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Mini review article

THE SIGNIFICANCE OF INFLAMMATION IN PATHOGENESIS, DIAGNOSIS AND TREATMENT OF ATHEROSCLEROSIS

ZNAČAJ ZAPALJENJSKOG PROCESA U PATOGENEZI, DIJAGNOZI I TERAPIJI ATEROSKLEROZE

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Abstract

Atherosclerosis is a chronic disease whose progression starts from birth. The basic substrate of an atherosclerotic lesion is the deposition of lipids in the intima of the blood vessel, which results in gradual narrowing and impaired blood flow through the tissues. The clinical manifestations of the atherosclerotic process are numerous and they depend on the degree of narrowing and the speed of the stenosis. The most common clinical manifestations are the result of rupture of the atherosclerotic plaque, which activates the process of thrombogenesis and leads to the development of blockage of blood vessels. The most important risk factors for atherosclerosis are elevated LDL, decreased HDL, cigarette use, hypertension, type II diabetes mellitus, age, family history of cardiovascular disease in first-line relatives (men younger than 55 years, women younger than 65 years). When mild forms of atherosclerosis are observed, they generally do not have any symptoms. With advanced atherosclerosis, there are visible and noticeable symptoms that vary depending on which artery is affected by atherosclerotic process. Some of them are: chest pain - caused by atherosclerosis in the arteries of the heart, acute brain ischemia or stroke - caused by atherosclerosis in the arteries leading to the brain, difficulty walking - narrowed arteries in the legs, high blood pressure, kidney failure, erectile dysfunction in men and painful relations in women - atherosclerosis in the arteries of the genital tract. The aim of this paper is to adequately investigate and show the connection between inflammation and atherosclerosis.

Keywords:
inflammation,
atherosclerosis,
disease,
blood vessels

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Sažetak

Ateroskleroza je hronična bolest čija progresija kreće od rođenja. Osnovni supstrat aterosklerotske lezije je deponovanje lipida u intimu krvnog suda, što za rezultat ima postupno suženje i otežan protok krvi kroz tkiva. Kliničke manifestacije aterosklerotskog procesa su brojne i zavise od stepena suženja i brzine nastanka stenoze. Najčešće kliničke manifestacije su posledice rupture aterosklerotskog plaka, što aktivira proces trombogeneze i dovodi do razvoja začepljenja krvnih sudova. Najznačajniji faktori rizika za aterosklerozu su povišeni LDL, smanjeni HDL, upotreba cigareta, hipertenzija, tip II dijabetesa, starost, porodična anamneza koja se vezuje za kardiovaskularne bolesti kod rođaka u prvoj liniji (muškarci mlađi od 55 godina, žene mlađe od 65 godina). Kada se posmatraju blagi oblici ateroskleroze, uglavnom nemaju nikakve simptome. Kod uznapredovale ateroskleroze javljaju se vidljivi i uočljivi simptomi koji variraju u zavisnosti od toga koja je arterija zahvaćena aterosklerotskim procesom. Neki od njih su: bol u grudima - izaziva ga ateroskleroza u srčanim arterijama; akutna ishemija mozga ili šlog - izaziva ih ateroskleroza u arterijama koje vode do mozga; poteškoće sa hodanjem - sužene arterije u nogama, povišen krvni pritisak, bubrežna slabost; erektilna disfunkcija kod muškaraca i bolni odnosi kod žena - ateroskleroza u arterijama genitalnog trakta. Cilj ovog rada jeste adekvatno istražiti i prikazati vezu između inflamacije i ateroskleroze.

Ključne reči:
inflamacija,
ateroskleroza,
bolest,
krvni sudovi

Introduction

Atherosclerosis is the most common cardiovascular disease. It is a chronic progressive diffuse disorder characterized by lipid accumulation and inflammation in the walls of large and medium sized arterial blood vessels.

The word atherosclerosis is a combination of two Greek words, *athero* (meaning gruel, porridge) and *sclerosis* (hardening) indicating major macroscopic features of affected arteries (thickening of the intimal layer of arteries, lipid accumulation).

The arterial wall consists of three layers surrounding the arterial lumen. The layer closest to the lumen is the intima, made up of one layer of endothelial cells and layer of glycosaminoglycans and collagen. The middle layer is mostly made of smooth muscle cells and is known as the media, while the outermost layer is called the adventitia.

The development of an atherosclerotic lesion passes through several stages (fatty streaks, atheroma, plaque), characterized by accumulation and transformation of extracellular and intracellular lipids, smooth muscle cells, inflammatory cells and necrotic debris in the subendothelial space in the intima of the affected arteries and disturbing the balance of pro and anti-inflammatory mechanisms.

Fully developed lesion (plaque) has a lipid core, covered by a fibrous cap. The formation of atherosclerotic plaques leads to reduction of the arterial lumen and changes in the blood flow, leading to thrombosis, while, in some occasions, plaques can become unstable or rupture, consequently creating thromboembolic complications in near or distant blood vessels (**figures 1, 2 and 3**) (1, 2).

Hypotheses on atherosclerosis initiation and development included three potential principles, response-to-injury, response-to-retention, and oxidative modification. According to the response to injury theory (3), the initial step in atherosclerosis is endothelial injury leading to loss of endothelial cells and increased permeability to lipoproteins and leucocytes. The second approach considered lipoprotein retention at the artery bifurcation as the initial step (4, 5).

Finally, the oxidative modification theory (6) asserts that oxidation of low-density lipoproteins (LDL) is a starting point in atherogenesis activating the differentiation of monocytes into macrophages and creating „foam cells".

Nowadays, atherosclerosis is considered as an inflammatory disease, considering the significant role of inflammation during the stage of formation and development of atherosclerotic lesion. The initiation is connected to antigens from oxidized low-density lipoprotein while progression is related to the release of antigens from cells in plaques going through apoptosis (7).

