New risk factors for atherosclerosis

Introduction

Atherosclerosis and atherothrombosis are endemic diseases in the developed world and at the top of mortality statistics.
Cardiovascular diseases are the main causes of death in both men and women aged above 45 and 65, respectively. In Hungary, this results in 720's death out of 100 000 inhabitants a year. Taking early death and disability into account, atherosclerotic vascular diseases are remarkable public health problems.
Atherosclerosis is a multifactorial illness; its pathogenesis can only partly be explained by the conventional risk factors, e.g. smoking, hypertension, age, sex, high cholesterol and triglyceride levels, obesity and diabetes. Therefore the attention has focused on seeking further ones in the past decades.
Atherosclerosis has recently been regarded as an inflammatory disease. (58) The altered function of the endothelium plays the most important role in its development. As a principle, Ross formulated the theory of „response to injury" in 1976, according to which the process starts with endothelial dysfunction and injury followed by inflammation.
More and more data confirm that local and systemic inflammation also has an impact on the development of acute coronary syndrome. (47) To the effect of inflammatory factors (cytokines, adhesive molecules), the plaque becomes vulnerable and local vasoconstriction is also activated.
Most evidences available are on the role of oxidized low density lipoprotein (oxLDL) and heat shock proteins in the inflammatory and immunologic processes. (33, 61)
Heat shock proteins can be found in all creatures from bacteria to humans and provide the integrity of the cell. Their structures bear a close resemblance in the different species. Against bacterial ones, considerable antibody formation begins in the organisms, and these antibodies are strongly cytotoxic in effect.
Research done till now has proved the connection between the HSP 60-65 and anti-HSP 60-55 antibody levels and the severity of atherosclerosis. (67)
As the role of chronic infections and autoimmune processes in the atherosclerosis becomes hardly arguable, numerous studies had been planned to decide what effects on the development of symptomatic, manifest, treatment requiring coronary disease they have together. They revealed that jointly elevated anti-Chlamydia pneumoniae and anti-HSP 60-65 antibody levels significantly increase the risk of atherosclerosis occurring in the coronary and peripheral arteries. (28, 10)
CRP is traditionally known to be an aspecific inflammatory marker. Besides it is a sign of inflammation, thus a characteristic feature of the atherosclerosis considered to be an inflammatory condition, can cause the further progression of the induced process per se.
In response to cytokines and adhesive molecules released in a higher grade the production of CRP (acute phase protein) is increased in the liver, and, as a „circulus vitiosus", CRP itself is

also able to enhance the ICAM, VCAM, IL-6, E-selectin formation of the activated endothelial cells, which can cause the progression of the disease. (17, 55, 65)
Refining the techniques of determining CRP concentration, exact measurements become possible in low ranges, too. This led to the recognition that in case of raised CRP levels, the severity of atherosclerotic vascular and coronary artery impairments, therefore the CRP level is regarded as a risk factor of the cardiovascular diseases, but the mechanism is not clear yet. (65)
There is a consensus on increased CRP and HDL cholesterol levels together being the main risk factors of atherosclerotic vascular diseases. (17)
Numerous data confirm that there is a relationship between chronic infections and the progression of atherosclerosis.
In recent years the aims of research were to reveal the causal connection between chronic infections and atherosclerotic vascular diseases. The studies can be divided into 5 groups: (61)

1. Seroepidemiologic studies.
2. Animal and human pathologic tests attempting to detect the pathogen itself (with electron microscope or culture) or its characteristic cell components (with immunohistochemistry or PCR) from the atherosclerotic lesion.
3. Possible mechanisms induced by the pathogens, mainly molecular changes in infected cells examined „in vitro".
4. Animal research to reveal whether pathogens can induce or enhance the process of atherosclerosis, and whether these changes can be prevented or stopped by treating the infected animals with antibiotics.
5. Clinical trials on vascular- and coronary-ill patients, during which the effect of antibiotics on the progression of the vascular disease and the development of the next coronary event was examined. (62)

