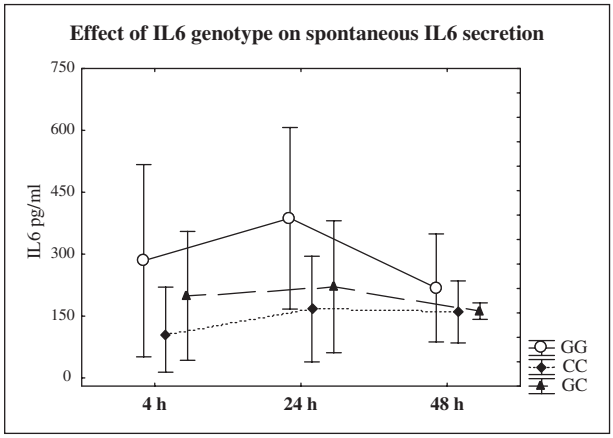

Figure 1
Effect of IL6 genotype on spontaneous IL6 secretion.
WBCC cultures were set up for the following number of patients: GG n = 70; CC n = 55; GC n = 120. The differences between genotypes in IL6 secretion after 4 h (p = 0.04), 24 h (p = 0.03) and 48 h (p = 0.04) were statistically significant (Kruskal-Wallis ANOVA). The values represent arithmetic means ± S.D.

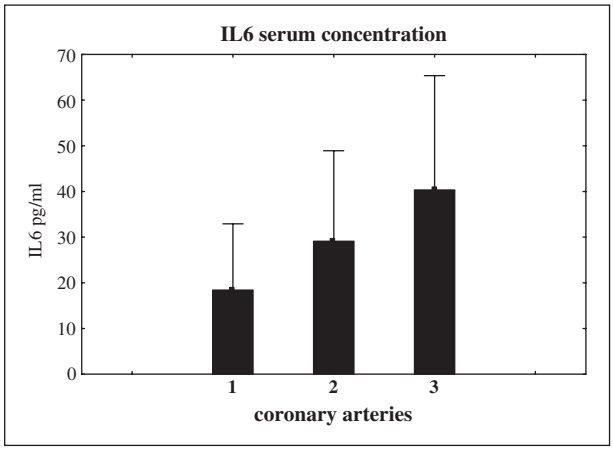

Figure 2
IL6 serum concentration.
IL6 serum level was measured in all patients. Forty six patients were affected with one-vessel disease, 130 with two-vessel disease and 144 with three-vessel disease. The differences between IL6 concentrations were statistically significant (ANOVA; p = 0.0001).
The values represent arithmetic means ± S.D.

(with ≥ 75% stenosis of the LAD, RCA and CX) than patients heterozygous and homozygous for the -174C allele. Moreover, serum IL6 concentrations as well as spontaneous, *in vitro* IL6 secretion remained significantly higher in the -174GG genotype carriers than in the -174GC and -174CC genotypes. These findings were complemented by the results of *in vivo* and *in vitro* IL6 secretion

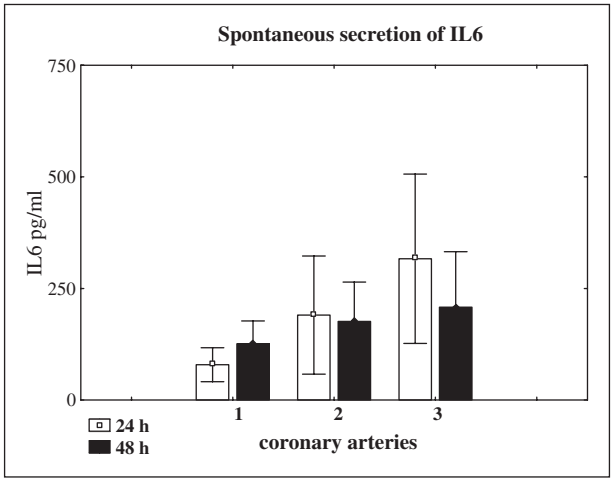

Figure 3
Spontaneous secretion of IL6.
WBCC cultures were incubated without stimulants and IL6 measured in supernatants. There were statistically significant differences between IL6 concentrations in cultures of patients with differing numbers of affected coronary arteries at 24 h (p = 0.03) and 48 h (p = 0.03) (Kruskal-Wallis ANOVA). The values represent arithmetic means ± S.D.

profiles. Both the IL6 serum concentrations and spontaneous IL6 secretion from *in vitro* WBCC cultures were directly related to the number of severely stenosed (≥ 75%) coronary vessels *i.e.* left anterior descending coronary artery, right coronary artery and circumflex artery.

