Bacterial burden of the patients with coronary artery disease treated surgically with coronary artery bypass grafting

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Submitted: 31 January 2005
Accepted: 19 March 2005

Arch Med Sci 2005; 1, 1: 13-17

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Abstract

Background: Infection with *Chlamydia pneumoniae*, *Helicobacter pylori* and *Mycoplasma pneumoniae* is taken into consideration as a factor influencing development of atherosclerosis.
Objective: The aim of the study was an assessment of infectious burden with selected bacterial microorganisms in the patients with advanced atherosclerotic process.
Material and methods: The study included 40 patients with three-vessels stable coronary arteries disease referred to the surgical revascularization. Plasma levels of IgG class antibodies anti-*Chlamydia pneumoniae*, anti-*Helicobacter pylori* and anti-*Mycoplasma pneumoniae* were determined with the tests based on the ELISA technique (Enzyme linked Immunosorbent Assay).
Results: In the study group, plasma IgG class antibodies anti-*Helicobacter pylori* ($n=35$, $f=0.875$, $p<0.05$) and a combination of antibodies anti-*Helicobacter pylori* and anti-*Chlamydia pneumoniae* ($n=17$, $f=0.81$, $p<0.001$) were significantly more frequently detected in comparison with the other antibodies.
Conclusions: The results of the study may suggest a participation of bacterial factor in the atherosclerotic process developing in coronary arteries. In stable advanced three-vessels coronary arteries disease the infection with two kinds of pathogens was significantly more frequently detected. However, our findings did not allow to determine a role of bacterial infection in etiopathogenesis of atherosclerosis

Key words: Chlamydia pneumoniae, Helicobacter pylori, Mycoplasma pneumoniae, atherosclerosis, coronary artery by-pass grafting.

Background

Recently, the role of infection and inflammatory processes in the pathogenesis of atherosclerosis has been widely discussed. It stays unclear whether the infections initiate atherogenesis or they only accelerate its natural course. Current studies suggest that the infections with *Chlamydia pneumoniae*, *cytomegalovirus* (CMV) and *Herpes simplex virus* (HSV) take a part in the atherosclerosis [1]. The presence of *Chlamydia pneumoniae*, CMV [2] and *Helicobacter pylori* [3] was found in the structures of atheromatous plaque whereas living *Chlamydia pneumoniae* were isolated from atheromatous lesions in coronary arteries [4]. There is also evidence that the macrophages from atheromatous plaques, infected with *Chlamydia pneumoniae* have a strong coagulative activity [5]. Besides, it was observed that the majority of the patients with coronary artery disease had antibodies

against *Chlamydia pneumoniae*, suggesting a past infection with this microorganism [6].

The attention is also paid to the potential relationship between a prevalence of infections with *Mycoplasma pneumoniae*, *Hemophilus influenzae* and *Hepatitis A virus* (HAV) and a presence of ischemic heart disease [7].

Among potential mechanisms of atherogenic action of microorganisms, their direct effects on the vessel wall and systemic activation of inflammatory processes, including chronic inflammation, are considered. However, the causality of bacterial and viral infections and development and progression of atherosclerosis is still not definitely clear. Recently, the burden with various pathogens is emphasized rather than the causality of monoinfection [8]. The aim of this study was to assess the infectious burden with selected microorganisms in the patients with advanced atheromatous process referred to direct myocardial revascularization.

Material

The study group comprised 40 patients (32 men and 8 women) aged from 32 to 67 years (mean age 54.2±7.6 years) with clinical presentation of stable angina caused by three-vessels coronary artery disease, referred to coronary artery bypass grafting (CABG) on cardiopulmonary bypass. The family history of cardiovascular diseases was positive in 26 patients (f=0.65), arterial hypertension was observed in 24 patients (f=0.6) and 5 patients had diabetes (f=0.125). Fourteen patients (f=0.35) had a history of transmural myocardial infarction. Left ventricular ejection fraction (LVEF) in the study group was from 40% to 79%, mean 55.8±9.5%. Peptic ulcer was previously diagnosed in 10 patients (f=0.25) but only one of them underwent the eradication. All these patients had a gastroscopy performed preoperatively to exclude the acute process.