In the atherogenesis, the causative agents are suspected to belong to viruses and bacteria. The mostly examined pathogen is Chlamydia pneumoniae.
In the beginning, the summation of the results of many retrospective seroepidemiologic studies (13, 18, 37) showed in accordance that C. pneumoniae seropositivity increases at least twice the risk of coronary diseases compared to the seronegative control cases. Now it is widely accepted that in the evaluation of seroepidemiologic studies, many factors and methodical deficiency have to be taken into account. To our present knowledge, they are not suitable to determine the aetiology of the infection, but they are of prime importance, because they can focus the attention on the possibility of correspondence and its clinical consequences.
There are many techniques for the detection of C. pneumoniae from the atherosclerotic tissue sample. Bacterial particles can be observed with electron microscope, the antigens of the pathogen can be determined by immunohistochemistry and bacterial DNA can be showed by "in situ" hybridization or PCR from the atheroma.
Chlamydia pneumoniae is able to infect the cells relevant to atherosclerosis (monocytes, macrophages, endothelial cells, smooth muscle cells of the vessel wall) and facilitates the changes leading to its progression.
Chlamydia pneumonia directly and indirectly destructs the vessel walls.

The direct effects of infection are smooth muscle cell proliferation in the artery walls and endothelial injury, which is accompanied by endotoxin and hypercoagulability. (13) As the consequence of the indirect effects, such molecular processes occur, that resulted in the production of inflammatory cytokines, adhesive molecules and modified LDL ones with their cytotoxic effect, too.
Many experimental studies could prove the pathogenic role of C. pneumoniae in the atherosclerosis according to the Koch's postulates.
Evidences on the fact that C. pneumoniae infection can enhance atherogenesis led to the course of clinical trials, in which antibiotics were applied in humans suffering from coronary artery disease and the changes in coronary events and the possibility of secondary prevention were examined.
The result of a meta-analysis of randomized and controlled trials was published in JAMA in 2005. Between 1996 and 2005, 110 studies were carried out and 11 were counted as randomized and controlled and 19 217 patients were involved. 7 studies examined patients with acute coronary syndromes, 4 ones with stable coronary illnesses and the antibiotics used for treatment were roxithromycine, azythromycine and clarithromycine. There was no difference between the treated and untreated groups in the view of mortality (4.6 % vs. 4.7 %), myocardial infarction (5.0 % vs. 5.4 %) and the two combined end-points (instable angina pectoris and myocardial infarction) (9.2 % vs. 9.6 %).
Consequently, antibiotic therapy is not indicated in any forms of coronary artery diseases, to our present knowledge. (5)
According to the new conception of Zhu et al., all the infections affected the patient till that time have a role in the pathogenesis of atherothrombosis, not a alteration alone induced by one pathogen. This is the theory of „cumulative pathogen burden", which says that all of the chronic infections induced by the pathogens increase the risk of the development of atherosclerosis via facilitating inflammatory response. (71)

Aims

In my studies carried out between 1997 and 2004, I searched for the connection between the development of atherosclerosis and infectious and autoimmune processes, examining the new risk factors of atherosclerosis and their relation to each other.
During the 7-year period – very fast – more and more problems emerged, new aspects came to the fore, that I tried to keep up with, therefore my studies are wide-ranging and tried to contribute to clarify the different viewpoints of atherogenesis.
Hereunder, because of the notably different aspects and methods of my trials – out of the ordinary way – I am going to discuss them simultaneously in favour of making them more transparent.

1. study

Aims:

The aims of the first study were to examine the relationship between the anti-Chlamydia pneumoniae antibody levels and the prevalence of coronary artery diseases among cardiopathic patients in Hungary.

