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Association of C-Reactive Protein with the Presence and Extent of Angiographically Verified Coronary Artery Disease

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Memon, L., Spasojević-Kalimanovska, V., Bogavac-Stanojević, N., Kalimanovska-Oštrić, D., Jelić-Ivanović, Z., Spasić, S. and Topić, A. *Association of C-Reactive Protein with the Presence and Extent of Angiographically Verified Coronary Artery Disease*. Tohoku J. Exp. Med., 2006, **209** (3), 197-206 —— Prospective studies have demonstrated that markers of inflammation, such as high-sensitivity C-reactive protein (hsCRP) and fibrinogen, predict future cardiovascular disease risk. However, the association between the hsCRP and fibrinogen levels and the extent of coronary stenosis in patients with coronary artery disease (CAD) remains controversial. The aim of our case-control study was to assess the association of inflammatory markers with the occurrence and extent of CAD. Serum hsCRP and plasma fibrinogen levels were measured in 138 patients with angiographically assessed CAD and in 183 healthy subjects matched according to age and gender. According to the number of significantly stenosed ($\geqq$ 50%) vessels, the patients were classified in four groups: those without stenosis (0-vessel disease) and those with 1, 2 or 3-vessel disease. The hsCRP and fibrinogen levels were significantly higher in patients than in controls ($p < 0.001$). Although the hsCRP and fibrinogen levels tended to increase with the number of stenotic vessels, the differences were only significant for hsCRP ($p < 0.01$). Regression analysis indicated hsCRP as an independent predictor for the presence (OR = 3.573, $p < 0.05$) and extent of CAD ($\beta = 1.095$, $p < 0.05$). In conclusion, the present study is the first report concerning the frequency distribution of hsCRP in Serbian healthy subjects and CAD patients. We have shown that elevated levels of hsCRP are associated with the presence and extent of CAD. ———— atherosclerosis; inflammation; fibrinogen; hsCRP; coronary artery disease

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With improved understanding of the critical role of inflammation in atherothrombosis, attention has lately been focused on the circulating markers of inflammation, such as C-reactive protein (CRP) and fibrinogen (Ross 1999; Jousilahti et al. 2001; Lind 2003). Evidence has been accumulated from several prospective studies that high levels of high-sensitivity CRP (hsCRP) and fibrinogen were associated with elevated risk of coronary, cerebral and peripheral vascular disease (Danesh et al. 1999; Maresca et al. 1999; Tamam et al. 2005).

Compared with other studied circulating inflammatory markers, CRP seems to have the most consistent relation to the risk of cardiovascular diseases in a variety of clinical settings, including healthy subjects, selected high risk subjects with traditional risk factors, and patients with cardiovascular diseases (Danesh et al. 1999, 2004). Because atherosclerosis represents a low-grade inflammatory process in the vascular bed, high-sensitivity (hs) assays are needed when using circulating CRP concentrations for risk prediction in cardiovascular diseases (Rifai et al. 2001).

Systemic markers of inflammation, such as serum hsCRP and plasma fibrinogen, are elevated in patients with coronary atherosclerosis, and are increased significantly in patients with unstable angina or acute myocardial infarction. Serum hsCRP levels are also powerful predictors of cardiac complications and death in patients with unstable coronary syndromes as well as in healthy men and women without prior cardiac history (Rifai et al. 2001). A potential explanation for the high event rate in patients with elevated hsCRP and fibrinogen levels is that a high hsCRP or fibrinogen level is a marker for more severe and extensive coronary artery disease. However, the association between hsCRP levels and the severity or extent of coronary stenosis in patients with coronary artery disease remains controversial. Some previous studies have shown an association between hsCRP and fibrinogen with the severity and extent of coronary artery disease (CAD) (Tataru et al. 2000; Zebrack et al. 2002), whereas others have not found a correlation (Azar et al. 2000; Hoffmeister et al. 2001).