Table I. Clinical characteristic of the study group

	Number of patients	Fraction
Gender (M)	32	0.8
Positive family history	26	0.65
Previous myocardial infarction	14	0.35
Diabetes	5	0.125
Hypertension	24	0.6
Cigarette smoking	35	0.875
History of peptic ulcer	10	0.25
BMI >25<30/Overweight	24	0.6
BMI >30/Obesity	5	0.125

BMI – Body Mass Index

Nearly 75% of the patients (n=29, f=725) had improper, elevated body mass index (BMI). Five of them were obese (f=0.125) and 24 had overweight (f=0.6). In the study group, mean BMI was 28±3.6 kg/m^2. Detailed clinical characteristics of the study group are shown in Table I.

The majority of the patients had a history of smoking (n=35, f=0.875) and 14 of them (f=0.35) did not give up the habit until the operation. The intensity of smoking in the study group is presented in Table II.

The patients from the study group had a various intensity of lipid disorders. Almost all of them (n=37, f=0.925) were chronically (three months or longer) treated with statins. The values of each lipid fractions are shown in Table III.

The exclusion criteria:
1. Presence of infectious disease within last three months
2. Fever
3. Use of immunosuppressants
4. Neoplastic diseases
5. Immunological diseases
6. Acute coronary event within last three months

Methods

The data on positive family history, past and current co-morbidities, cigarette smoking and drugs use of the patients were taken from the anamnesis. Body mass index (BMI) was calculated from the following formula:

BMI=body mass (kg) /height (m^2)

According to the World Health Organization (WHO) recommendations an overweight was diagnosed in the patients with BMI higher than 25 kg/m^2 and an obesity was diagnosed in those with BMI higher than 30 kg/m^2.

The progression assessment of atheromatous lesions in coronary arteries was made based on the analysis of selective coronary angiograms.

The left ventricular systolic function was estimated on the basis of ejection fraction taken from echocardiography (Hewlett Packard SONOS 2000, 3.5-2.7 MHz sound).

The IgG class antibodies against *Helicobacter pylori* were determined using an immunoenzymatic test based on the ELISA (Enzyme Linked Immunosorbent Assay) method (DIESSE Diagnostic Senese, Siena, Italy). The antibodies level measurements were taken twice from each blood sample, then an arithmetical mean was calculated. According to the manufacturer's recommendations the assay result was considered positive when higher than 1.1 AU (arbitrary units).

Table II. Intensity of smoking habits in the study group

			Number of cigarettes/day	Years of smoking	Years from cessation of smoking
	n	f	±SD (MED)	±SD (MED)	±SD (MED)
History of cigarette smoking	35	0.75	24.43±11.93 (20)	25.69±8.73 (28)	–
Current smokers	14	0.35	25.36±11.68 (22.5)	27.14±8.06 (30)	–
Ex-smokers	21	0.525	23.81±12.34 (25)	24.71±9.21 (20)	10.05±8.21 (11)

n – number of patients
f – fraction
MED – median

The IgG class antibodies against *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were determined with immunoenzymatic method EIA (Enzyme Immunoassay – Institute Virion Ltd., Zürich, Switzerland). Analogically to *Helicobacter pylori* a mean was calculated from two measurements. The results were considered positive when higher than 9 U (units).

In statistic analysis location and dispersion measures (arithmetic mean, Maximal and minimal values, median and standard deviation) were involved. The comparisons were performed using t-Student test. P value lower than 0.05 was considered the significance level.