Patients and methods:
The study was planned to be a prospective and comprehensive one. 56 patients with coronary artery disease were involved. Control group consisted of 59 patients suffering from other heart diseases (not from IHD), e.g. not ischemic dilated cardiomyopathy, valvular heart diseases, arrhythmias, hypertension.
The occurrence of conventional IHD risk factors (weight, smoking, blood pressure, serum cholesterol and blood sugar levels) and the frequency of accompanying conditions were also registered and compared. Chlamydia pneumoniae specific IgA and IgG antibody titres were determined in a dilution range of 1/16 to 1/4096 with immunofluorescence.
Results:
To sum up, higher anti-Chlamydia pneumoniae antibody levels in coronary-ill patients (in higher dilution, antibodies were detected in 64 % of IHD patients, while this number was 42 % in the control group) mean that their presence is a risk factor of the coronary artery disease. Higher cholesterol levels were measured in the study group than in the control one (6.1 vs. 5.5 mmol/l) contrary to the fact that they took cholesterol-decreasing drugs in a higher portion. At the onset of the illness (before taking medicine), the patients' cholesterol level could be calculated as higher, than the one measured by us, and this had obviously a role in the manifestation of the disease.
In case of higher anti C. pneumoniae IgG titre, higher cholesterol level was detected, as an explanation of the fact that both parameters are the risk factors of the coronary artery disease. Implicitly, both parameters have higher values in case of the disease. Therefore, screening the whole population, more coronary-ill patients were found with high cholesterol and antibody levels than if these levels were low. Furthermore, molecular changes induced by anti C. pneumoniae antibodies themselves unfavourably influence the lipid profile. So, the two processes, separately and jointly, increases the chance for the development of coronary artery diseases.

2. study:
Aims:
In this study, we sought the answers to the following questions. In what portion inflammatory and autoimmune processes together are present in stable and acute coronary diseases and how both factors influence the risk of myocardial infarction and stable angina pectoris.
Patients:
129 patients were involved at the MÁV Hospital's Cardiology Department in 2001 and 2002. 1. group consisted of 40 patients treated with acute myocardial infarction and the 43 patients with stable effort angina (SEA) belonged to the 2. group. 46 patients were in the control group, having no cardiac illness.
Methods:
Anti-Chlamydia pneumoniae antibodies were detected by micro-immunofluorescence (MIF), anti-CMV, -HSV-1, -hHSP 60 and -mHSP 65 antibodies by ELISA. Statistical calculations were done with Windows SPSS version 9.0.
Results:
To sum up the findings of the study, among the pathogens mentioned only the anti-Chlamydia pneumoniae antibody level proved to be a risk factor in both stable (SEA) (just tendency can

be observed, not significant variance) and instable coronary diseases (AMI) (OR: 2.8; p=0.03). Among the anti-HSP antibodies (as an easily measurable parameter) characteristic of autoimmune processes, hHSP 60 and mHSP 65 significantly increased the chance of the development of AMI (OR: 4.7; p=0.012) and SEA (OR: 3.2; OR: p=0.04), respectively. If anti-Chlamydia pneumoniae and HSP antibody levels – separately being risk factors – are both present, they increased the chance of the development of AMI (hHSP 60) (OR: 15.5; p=0,016) and SEA (mHSP 65) (OR: 7.8; p=0.014) more than ten times. According to statistical calculations the two types of antibodies are independent risk factors. Therefore, if chronic infections and autoimmune processes playing a role in the progression of atherosclerosis simultaneously occur, we have to expect that they enhance each others harmful effects and remarkably raise the chance of the manifestation of the acute disease.

3. study

Aims:

In this study the question was percutaneous intervention as a rough, mechanic impact affecting atheromatosis plaques in the coronary arteries is able to activate the pathogens likely persisting in them, and if so, whether it is related to the enhanced production of CPR, IL-6 and hHPS 60, and these processes can influence the formation of restenoses in the future.