This case-control study was designed to investigate the association of the two most extensively studied inflammatory markers, fibrinogen and hsCRP, with the presence and extent of CAD assessed by coronary angiography. The same markers were also measured in the healthy control group, matched according to age and gender. The frequency distribution of hsCRP in healthy individuals represents the first report on the hsCRP levels in the Serbian population.

Subjects and Methods

Subjects

The patient group consisted of 138 subjects (89 men and 49 women, aged from 31 to 71 years), who were undergoing coronary angiography for suspected coronary artery disease at the Institute of Cardiovascular Diseases, Clinical Center of Serbia, Belgrade. All patients with myocardial infarction within the last 6 months, those with unstable angina who had anginal pain at rest within 1 month, or those with a history of prior coronary revascularization were excluded. All patients had a history of stable angina defined on the presence of chest pain that did not change its pattern during the preceding 2 months. The control group 183 (103 men and 80 women) consisted of healthy individuals who underwent regular annual medical check up at the Health centers in Belgrade, with no history of cardiovascular disease, matched to the patients according to age and gender.

The following data were recorded for all the subjects included in the study: age, gender, height, weight, blood pressure, and the assessment of risk factors including the history of myocardial infarction, arterial hypertension, diabetes mellitus, smoking and hyperlipidemia. Cardiac medications taken at study entry were recorded. A standardized questionnaire was applied to all cases and controls by the same trained interviewers. Hypertension was defined as systolic blood pressure $\geqq$ 140 mmHg, diastolic blood pressure $\geqq$ 90 mmHg, or the use of any antihypertensive medication. Diabetes mellitus was diagnosed when the subject was medicated for diabetes or the fasting blood glucose exceeded 7.0 mmol/L. Hyperlipidemia was defined for subjects with low density lipoprotein cholesterol (LDL-C) $\geqq$ 4.13 mmol/L and triglycerides $\geqq$ 2.25 mmol/L. Neither the patients nor the control subjects included in this study showed any evidence of ongoing systemic or cardiac inflammatory disease. To avoid confounding factors, we excluded

patients with the history of recent clinical infection; concurrent major renal, hepatic and malignant disease; surgery or major trauma in the previous month and obvious signs and clinical evidence of hospital-acquired infection. We excluded individuals with hsCRP ≧ 10 mg/L, a level considered to be indicative of a clinically relevant inflammatory condition (Pearson et al. 2003). The study was planned according to the ethical guidelines following the declaration of Helsinki. All patients gave written informed consent before the study entry and the Ethics Committee at Faculty of Pharmacy, University of Belgrade, approved this study.

Coronary artery disease was defined as at least one coronary artery having ≧ 50% luminal diameter stenosis. To evaluate the angiographic extent of CAD, the patients were classified according to the number of stenosed vessels. Patients with lesions of less than 50% luminal narrowing were referred to as having no clinically significant stenosis or 0-vessel disease (n = 37). Luminal narrowing of 50% or more was defined as a significant lesion and the patients were here referred to as having single (n = 40), double (n = 26) or triple (n = 35) vessel disease. As the group 0 consisted of patients with less than 50% luminal narrowing, who could not be declared as healthy, stenosis-free subjects, a control group of 183 healthy subjects was included in the study. Two experienced cardiologists, unaware of the patients' clinical history and biochemical results visually reviewed all angiographic images to assess the extent of CAD. Agreement was reached by consensus.