The hyper-responsiveness of the –174GG patients, found in our study, is consistent with molecular studies on IL6 gene promoter regulation. The –174G>C polymorphism has been regarded as a key regulator of downstream IL6 production. IL6 mRNA expression requires a synergistic interaction of a number of transcriptional factors, including NF-κB, NF-IL6 and C-JUN/AP-1. The -174 polymorphic IL6 gene region is located in close proximity of such transcription factors as NF-κB, NF-IL6, C-JUN/AP-1. The G>C nucleotide substitution creates a potential binding site for the transcription factor NF-1 [7, 12, 13]. The -174G allele has been found to have much higher promoter strength than the C allele both in the *in vitro* model of transfected cells [7] and in *ex vivo* IL6 production by whole blood cell cultures [12]. The hyper-responsiveness of the –174GG patients compared to the other genotypes has been noted in recent clinical analyses which revealed the highest IL6 plasma levels in patients awaiting CABG, and in coronary angioplasty patients [8, 9].

Our results document a significant and direct relationship between serum IL6 levels, spontaneous *in vitro* IL6 production and the number of severely occluded coronary

vessels. Past papers have failed to document a significant relationship between different inflammatory markers such as CRP, IL1β, IL6, IL8, SAA, fibrinogen, sICAM-1, VCAM-1, E-selectin, WBCC count and the number of stenosed coronary arteries, as assessed by different quantitative methods [17, 19, 20]. The discrepancy between our and previous results may be due to a different study model. We have analyzed more numerous groups and patients with more advanced coronary artery stenosis. In contrast to other authors, we applied a sensitive bioassay with the IL6-dependent B9 hybridoma plasmocytoma cell line, destined for detection of only bioactive IL6 [21]. This assay measures IL6 both free and complexed with sIL6R; the forms which are able to bind to cellular receptors and induce a specific biological response [22]. The immunoreactive IL6, which is measured using ELISA-based methods, detects active IL6 complexes as well as IL6 plus the sgp130 molecule. The IL6-sgp130 complex, by competing with IL6 receptors, inhibits IL6 biological activity [23].

The wide variety of IL6 values might have been due to the seasonal variability of IL6 [24] or the depressive mood [25] of patients, who were recruited consecutively over several months and with blood taken the day prior to surgery.

The associations between the -174G>C polymorphism and the number of stenosed vessels, and between IL6 secretion and affected coronary arteries, were independent of the classical risk factors. Such a tendency is in line with other clinical reports revealing the absence of a link, between either IL6 gene polymorphisms or plasma IL6 level and traditional cardiovascular risk factors [19, 26].

It might be speculated that the –174GG IL6 hyperproducers are more endangered, than other genotype carriers, with unwanted consequences. IL6, by virtue of its inflammatory activity, may promote new lesion formation and may enable an enlargement of the already existing ones. This effect may be mediated by activated monocytes [27], which were the prevalent IL6 producers in our study, and the clonal expansion of pathogenic T helper cells [28]. Such an assumption may stem from animal experiments. IL6-treated atherosclerosis-prone mice have been found to develop more and much larger lesions than did non-treated animals [29]. IL6 may enhance coagulation and thrombosis by stimulation of platelet aggregation, expression of tissue factor and synthesis of fibrinogen [30]. Finally, high IL6 plasma levels may be responsible for an alteration of plaque morphology from the stable to the vulnerable form. The latter is the main cause of acute cardiovascular events [31].