Patients:

28 patients were chosen at the MÁV Hospital's Cardiology Department in 2003. Blood sample was taken from every patients undergoing elective PCI during that period, and those patients' data were processed who had coronarography again 2 to 8 days after the previous one, and re-PCI in case of a restenosis greater than 50 %

Methods:

Blood was taken before and 4 and 14 days after the elective PCI.

DNA was detected from the sample by PCR technique. Serum antibody (HSV, HCMV, Cpn., EBV, HSP), CRP and IL-6 levels were determined by ELISA. Statistical calculations were done with Windows SSPS version 13.0.

Results:

Summarizing the results of the study, we found increased inflammatory parameters 2 weeks after the PCI (CRP >3 mg/l 46.4 % before and 85.7 % after PCI; p=0.002; IL-6 > 5 pg/l 14.2 % before and 39.3 % after PCI; p=0.042), that cannot be explained by the acute phase protein response of the body to the intervention because of the time passed. As the formation of the atherosclerotic plaques is supposed to be regulated by inflammatory factors, too, this change is considered to be a local inflammatory process activated by the mechanic impact (PCI) on the plaque, and deteriorates atherosclerosis further.

Pathogen DNA could be detected from more (8) sample after PCI, which refers to the fact, that the reactivation and reproduction of the pathogens had happened in some patients, so the intervention is not indifferent from the view of the pathogen in the atherosclerotic plaque.

There were no relation between restenoses and the elevated inflammatory parameters. The site of restenosis was not where pathogen DNA was detected. Its reason was the low number of patients having restenosis; therefore prognostic consequences cannot be drawn from these data.

4. study

Aims:
We examined the connection between the baseline CRP level in STEMI and the severity of the coronary status on the coronarogram.
Patients:
Data of the patients presenting with ST-segment elevation myocardial infarction in the Bajcsy-Zsilinszky Hospital in 2005 and 2006 were processed retrospectively. In the 1. group (243 patients) CRP was ≤ 5 mg/l, in the 2. one (204 patients) CRP was 5 to 30 mg/l.
Methods:
Lab tests were performed in the hospital's main laboratory, under standard lab circumstances. Windows SPSS version 13.0 was used for statistical calculations.
Results:
To sum up, we drew the consequence, that higher CRP level suggest more severe coronary status. Beside the thrombotic coronary occlusion in every patient, as stated in the criteria, significantly more patients had three-vessel disease with higher CRP level (high CRP: 39.22%, low CRP: 15.64 %; p=0.00001). Remarkably more patients had one-vessel disease with low CRP level (high CRP: 28.9 %, low CRP 53.9 %; p=0.000001). Our results are in accordance with the already accepted opinion, that high CRP level is a risk factor of coronary artery diseases and a prognostic factor, too.

Discussion:

Research on the role of inflammatory, autoimmune and infectious processes in the pathogenesis of atherosclerosis have been carried out with great interest for nearly two decades. First, the effect of chronic infections was widely examined.
Our study also revealed that Chlamydia pneumoniae infection does not relates generally to the sickness of the heart, but to coronary artery diseases. It is a risk factor of them, like the classical ones (smoking, hypertension, diabetes, high cholesterol level, weight).
Evidences on the C. pneumoniae infection enhancing the atherogenesis set off a series of clinical trials, during which antibiotics were applied to coronary-ill patients and the changes in coronary events were examined. Considering the results of some bigger trials and meta-analysis, it can be declared that we cannot influence the effect of chronic infections on the progression of atherosclerosis with antibiotic therapy.
Nowadays, atherosclerosis is regarded as an inflammatory disease. Most data are on the function of heat shock proteins in the inflammatory and autoimmune processes. Studies done till now examined the relationship between the anti-HSP 60-65 antibody levels and the severity of atherosclerosis (67) and they showed positive correlation.
We studied the impact of anti-Chlamydia pneumoniae and anti-HSP antibody levels' parallel elevation on the patients with myocardial infarction, stable effort angina and the control group.
According to our results, hHSP 60 level was significantly higher in myocardial infarction, and mHSP 65 level in stable effort angina as in the control group, so the anti-HSP antibody unambiguously relates to the development of coronary artery diseases.