Results

Nearly one hundred per cent (n=39, f=0.975) of the study group patients were seropositive considering the determined antibodies. The presence of antibodies against one or two of the pathogens was observed in over three fourths of the patients (n=33, f=875). The antibodies against *Helicobacter pylori* were detected the most frequently (n=35, f=0.875), next the antibodies against *Chlamydia pneumoniae* (n=25, f=0.625). The antibodies against *Mycoplasma pneumoniae* were found in one third of the patients (n=12, f=0,3). The above differences were statistically significant. In the assessed material, the antibodies against two of the pathogens were detected significantly more frequently (n=21, f=0.525) than against one (n=12, f=0.3; $p<0.05$) or three (n=6, f=0.15; $p<0.01$) of the microorganisms. In the subset with the positive antibodies against only one pathogen, those against *Helicobacter pylori* were predominant (n=9, f=0.75) and in the subset with positive antibodies against two pathogens the combination of anti-*Helicobacter pylori* and anti-*Chlamydia pneumoniae* were the most common (n=17, f=0.81). These differences were also statistically significant. The profile of antibodies present in the study group is shown in Table IV.

In the subset with positive history of peptic ulcer all patients (n=10) were seropositve as far as the antibodies against *Helicobacter pylori* were concerned. Half of them (n=5, f=0.5) had also positive antibodies against *Chlamydia pneumoniae* and in two (f=0,2) the antibodies against *Mycoplasma pneumoniae* were detected. One patient from this subset had the antibodies against all three assessed pathogens. The characteristics of the antibodies present in the subset with positive history of peptic ulcer are presented in Table V.

Discussion

On the basis of the current experimental and clinical studies, the detection of the microorganisms presence in atheromatous plaque structures and the results of serological analyses, the hypothesis was made that some of the pathogens may play a significant role in atherogenesis, and the others may contribute to the disease development by systemic inflammatory process. Significantly increased concentrations of the inflammatory markers were observed only in 30% of the patients with stable three-vessel coronary artery disease [9]. It results from the epidemiological data that the infection with *Chlamydia pneumoniae* is widespread as respiratory tracts inflammation with a tendency to frequent recurrences. *Chlamydia pneumoniae* is an intracellular microorganism and it multiplies in all histological structures related with the atheromatous process: in macrophages, endothelial and smooth muscle cells. It may release cytokines from infected cells and stimulate the immunological system of the

Table III. Lipid profile the study group

	Range (mg/dl)	Average ±SD (mg/dl)	MED (mg/dl)
TCH	110–336	204.95±56.66	206.5
HDL	30–85	52.2±12.28	53
LDL	44–197	120.18±44.92	123.5
TG	60–678	163.75±122.37	129.5

TCH – total cholesterol
HDL – HDL cholesterol
LDL – LDL cholesterol
TG –triglycerides
MED – median

Table IV. Presence of anti-*Helicobacter pylori* (H), anti-*Chlamydia pneumoniae* (C) and anti-*Mycoplasma pneumoniae* (M) antibodies in the study group

	n – *number of patients* (fraction)	1 *pathogen* n=12 (0.3)	2 *pathogens* n=21 (0.525)	3 *pathogens* n=6 (0.15)
H	35 (0.875)	9 (0.75)	H–C 17 (0.81)	
C	25 (0.625)	2 (0.17)	H–M 1 (0.05)	
M	12 (0.3)	1 (0.08)	M–C 3 (0.14)	
	p<0.05 H vs C, C vs M p<0.001 H vs M	p<0.05 H vs C, H vs M p>0.05 C vs M	p<0.001 H–C vs H–M, H–C vs M–C p>0.05 H–M vs M–C	

n – number of patients
f – fraction

host. These data established the role of *Chlamydia pneumoniae* in the inflammatory theory of atherosclerosis. However, the prevalence of antibodies against *Chlamydia pneumoniae* in the general population is high, as well as those against *cytomegalovirus* and *Helicobacter pylori*, and this fact diminishes the value of serological findings in the analyses of one pathogen causality. Zhu et al. [8] presented the conception of total pathogens burden. Determinig the IgG class antibodies against mixed bacterial and viral flora (*Chlamydia pneumoniae*, CMV, HSV-1, HSV-2 and HAV) in the group of patients who underwent coronary angiography, Zhu et al. found that the prevalence of coronary artery disease grew with the number of microorganisms. They documented the presence of coronary artery disease in as much as 85% of patients with antibodies against five pathogens and in 48% of patients with antibodies against two or less pathogens.

