REVIEW

Prediction of coronary vessel involvement on the basis of atherosclerosis risk factor analysis

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Abstract: The prediction of coronary vessel involvement by means of noninvasive tests is one of the fundamental objectives of preventive cardiology. This review describes the current possibilities of coronary vessel involvement prediction by means of ultrasonographic examination of carotid arteries, analysis of polymorphisms in the genes encoding enzymes responsible for production of nitric oxide and carbon monoxide and assessment of levels of certain proinflammatory cytokines. In the presented work these noninvasive markers are correlated with the extent of coronary vessel involvement as assessed by coronary angiography, intravascular ultrasound and virtual histology *(Fig. 5, Ref. 40)*. Full Text in PDF *www.elis.sk*.
Key words: coronary artery disease, atherosclerosis, intravascular ultrasound, gene polymorphism, proinflammatory cytokines, intima-media thickness.

Coronary artery disease (CAD) is the most frequent cause of death in the developed countries. In the USA, CAD is diagnosed in 12 % of the population, leading to approximately 4 million hospital admissions per year (1). More than 50 % of acute coronary syndrome (ACS) cases occur in patients without any previous symptoms because only 20 % of acute coronary vessel occlusions are caused by stenoses of over 75 % (2). This is one of the fundamental subjects of preventive cardiology, since ACS leads to substantial personal and social consequences, including sudden death and chronic heart failure with the need for lifetime pharmacologic and possibly nonpharmacologic treatments (cardiac resynchronization therapy, implantable cardioverter-defibrillators), which have a negative impact on work and social assertion.

Therefore, in recent years, much attention has been focused on the development of new methods capable of detecting asymptomatic patients at high risk for ACS before it occurs. The pathophysiology of ACS is currently understood as an interplay of factors that include an unstable patient (accumulation of risk factors), high-risk blood (prothrombogenic state) and a high-risk plaque. The following types of plaques are considered unstable: eccentric

plaques, bulky plaques compensated for by positive remodeling (an adaptive reaction of the artery to plaque progression which maintains sufficient vessel lumen) (3, 4) and ruptured plaques with superimposed intracoronary thrombosis (5).

Possibilities diagnosing high-risk coronary vessel involvement

The gold standard for coronary vessel evaluation is the coronary angiography (CAG). However, this technique does not visualize the atherosclerotic plaque per se. This visualization is possible with the use of intravascular ultrasound (IVUS). During this examination, a probe that allows for the visualization of the vessel wall and the atherosclerotic plaque is inserted into the vessel lumen (Fig. 1), followed by the subsequent evaluation of the above-mentioned high-risk morphological features (6) (Fig 2, 3). An additional risk factor for the development of ACS is plaque composition. Plaques leading to ACS contain a higher amount of lipid and necrotic matter (7). In vivo, plaque composition can be evaluated by means of virtual histology (VH), which correlates well with actual histology (8). Virtual histology is an imaging technique that is based on a different way of analyzing the signal acquired during the IVUS examination**.** In contrast to IVUS examination, which assesses the amplitude of the ultrasound signal, VH assesses the frequency of the reflected ultrasound signal, which is specific for different plaque components (calcification, fibrous and fibrolipid tissue, necrotic core) (Figs 4, 5). A newly recognized type of plaque is the so-called thin-cap fibroatheroma (TCFA). Since one of the criteria for TCFA is the width of the fibrous cap of less than 65 μm, a value below the resolution capacity of the IVUS, TCFA is classified as either TCFA (a pathological-anatomical description of the lesion) or VH-TCFA (TCFA diagnosed by virtual histology). The criteria for VH-TCFA is a plaque comprised of more than 10 % necrotic tissue in at least three consecutive cross-

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Fig. 1. IVUS probe on an intracoronary guidewire.
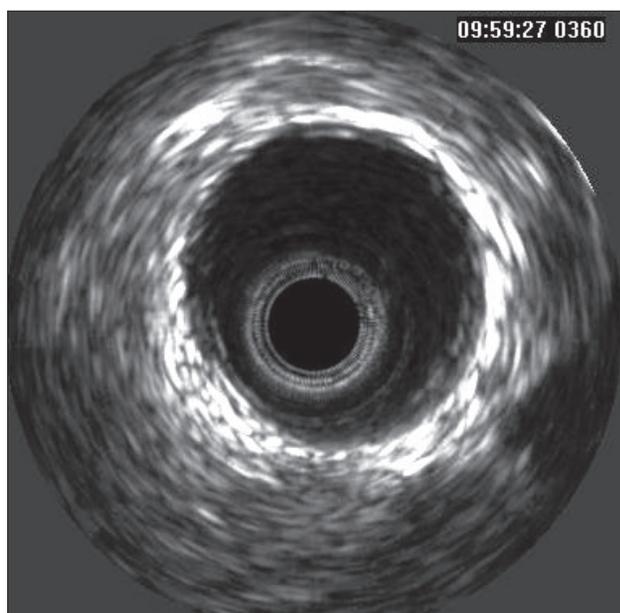
Fig. 2. IVUS picture of a normal coronary artery.