High levels of anti-Chlamydia pneumoniae and anti-hHSP 60 antibodies together remarkably increased the risk of acute myocardial infarction, statistically correcting the results to any parameters; odd ratio was always in the range of 12.0 to 21.1.
The situation was the same in case of the anti-mHSP 65 antibody levels and stable effort angina with an OR of 6.2 to 10.2.
Some researches were conducted to discover the connection between the anti-pathogen antibody levels and restenoses following PTCA.
Reviewing the references, we have not found any studies on detecting the pathogens in a higher portion after the intervention, serving as a basis to the presumption that the intervention can reactivate the pathogens and via this, can influence subsequent cardiac events.
In accordance with our results, Chlamydia pneumoniae and cytomegalovirus DNA can be found in a higher portion in the peripheral blood after PCI, than before, although DNA could always be detected in only one out of the 5 parallel samples, referring to its amount. In our study, pathogen DNA was detected in the blood sample of 2 patients (7.1 %) by PCR technique before the intervention, and in 8 ones (28.6 %) after it, so during percutaneous intervention, there is a possibility for the pathogens to reactivate and induce atherogen processes.
We discovered that CRP and IL-6 levels significantly elevate after PCI, which shows the involvement of inflammatory factors in the atherogenesis and restenoses after PCI, while unchanged level of HSP refers to the fact that autoimmune processes are not activated during percutaneous intervention. (59)
CRP, as an acute phase protein and the aspecific marker of the inflammation is a parameter used in clinical practice for years now. However, the molecule itself also can cause the progression of the atherosclerosis, as it facilitates the production of cytotoxic mediators. (17, 55, 65)
Every study revealed some kind of connection between CRP level and the development and progression of the coronary artery and peripheral vascular diseases. (16, 50)
Referring to our study, we can declare that in the form of acute coronary syndrome demanding the fastest treatment, ST-segment elevation myocardial infarction, the baseline CRP level showed a correlation to the coronary artery status found on coronarography. In case of higher CRP level other significant coronary stenoses were also found beside the occluded artery. The coronary artery disease was more extensive. This aspect has not yet been discovered by any other researchers to our best knowledge.

Consequences and new findings:

In our studies, in accordance with international data, we found that

- a high anti-C. pneumoniae antibody level indicating previous infection is a risk factor of coronary artery diseases.
- it is related to lipid parameters, influencing them unfavourably, making the coronary artery disease more severe.

- it was significantly higher not only in coronary artery diseases as general but also in stable effort angina and myocardial infarction, respectively, comparing to the values in the control group.
- increased hHSP 60 level is the risk factor of myocardial infarction, while mHSP 65 raises the possibility of the development of stable effort angina.
- anti-Chlamydia pneumoniae IgG level jointly increased with hHSP 60 one raises the risk of myocardial infarction more than ten times, while with mHSP 65 level the development of stable effort angina more than seven times.
- as a new result, we detected the pathogen DNA knowingly persisting in coronary plaques in the blood more times after PTCA, than before the intervention, so we found evidence on the fact, that percutaneous intervention reactivates the pathogens.
- furthermore, coronary angiography reveals more extensive coronary artery disease in STEMI in case of higher CRP levels.
- taking the unambiguous results related to the therapy of chronic infections into account, according to which antibiotics do not influence atherosclerotic vascular diseases deteriorated by chronic infection, the clinical benefit of our results is in screening.
- in my opinion, determination of anti-Chlamydia pneumoniae and HSP IgG titres in certain patients on the occasion of routine blood taking can help to screen high risk coronary ones, resulting these patients' special therapy and check-up till the performance of the invasive interventions if needed.