Biochemical measurements

Blood samples were obtained from the patients before coronary angiography after overnight fasting. Peripheral venous blood was drawn into collection tubes with ethylene diamine-tetraacetic acid (EDTA), citrate, or serum separator gel. Lipid and apolipoprotein parameters were determined in EDTA plasma, hsCRP in sera and fibrinogen in citrate plasma. EDTA plasma and serum were separated and multiple aliquots of each sample were stored at –80°C until assayed. High sensitivity serum CRP levels were measured by the latex-enhanced immunoturbidimetric method (Quantex hsCRP kit, BIOKIT, Barcelona, Spain) on ILab 600 analyzer (Instrumentation Laboratory, Milan, Italy). Fibrinogen was measured in citrate plasma by Clauss method on ACL 200 Instrumentation laboratory and original reagents. Total cholesterol and triglycerides were assayed by routine enzymatic methods, using ILab 600 analyzer and Randox Laboratories Ltd., (Randox, Armdore, United Kingdom) reagents. High-density lipoprotein (HDL) cholesterol was measured using the same enzymatic method after precipitation of the plasma with phosphotungestic acid in the presence of magnesium ions. The concentration of LDL-cholesterol was calculated with the Friedwald formula. Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured by immunoturbidimetry using the ILab 600 analyzer and Dialab (Vienna, Austria) reagents.

Statistical analysis

Results are presented as mean ± S.D. for continuous normally distributed variables, as median (interquartile range) for non-normally distributed data, and as percentages for categorical data. Frequency distribution of hsCRP values within men and women were compared with the Kolmogorov-Smirnov test. Comparisons between normally distributed continuous variables were performed by the Student's t-test and analysis of variance (ANOVA) test. In cases of asymmetric continuous variables (hsCRP and triglycerides), the hypotheses testing were based on the calculations by the nonparametric Mann-Whitney's U-test and Kruskal-Wallis test. Association between categorical variables was tested by the chi-square test for contingency tables.

An ordinal regression analysis was used to elucidate the association between the extent of CAD and the hsCRP levels. The dependent variable was the extent of CAD coded as 0-, 1-, 2- and 3-vessel disease. Independent variables were coded as the following dummy variables: sex, 0 for female and 1 for male; body mass index (BMI), 0 for less than 28 kg/m^2 and 1 for equal to more than 28 kg/m^2; hyperlipidemia, 0 for normolipidemic and 1 for hyperlipidemic; cigarette smoking, 0 for nonsmokers and 1 for smokers; hypertension, 0 for normotensive and 1 for hypertensive; diabetes mellitus, 0 for absence and 1 for presence; high total cholesterol, 0 for less than 6.2 mmol/L and 1 for more than 6.2 mmol/L; low HDL-C, 0 for more than 0.91 mmol/L and 1 for less than 0.91 mmol/L; high LDL-C, 0 for less than 4.13 mmol/L and 1 for more than 4.13 mmol/L; high triglycerides (TG), 0 for less than 2.25 mmol/L and 1 for more than 2.25 mmol/L; low apolipoprotein A-I (apoA-I), 0 for more than 1.2 g/L and 1 for less than 1.2 g/L; high apolipoprotein B (apoB), 0 for less than 1.2 g/L and 1 for more than 1.2 g/L; high fibrinogen, 0 for less than 4.5 g/L and 1 for more than 4.5 g/L; high hsCRP, 0 for less than 1 mg/L and 1 for more than 3 mg/L. To examine

the independent predictors of significant CAD (≧ 50%), multivariable logistic analysis was performed. Significance was accepted at $p < 0.05$.

Results

Basic characteristics and the laboratory findings in the patients and the control subjects are listed in Table 1. There were well-expected differences in the physical findings, habits and lipid parameters between patients and control subjects. Tendency towards obesity (mean BMI, 27.8 kg/m^2) was clearly apparent in the patients. In particular, the patients had higher blood pressure, more frequent smoking habits; a higher percent of them had diabetes mellitus, and less favorable lipid profile. The patients had significantly higher values of triglycerides (+47%). Values for HDL-C and apoA-I were lower (–28% and –24%) within the patients with a highly significant difference ($p < 0.001$). There was no significant difference between the patients and the controls for total cholesterol and LDL-C.