How may we reconcile our results with those for Italian MI patients, which indicate a predictive strength of the -174CC genotype and -174C allele for acute MI [14, 15]? These two sets of results do not seem to be mutually exclusive. The association found in our paper between the -174GG genotype, IL6 secretion and stenosed coronary arteries refers to advanced CHD, of 7-9 years disease duration, and with severe lesions in two or three main coronary arteries. About 70% of these patients had suffered from one to four myocardial infarctions. So, a chronic, long-term inflammatory process may not be the equivalent, in terms of pathogenesis, of an acute MI, which is an athero-thrombotic event. This predictive role of the -174GG genotype for severe coronary artery stenosis may become more similar in the Italian group, as their disease progresses. There is also another explanation for the discrepancy. A approximate analysis suggests that the distribution of the -174G>C genotypes in Italian and Polish populations may be different. So, the functional impact of the -174G>C genotype may be dissimilar. Such hypotheses however, need to be substantiated.

The contemporary anti-inflammatory therapies for CHD are targeted against CRP and other acute phase reactants. Meanwhile, we and others [17, 19, 20] did not find a relationship between the number of stenosed coronary arteries and blood CRP levels. The relationship found in our study inclines us against high IL6-targeted therapy. Such a therapy should, be important for the -177GG genotype CHD carriers in particular. For this purpose, the ASA preparations should be strongly recommended as they exert a prominent and broad anti-inflammatory effect, including reduction of IL6 activity [16]. Recent papers indicate that the rate of atherosclerotic plaque initiation and growth, as well as their switch from stable to vulnerable forms, is dependent on angiotensin II, and this process is mediated by IL6 [31, 32]. A selective AT1 antagonist appeared to be the choice for reducing IL6 synthesis [32]. The blockade of AT1 receptor appeared to be superior to the ACE inhibitors in the reduction, not only CRP but also IL6 levels in CHD patients [33]. In searching for strategies for preventing atherosclerotic plaque development, an additive effect in $APOE^{-/-}$ mice fed a high-fat diet, has been obtained with a combined RAS blockade plus HMG-CoA reductase inhibitors [34]. The combination of RAS blockade with simultaneous ASA treatment recently tested in patients appears to be a promising anti-inflammatory approach [35]. Statins, known from their potent ability to reduce acute reactants such as CRP and SAA appeared not to influence IL6 production [36, 37].

Understanding the effect of anti-inflammatory drugs could help to explain why an expected correlation between IL6 and CRP levels was not noticed in our paper, and patients with advanced coronary lesions were characterized by relatively low CRP values. This was the result of long-term, combined anti-inflammatory treatment with ASA, ACEI, β-blockers and statins. This therapy, as discussed above, was preferentially directed towards CRP and did not appear to have a significant effect on IL6 production. How then, in light of numerous observational cohort studies performed by Ridker and his group, should CRP and IL6 levels be considered in relation to patient prognosis? Numerous papers from the Ridker's study group revealed that blood CRP concentration is an important, independent cardiovascular risk factor in healthy populations of both sexes [38]. These results do not match those for our patients with advanced CHD. New studies placed CRP as an independent constituent of the global risk prediction model in healthy populations [39, 40]. The conclusions of these studies cannot be valid for the patients examined in our paper. Recently, CRP levels appeared to be an independent predictor of ischemic stroke in patients with pre-existing cardiovascular disease [41]. According to the stratification model in this paper, our medically controlled patients have a low risk of future stroke. Interleukin 6 can also serve as an independent risk factor for cardiovascular events in healthy men [42] and women [43], as well as in patients with pre-existing cardiovascular disease [44]. However, confirmation of a stratification risk for our patients requires longer observational study.

Our results, indicating a relationship between the number of severely stenosed coronary arteries and IL6 secretion, in combination with the -174G>C polymorphism, emphasize the role of IL6 as an important, non-classical factor that contributes to the development of severe atherosclerosis. They also suggest the need for targeted therapy, specifically directed against high IL6 levels.

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