sections and direct contact of the necrotic core with the vessel lumen (since the thin fibrous cap cannot be directly visualized) (9).

It has been established that during the gradual process of plaque enlargement, the absolute amounts of fibrous and lipid components of the plaque increase. That means that calcification and especially necrotic tissue are related to the development of an unstable plaque (9). Marso et al published an interesting study this year in which they evaluated the relationship between the type of coronary vessel involvement and the Framingham risk score. The type of coronary vessel involvement was assessed by IVUS and VH. The data from 531 patients included in a worldwide prospective VH-IVUS registry from 37 participating centers were evaluated in this study. Positive correlations were observed between the Framingham score and the atherosclerotic plaque volume and the presence of VH-TCFA. This finding corresponds well with the results from previous studies (1).

In the PROSPECT trial, including 697 patients who underwent a very detailed assessment of all three main coronary vessels, including IVUS and VH, in the acute phase of ACS were prospectively followed for three years. The goal of the study was to ascertain whether there are certain plaque characteristics that allow for the discrimination between plaques that will subsequently lead to the development of coronary syndromes and those that are not dangerous to their carrier. In a multivariate analysis, it was established that lesions leading to the development of coronary syndromes during the follow-up were bulkier (plaque burden >70 %; this parameter expresses the plaque percentual proportion at a certain vessel point), were more frequently the VH-TCFA type of plaque and had a lumen smaller than 4 mm^2 (2, 10).

The use of ultrasonographic examination of the carotid arteries in the prediction of coronary involvement

Ultrasonographic examination of the carotid arteries is a non-invasive method facilitating the measurement of vessel lumen size and the intima and media thickness (IMT) and the assessment of the presence and extent of atherosclerotic plaques. In the context of predicting coronary vessel involvement, it must be emphasized
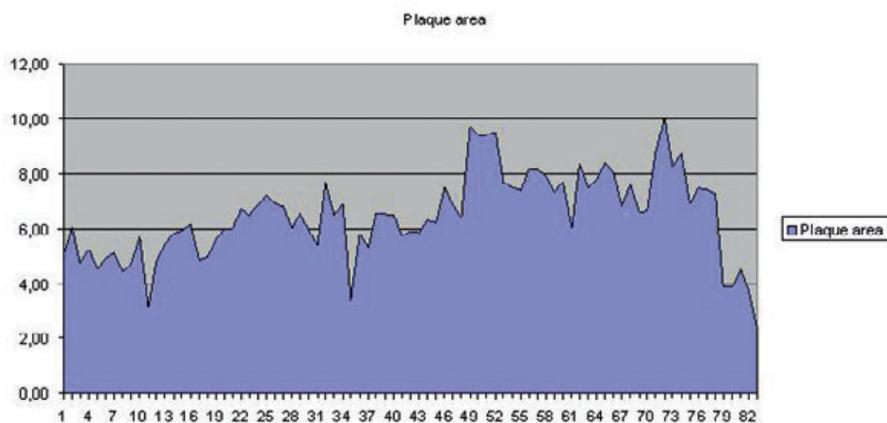
Fig. 3. Evaluation of plaque volume in an examined segment of a coronary artery.

that atherosclerotic involvement of the carotid arteries is a strong predictor of coronary vessel involvement. The IMT value in the area of the common carotid artery correlates with the severity of coronary vessel involvement (11). For the IMT values greater than 1.15 mm, there is a 94 % probability of the presence of CAD. Only 1.9 % of patients with triple vessel disease according to CAG have an IMT value of less than 1 mm (12). The presence of plaques in the common carotid artery and an increased IMT value correlates well with the results of myocardial perfusion scintigraphic stress testing in patients evaluated for suspected CAD as confirmed by the work of Hallerstam et al with 110 patients (13).