Inflammatory parameters hsCRP and fibrinogen in the patients and the healthy control group are presented in Table 2. The distributions of hsCRP for both men and women in our healthy control group were non-normal and highly skewed to the right. The frequency distributions of hsCRP values for these apparently healthy men and women were comparable ($p = 0.53$). Serum hsCRP and plasma fibrinogen levels in the patients were significantly higher than those in the healthy controls ($p < 0.001$). Patients showed a higher plasma fibrinogen level by 35%, whereas

Table 1. Baseline characteristics of patients and controls.

	Patients	Controls	p^a
Number (n)	138	183	
Age (years)	55.8 ± 9.3	55.6 ± 10.1	0.82
Male sex (%)	64.5	56.3	0.14
BMI (kg/m^2)	27.8 ± 3.8	26.5 ± 3.5	< 0.01
Waist-to-hip ratio	0.92 ± 0.01	0.89 ± 0.11	< 0.05
HTA (%)	86.2	49.7	< 0.001
Smokers (%)	45.9	30.5	< 0.001
Diabetes mellitus (%)	10.4	0.0	< 0.001
Hyperlipidemia (%)	53.6	35.5	< 0.01
TC (mmol/L)	5.44 ± 1.09	5.48 ± 1.01	0.72
Triglycerides (mmol/L)*	2.00 (1.60-2.64)	1.36 (1.02-1.93)	< 0.001
HDL-C (mmol/L)	0.84 ± 0.21	1.17 ± 0.32	< 0.001
LDL-C (mmol/L)	3.71 ± 0.98	3.65 ± 0.95	0.61
ApoA-I (g/L)	1.40 ± 0.29	1.85 ± 0.42	< 0.001
ApoB (g/L)	1.38 ± 0.34	1.33 ± 0.30	0.25
β-Blockers (%)	71.7	12.6	< 0.001
ACE inhibitors (%)	50.0	7.7	< 0.001
Calcium antagonists (%)	12.3	10.3	0.58
Statins (%)	6.5	0	< 0.001
Antiplatelet therapy (%)	71.0	4.4	< 0.001

*Values are given as median values and interquartile ranges; [a] Mann-Whitney's U-test, Student's t-test or Chi-squared test. BMI, body mass index; HTA, hypertension; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; ACE, angiotensin-converting enzyme.

TABLE 2. Serum hsCRP and plasma fibrinogen levels in patients with coronary artery disease and control subjects.

		Patients	Controls	p^a
hsCRP (mg/L)				
All	Mean ± S.D.	3.28 ± 2.47	1.85 ± 1.90	< 0.001
	median, (25^{th} - 75^{th})	2.42 (1.55 – 3.78)	1.19 (0.62 – 2.35)	
	n	(138)	(183)	
Males	Mean ± S.D.	3.07 ± 2.28	1.98 ± 2.16	< 0.001
	median, (25^{th} - 75^{th})	2.02 (1.61 – 3.46)	1.10 (0.60 – 2.66)	
	n	(89)	(103)	
Females	Mean ± S.D.	3.67 ± 2.85	1.67 ± 1.50	< 0.001
	median, (25^{th} - 75^{th})	2.60 (1.50 – 5.64)	1.25 (0.63 – 2.18)	
	n	(49)	(80)	
Fibrinogen (g/L)				
All	Mean ± S.D.	5.16 ± 1.26	4.08 ± 1.16	< 0.001
	median, (25^{th} - 75^{th})	5.26 (3.44 – 5.64)	3.83 (3.22 – 4.90)	
	n	(129)	(180)	
Males	Mean ± S.D.	5.06 ± 1.25	3.78 ± 1.12	< 0.001
	median, (25^{th} - 75^{th})	5.23 (4.09 – 5.87)	3.53 (3.07 – 4.19)	
	n	(81)	(102)	
Females	Mean ± S.D.	5.35 ± 1.27	4.46 ± 1.12	< 0.001
	median, (25^{th} - 75^{th})	5.37 (4.43 – 6.22)	4.49 (3.69 – 5.19)	
	n	(48)	(78)	

[a] According to Mann-Whitney's U-test or Student's *t*-test.

hsCRP was even two times higher in the patients then in the healthy individuals. Gender specific hsCRP and fibrinogen levels are also shown, and very significant differences between patients and controls were found in both genders.