There were also attempts to establish the relationship between infective burden and the prevalence of cardiovascular events [10, 11]. The findings of these studies indicated for the positive relation between increasing infective burden and a risk of death or myocardial infarction. It should be emphasized that this risk was related to viral *herpes* infections [10, 11] because the investigators from the Zhu group did not find in their material the relationship between

Table V. Presence of anti-*Helicobacter pylori* (H), anti-*Chlamydia pneumoniae* (C) and anti-*Mycoplasma pneumoniae* (M) antibodies in the group of patients with peptic ulcer history (n=10)

	1 *pathogen* n=2 (0.2)	2 *pathogens* n=7 (0.7)	3 *pathogens* n=1 (0.1)
H	2 (0.2)	H–C 5 (0.71)	
		H–M 2 (0.29)	
		C–M 0	
p		>0.05 H–C vs H–M	

n – number of patients
f – fraction

increasing bacterial burden (*Chlamydia pneumoniae* and *Helicobacter pylori*) and the prevalence of myocardial infarction or cardiovascular death, in contrast to increasing viral burden [10]. Another group of researchers, the authors of the German AtheroGene Study [11] found a weak positive relationship between increasing burden with mixed flora, and a strong one between viral burden of *herpes* family (CMV, HSV-1, HSV-2) and the risk of coronary event occurrence. Whereas, in the AtheroGene study [7] they found that the infective burden, mostly the bacterial one, correlated with the extent of atheromatous process (involvement of two or more vascular regions).

The results of studies on the infective burden of patients with advanced atheromatous image of critical multivessel stenoses in coronary angiography, referred to the direct myocardial revascularization (CABG) can be found sporadically in the literature. The intraoperatively taken tissue samples were searched for the presence of genetic material of microorganisms [3, 12]. In one of the studies [3] serological tests were used. Our material concerns a similar group of patients. In the present study we analyzed the prevalence of antibodies against three bacteria, including two extracellular ones: *Mycoplasma pneumoniae* and *Helicobacter pylori*. In our material, the prevalence of *Helicobacter pylori* is similar to this presented in the AtheroGene Study, whereas the prevalence of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* is lower [7]. On the other hand, in our series the prevalence of *Helicobacter pylori* is higher than in the study of Kowalski et al. [3]. The results of the present study are partially different from the data in the literature. Probably it results from the differentiation of the analyzed groups and the number of pathogens determined. In our study we analyzed bacterial flora, whereas in the literature the studies concern mixed bacterial and viral one. In the present material the combination of two pathogens was found significantly more frequently. It can be caused by the choice of determined microorganisms. It seems that the global determination of antibodies against a number of bacteria and viruses of probable atherogenic action

and the assessment of total infective burden is a promising method that can allow to understand the complex atheromatous process and determine the prognosis of the patients. The study limitation is the small number of serological tests and the assessment of bacterial flora only. The issue requires further investigation directed at widening of serological determination with regard to virology.

Conclusions

1. The study results may indicate the participation of bacterial factor in atheromatous process of coronary arteries.
2. In the stable coronary artery disease with advanced three-vessels atheromatous lesions, the prevalence of combined two-pathogens infection was significantly more frequently found.
3. Nevertheless, the study findings do not allow to establish a role of bacterial infection in the pathogenesis of atherosclerosis.

Acknowledgment

The study was financed by the Medical University in Lodz, Poland through the intramural grant No 502-11-819

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