The IMT value also correlates with the incidence of acute myocardial infarction (AMI) (14, 15). The IMT itself, but also its irregularity in the examined segment, are predictors of an advanced atherosclerotic process, since a higher level of IMT irregularity can be observed in patients with more advanced CAD (16). Another factor that can be observed in the carotid arteries is the presence of positive remodeling. This term refers to a compensatory reaction of the vessel to plaque progression. By increasing the vessel diameter, the size of the vessel lumen is also increased, thereby compensating for the reduction caused by progression of the atherosclerotic plaque. Kato et al have demonstrated that positive remodeling, an increased IMT value and hypoechogenic and calcified plaques in the carotid arteries can be observed significantly more often in patients with multiple complex lesions of the coronary vessels (17).

Ultrasonographic examination of the carotid arteries allows not only for the assessment of

plaque morphology, but also of plaque echogenity. Less echogenic plaques contain higher amounts of lipids and necrotic matter and are therefore considered high-risk.

Ultrasonographic examination of the carotid arteries is being increasingly employed for the estimation of coronary risk because many patients evaluated during a theoretical examination 24 hours before the occurrence of ACS would have been judged to be at low or intermediate risk solely on the basis of classical risk factors. The ultrasonographic examination of carotid arteries helps in the diagnosis of developed atherosclerotic lesions and may shift the risk profile of a patient from the low- or intermediate-risk to the high-risk group (18).

The use of gene polymorphisms in the prediction of coronary atherosclerosis incidence

Endothelial dysfunction plays a key role in the pathophysiology of ACS. A procoagulative and proinflammatory phenotype of the endothelium in combination with high vascular tone can facilitate plaque rupture and intraluminal thrombus formation. Nitric oxide (NO) and to a lesser extent carbon monoxide (CO) play a significant role in the regulation of vascular tone. Therefore, the role of pivotal genes involved in the metabolism of these gaseous molecules and also the significance of their selected polymorphisms will be mentioned. These genes encode endothelial nitric oxide synthase (eNOS) and hemoxygenase type 1 (HO1). The product of the first gene is an enzyme that synthesizes NO, a substance with vasodilatory, antithrombotic and antiproliferative properties. Many

polymorphisms in the eNOS gene that demonstrate a variable clinical impact have been identified; one of which is the Glu298Asp polymorphism whose significance has been extensively studied. In case of this polymorphism, which is localized in exon 7 of the eNOS gene, the substitution of guanosine (G) for thymidine (T) at position 894 leads to a change in the amino acid sequence of the protein with the substitution of glutamate (Glu) for aspartate (Asp). According to many studies, individuals homozygous for the risk allele (Asp/Asp) are at an increased risk for development of arterial hypertension and coronary atherosclerosis (19, 20). The enzyme HO1 catalyzes the reaction in which the heme is degraded to iron (Fe), CO and biliverdin, which is subsequently converted to bilirubin. CO and bilirubin are substances with vasodilatory, antioxidative, angiogenic and anti-inflammatory properties and thus lead to suppression of atherogenesis. The activity of this inducible enzyme is determined by the number of guanosine-thymidine (GT) dinucleotide repeats in the gene promoter. As the number of dinucleotide repeats increases, transcription of the gene, and thus the enzymatic activity, decreases. Many studies have identified a positive correlation between the number of dinucleotide repeats and the development of diseases in which oxidative stress is the underlying pathogenetic mechanism, including CAD (21, 22). Conversely, gene variants with a low number of GT repeats react to oxidative stress with increased transcriptional activity and thus act to protect against the development of atherosclerosis (23).

Proinflammatory cytokines and atherosclerosis

Vascular cellular adhesive molecules (VCAM-1), intercellular adhesive molecules (ICAM-1) and selectins (E-endothelial, P-platelet) belong to a group of soluble adhesive molecules, which is a subgroup of the superfamily of immunoglobulins. They are considered to be a marker and mediator of endothelial vasomotor dysfunction and contribute to atherosclerotic plaque progression (24). Their increased expression is believed to correlate with increased cardiovascular risk (25, 26, 27), and they are thought to participate in all the above-mentioned early processes of atherosclerosis (28). The expression of adhesive molecules on the surface of the endothelium is especially increased in the primarily altered (dysfunctional) endothelium and is influenced by many mechanisms. One of the principal mechanisms with regard to atherogenesis is the regulation of adhesive molecule expression by oxidized LDL particles. Another key mechanism is the regulation by mechanical action of the blood stream, or more precisely by changes in the vessel wall strain (the so-called shear stress). The possibility for noninvasive detection of the atherosclerosis burden and risk stratification by means of adhesive molecule level assessment is currently under cautious debate (29). In particular, ICAM-1 is probably a marker of early atherosclerotic changes. However, according to published data, is rather not an ideal marker for the stratification of ACS risk (30, 31). VCAM-1 appears to be more specific and prognostically significant. A significant correlation with plaque progression and cardiovascular changes, along with a significant increase in its levels during the course of an acute coronary syndrome have been described in VCAM-1 (32).