We explored the association of hsCRP and fibrinogen concentrations with the extent of CAD by dividing the patients according to the number of stenosed coronary vessels. Patients with stenosis < 50% had lower hsCRP concentrations than patients with more extensive disease (Fig. 1A). The hsCRP levels tended to increase depending on the number of ≧ 50% stenotic vessels: 1.85 mg/L in 0-VD, 2.03 mg/L in 1-VD, 2.60 mg/L in 2-VD and 2.76 mg/L in 3-VD, and the differences among the four groups were significant ($p < 0.01$). Statistically significant differences were observed for: 0-VD vs 2-VD ($p < 0.05$), 0-VD vs 3-VD ($p < 0.01$) and 1-VD vs 3-VD ($p < 0.02$). Plasma fibrinogen concentrations were higher in the group with one (5.25 g/L), two (5.62 g/L) and three (5.31 g/L) vessel disease than in the group with no (4.81 g/L) diseased vessel (Fig. 1B), but the difference was not statistically significant ($p = 0.41$). Since lipid-lowering drugs (statins) can affect the serum hsCRP levels or may cause regression of coronary stenosis, 9 patients who were treated with statins were excluded. Exclusion of these subjects only slightly modified the differences in median hsCRP levels among the four groups (1.85 mg/L in 0-VD, 1.94 mg/L in 1-VD, 2.87 mg/L in 2-VD and 2.76 mg/L in 3-VD; $p < 0.01$). After the exclusion of patients taking statins, a step-wise increase in fibrinogen levels was found depending on the number of ≧ 50% stenotic vessels: 4.84 g/L in 0-VD, 5.19 g/L in 1-VD, 5.62 g/L in 2-VD and 5.31 g/L in 3-VD. There was no demonstrable difference in fibrinogen levels among the four groups of patients ($p = 0.61$).

To establish the independent value of serum hsCRP in predicting the extent of CAD, we performed the ordinal regression analysis (Table 3). The present analysis revealed that hsCRP level

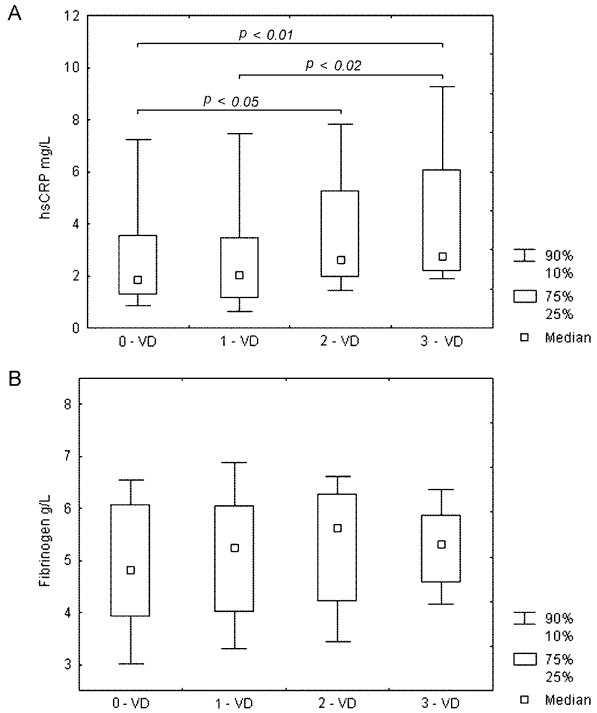

Fig. 1. Serum and plasma concentrations of inflammatory parameters: hsCRP (A) and fibrinogen (B) in patients with different numbers of significantly stenosed coronary arteries (0-VD, 1-VD, 2-VD and 3-VD). The levels of hsCRP and fibrinogen are shown as the box showing the 25th - 75th percentiles containing the median value and the lines showing the 10th - 90th percentiles.

was an independent predictor of the extent of CAD ($\beta = 1.095$, $p < 0.05$). To find out how hsCRP behaves as a predictor of CAD extent in the presence of other potential predictors, we included separately age, gender, BMI, lipid parameters, hypertension, hyperlipidemia, diabetes mellitus, current smoking, statin therapy, and fibrinogen levels in this model. Even after combination with other clinical and biochemical variables shown in Table 3 we found that hsCRP was independent predictor for the extent of CAD.

Finally, we estimated whether measurement of hsCRP had any potential in prediction of risk for development of significant coronary stenosis (≧ 50%). In Table 4 the odds ratio for the presence of significant coronary stenosis was 3.57; (95% CI: 1.082 –11.801) $p < 0.05$. Multiple logistic regression was applied to find how hsCRP behaves as a predictor of significant stenosis in coronary arteries in the presence of other potential predictors, so we included separately age, gender, lipid parameters, hypertension, diabetes mellitus, current smoking, statin therapy, in this model, as presented in Table 4. Association between hsCRP values and the presence of significant coronary stenosis remained strong even after adjustment for potential confounding variables.

Discussion

Numerous prospective studies in the past decade confirmed that atherosclerosis is an inflammatory disease and that blood markers of inflammation, hsCRP and fibrinogen, can be used to predict the risk of cardiovascular events (Danesh et al. 1999, 2004; Ross 1999). However, hsCRP distributions in apparently healthy adults in the general population must be known, before screening of individuals at risk can be recommended. Therefore, in this report we described the frequency distribution of hsCRP in 183 Serbian healthy adults. The distribution of hsCRP for both men and women were highly skewed to the right and very similar. This observation has a substantial clinical implication, because it clearly demonstrates that a single set of cut-off points for risk assessments of future coronary events can be used for both genders. Median hsCRP concentration was 1.19 mg/L in our healthy control group. This study shows that the frequency distributions, median, and ranges of hsCRP concentrations in our population from South-Eastern Europe are very similar to those previously described for other white populations from Northern Europe (Imhof et al. 2003) and the United States (Rifai et al. 2003).

In our case-control study, the obtained results for hsCRP and fibrinogen were significantly different between the healthy control group and patients with CAD. Patients had significantly higher levels of both inflammatory markers (Table 2). The patients in our study had median values 2.42 mg/L for hsCRP and 5.26 g/L for fibrinogen. To avoid the effect of most possible interfering factors, it would be best if we could have

TABLE 3. Associations of the extent of CAD with serum hsCRP levels and other risk factors.

	β Values	95% CI	p^a
hsCRP	1.095	0.090 – 2.096	< 0.05
hsCRP + gender	1.063	0.050 – 2.071	< 0.05
hsCRP + BMI	1.142	0.100 – 2.274	< 0.05
hsCRP + Smoking status	1.098	0.070 – 2.122	< 0.05
hsCRP + DM	1.065	0.060 – 2.072	< 0.05
hsCRP + hyperlipidemia	1.059	0.040 – 2.069	< 0.05
hsCRP + TC	1.417	0.395 – 2.440	< 0.01
hsCRP + TG	1.553	0.523 – 2.582	< 0.01
hsCRP + HDL-C	1.431	0.417 – 2.445	< 0.01
hsCRP + LDL-C	1.436	0.423 – 2.45	< 0.01
hsCRP + ApoA-I	1.124	0.070 – 2.175	< 0.05
hsCRP + ApoB	1.097	0.100 – 2.099	< 0.05
hsCRP + fibrinogen	2.339	1.008 – 3.671	0.001
hsCRP + statins	1.247	0.209 – 2.286	< 0.05

[a] According to ordinal regression analysis.