In patients with stable angina pectoris who have stable atherosclerotic plaques, the extent of coronary vessel involvement and its progression are better reflected by serum levels of E-selectine (33).

Aside from cytokine levels, the highly-sensitive C-reactive protein (hsCRP) has been proven to be a very useful marker for coronary atherosclerosis. It not only acts as a biomarker, but also plays a role in the development of coronary thrombosis (34). In summary, with regard to the complexity of the pathophysiological processes occurring in atherosclerotic plaques, one cannot assume that a universal biomarker of atherosclerosis capable of monitoring changes in plaque stability and progression and enabling the stratification of high-risk individuals will ever be discovered. In recent years, a more complex approach that combines imaging techniques and soluble marker assessment is being advocated (35, 36, 37).

Trial evaluation of the significance of noninvasive markers of coronary atherosclerosis

In spite of all the aforementioned findings regarding the prediction of coronary risk, our knowledge is still insufficient. In a theoretical examination of completely healthy patients 24 hours before the development of an ACS, many individuals would be judged to be at intermediate- or even low-risk. Therefore, we undertook a study that focused on current possibilities for coronary vessel involvement prediction. The goal of the project is to evaluate the possibilities for predicting coronary vessel atherosclerotic involvement and atherosclerotic plaque composition by means of a carotid arteries examination (IMT measurement and plaque echogenity assessment) in combination with an assessment of gene polymorphisms, which play key roles in the metabolism of gaseous molecules that regulate vascular tone, and an assessment of the levels of proinflammatory cytokines. Examination of the coronary vessels was carried out using the following methods: coronary angiography (assessing the extent and severity of atherosclerotic involvement), intravascular ultrasound (assessing the hemodynamic significance of borderline stenoses and plaque volume) and virtual histology (assessing the plaque composition).

Patients in whom CAG was indicated for any reason were enrolled in the trial. In individuals with a borderline stenosis (narrowing of the vessel diameter by 40–70 % as assessed by angiography), the IVUS and VH examinations were carried out to determine the hemodynamic significance. This is a routine examination since IVUS is an appropriate method for determining the hemodynamic significance of lesions (38). If the stenosis was judged to be hemodynamically significant, percutaneous or surgical revascularization was carried out; if not, the patient was treated conservatively. In patients with multiple lesions of the coronary vessels, the IVUS was carried out in the angiographically most significant lesion. In cases of similarly significant stenoses, the one found in the proximal third of one of the main coronary vessels (where plaques most frequently causing ACS are localized) was chosen for IVUS examination. Subsequently, ultrasonographic examination of the carotid arteries was performed, and polymorphisms of the eNOS and HO1 genes and levels of the following proinflammatory cytokines were assessed: interleukin-6, VCAM-1, ICAM-1, tumor necrosis factor alpha (TNF-alpha), CD 40 ligand and hsCRP. The patients were also classified on the basis of classical atherosclerosis risk factors (smoking, arterial hypertension, diabetes mellitus, total cholesterol, LDL cholesterol, gender and age) according to the Framingham score and the Score risk stratification valid for the Czech population (39, 40). Currently, 107 patients for whom CAG and IVUS examinations were indicated have been enrolled in the trial. All patients underwent the aforementioned noninvasive tests. At the moment, the acquired data is being analyzed.

Conclusion

Based on trials comparing the ultrasonographic findings on the carotid arteries with coronary vessel involvement, it is known that a correlation exists between the atherosclerotic burden in both vessel territories. Likewise, studies have been conducted to evaluate the relationship between the degree of atherosclerosis development and polymorphisms of the genes regulating vascular tone. Finally, studies evaluating the significance of the increased levels of proinflammatory cytokines as markers of coronary atherosclerosis have also been carried out. To our knowledge, no trial to date has evaluated the possibility of coronary vessel atherosclerotic burden prediction by means of a complex examination of all of these risk factors along with "classic" population risks of the development of CAD. We hope that this study will provide valuable information for the noninvasive risk stratification of CAD.

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