CI, Confidence interval; hsCRP, high-sensitivity C-reactive protein; BMI, body mass index; DM, diabetes mellitus; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

TABLE 4. Multiple logistic regression analysis for hsCRP and other risk factors.

	OR	95% CI	p
hsCRP	3.573	1.082 – 11.801	< 0.05
hsCRP + gender	3.846	1.135 – 13.027	< 0.05
hsCRP + HTA	3.756	1.090 – 12.939	< 0.05
hsCRP + Smoking status	4.067	1.185 – 13.958	< 0.05
hsCRP + DM	3.588	1.084 – 11.879	< 0.05
hsCRP + TC	3.200	0.944 – 10.850	0.060
hsCRP + TG	3.579	1.047 – 12.232	< 0.05
hsCRP + LDL-C	3.744	0.985 – 11.052	0.053
hsCRP + ApoA-I	3.851	1.097 – 13.510	< 0.05
hsCRP + ApoB	3.610	1.067 – 12.220	< 0.05
hsCRP + statins	3.818	1.141 – 12.771	< 0.05

OR, odds ratio.

compared the values obtained in the patients with the corresponding values found in a group of stenosis-free patients with the same characteristics, instead of the group of healthy controls. However, as coronary angiography is an aggressive method, it is never performed without a good reason, and it was practically impossible to recruit such a group of patients. Among the patients involved in this study, the individuals with the lowest extent of CAD were classified as group 0, which consisted of 37 individuals with luminal narrowing less than 50%. However, only few of them were without any stenosis at all, so too small group to perform any meaningful

statistical analyses.

The association of increased hsCRP and fibrinogen levels with coronary heart disease risk has been shown in many cross-sectional, prospective and case-control studies (Danesh et al. 1999; Maresca et al. 1999). Our results, concerning hsCRP values for patients with chronic stable angina, agree with the previous conclusion of Ikonomidis et al. (1999) that the steady-state hsCRP concentrations are approximately 2-fold higher in patients with chronic coronary heart disease compared to control group.

The role of immune system and inflammatory pathways in the development of atherosclerotic disease is well established, and systemic markers of inflammation appear useful for indicating elevated cardiovascular disease risk. However, the current evidence is insufficient because of the relatively few and controversial studies targeting association between the extent of coronary artery disease and inflammatory markers. In our case-control study, we found a significant association between the levels of hsCRP and the extent of CAD defined by the number of stenosed vessels. In contrast to hsCRP, there was no association between fibrinogen levels and the extent of coronary artery disease. Although, the increased number of stenosed vessels was followed by an increase in fibrinogen, the difference did not reach statistical significance (Fig. 1B). Median values of fibrinogen in these groups were similar and the confidence intervals largely overlapped. In the present study, it is interesting to note that ordinal regression analysis showed a strong independent association between circulating hsCRP levels and the extent of CAD. This result also provides evidence that the association between hsCRP and the extent of CAD was not affected by the potential confounding effects of other clinical, biochemical and therapeutic features of the study participants (Table 3). Our results confirm previous findings that C-reactive protein is an independent predictor of coronary artery disease in patients with chronic stable angina (Hoffmeister et al. 2001; Yin et al. 2004). However, adjusting the effect of hsCRP for other risk factors did not diminish the magnitude of the hsCRP association with significant coronary stenosis (Table 4). Quantification of hsCRP as an independent predictor may provide a valuable tool to assess the presence of CAD and the extent of coronary atherosclerosis.

Our results for fibrinogen and hsCRP are in agreement with some previous studies (Erren et al. 1999; Tataru et al. 2000; Zebrack et al. 2002) and opposite with others (Azar et al. 2000; Hoffmeister et al. 2001; Arroyo-Espliguero et al. 2004). This difference between their findings and the results of the present study could be attributed to different study design and patient selection. Hoffmeister et al. (2001) in their case-control study reported no correlations between inflammatory markers and any of the applied scores, but some patients were on statin therapy, and 62% of patients had myocardial infarction. These findings are in agreement with the results of Azar et al. (2000), who found no correlation of the hsCRP with the extent score and the number of stenosed ($\geqq$ 50%) vessels in patients undergoing coronary angiography. Arroyo-Espliguero et al. (2004) examined patients with chronic stable angina and patients with acute coronary syndromes separately. This study showed that the hsCRP did not correlate with the extent or severity of CAD, but the hsCRP levels predicted future cardiovascular events in patients with chronic stable angina independently of CAD severity. In the large ECAT study (1993) no association between fibrinogen, PAI-1 activity, leukocyte count and extension of coronary heart disease was found. Levenson et al. (1997) analyzed the prognostic value of fibrinogen in hypercholesterolemic patients. The value of fibrinogen increased according to the number of diseased sites and suggests that such an increase may be a marker of the extent of arterial disease. Investigations of Tataru et al. (2000) in survivors of myocardial infarction showed a significant association of hsCRP concentrations with angiographically detected degree of coronary heart disease defined as numbers of stenosed coronary vessels. Haverkate et al. (1997) demonstrated that hsCRP levels weakly correlated with the number of stenotic vessels in 2,121 patients undergoing coronary angiography, although 50% of patients were those with unstable angina. In

the last similar study by Taniguchi et al. (2005), hsCRP levels did not correlate with the number of stenotic vessels. After the exclusion of patients who were taking statins, the plasma hsCRP levels were found to be associated with the presence and extent of coronary atherosclerosis.

In order to exclude the possibility that the lower hsCRP concentration results from the excessive use of medications with a potential anti-inflammatory activity, we evaluated the percent of individuals taking such medications. Recent data suggest that statins have lipid independent anti-inflammatory properties (Albert et al. 2001). Only 6.5% of patients were taking statins, and after excluding these subjects only slightly modified values for hsCRP were obtained in the four examined vessel-disease groups. There were no demonstratable differences in fibrinogen levels in our study and the neutral effect on fibrinogen is consistent with reports from large-scale trials (Lowe et al. 2000).

Aspirin has the potential to reduce CRP levels and reduces cardiovascular events rates, suggesting the possibility that the benefit of aspirin may have been due, at least in part, to its anti-inflammatory effects. The percent of our patients taking aspirin is high (71%) but all of them were on low doses of aspirin (a daily dose of about 80 mg). At these doses the anti-inflammatory effect of aspirin is generally considered negligible. However, the greatest benefit of low-dose aspirin use is the inhibition of platelet aggregation, which reduces cardiovascular events by modifying the thrombotic response to plaque rupture (Ridker et al. 1997; Hannekens et al. 1997; Hobikoglu et al. 2005).

Controversial results of the association of hsCRP and fibrinogen with the extent and severity of coronary artery disease are in accordance with the unclear explanation of the exact role of hsCRP and fibrinogen in the atherosclerosis process (Yin et al. 2004). It has been discussed whether hsCRP is only an epiphenomenon of existing atherosclerosis as well as a marker of chronic infection without an independent role in the development of disease, or it is an active component in the development of disease and tissue damage. It has been hypothesized that hsCRP may directly interact with either atherosclerotic vessels or ischemic myocardium by activation of the complement system, and thereby promote inflammation and thrombosis. Fibrinogen is regarded as a marker of inflammation, but also a procoagulant factor in atherothrombosis (Levi et al. 2004).

In summary, the present study suggests that increased circulating levels of hsCRP and fibrinogen represent the inflammatory markers of advanced atherosclerotic disease in patients with CAD. In contrast to fibrinogen, the level of hsCRP was associated with the extent of coronary disease, indicating that this parameter is not only a marker of disease presence but also a quantitative indicator of disease extent. Although our results do not provide new insights into the pathogenesis of atherosclerosis, they do give further support to the increasingly accepted hypothesis, that the atherosclerosis may be a chronic inflammatory disease.

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