During the growth of atherosclerotic plaque, there is a continuous accumulation of macrophages and lymphocytes, proliferation of smooth muscle cells and accumulation of lipids in the intima. In the process of accumulation of local cellular fat, a significant part belongs to the creation of oxidized LDL particles. Changes in the intima and the formation of atheromatous plaque mainly result in progressive degeneration and weakening of the media and lymphocytic infiltration of the adventitia. Repeated damage to the endothelium is significant for the growth of atheromatous plaque, which further leads to platelet aggregation and the release of platelet growth factors, as well as the proliferation of vascular smooth muscle cells. Thickening of the intima in a progressive state leads to ischemia of the deeper layers of the plaque, also to the intima-media junction. Therefore, atherosclerosis represents an inflammatory process, the formation of which is a consequence of chronic damage to the walls of blood vessels by multiple risk factors, such as: aging, hyperlipidemia, hypertension, smoking, diabetes mellitus and exposure to some infectious agents (herpes viruses, *Chlamydia*) (8).

One should also be aware of the fact that almost 40% of atherosclerosis cases can be attributed to the genetic variations. Using data from large European and

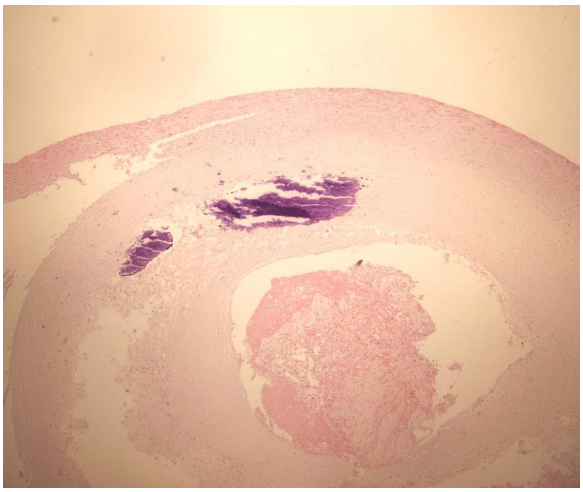

Figure 1. Coronary artery fibroatheroma, with thrombus in the lumen and dystrophic calcifications in the media (H&E staining, magnified 4x)

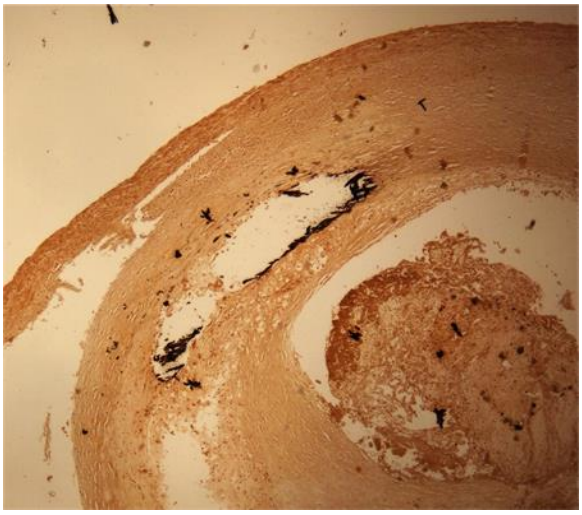

Figure 2. Von Kossa staining (calcium deposits colored black, magnified 4x)

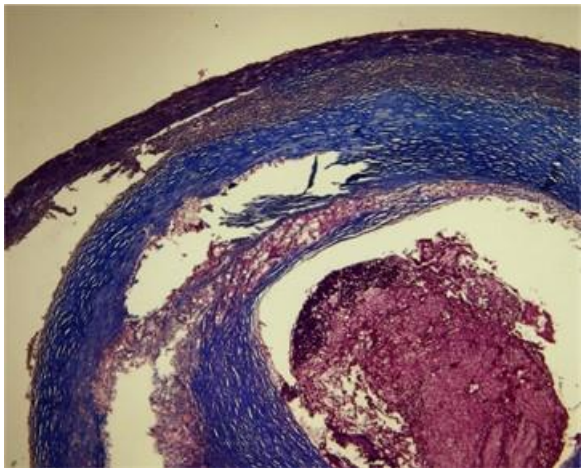

Figure 3. Masson's trichrome staining (blue color indicates connective tissue deposits, magnified 4x)

Japanese cohorts, meta-analyses identified more than 200 genetic loci directly related to the disease (9).

From the clinical aspect, atherosclerosis is manifested through several diseases with high morbidity and mortality, such as coronary heart disease (angina pectoris, heart attack, sudden cardiac death); cerebrovascular disease (stroke); peripheral vascular disease (intermittent claudication, gangrene) (10).

The aim of this paper is to analyze the role of inflammation in the development, diagnosis and treatment of atherosclerosis through a review of the previous literature.

The role of inflammation in the pathogenesis of atherosclerosis

The classical approach to atherosclerosis pathogenesis was associated with cholesterol accumulation due to uptake of lipoproteins (especially LDL) in the intima of the arteries, forwarded by an immune response. In 1999, Ross indicated that atherosclerosis should be considered as a chronic inflammatory disease, since inflammation is present from the earliest stages (monocyte infiltration from circulation to fatty streaks) (11). The discovery of vascular cell adhesion molecule-1 (VCAM - 1), as a factor whose overexpression on the surface of endothelial cells leads to leukocyte recruitment, confirmed the role of inflammation in the process. In the later stages, researchers identified the role of pro-inflammatory cytokines such as interleukin -1 and tumor necrosis factor, as well as different types of white blood cells in atherosclerotic lesions.

The use of monoclonal antibodies has shown that both innate and adaptive immune response have their part in the process of atherosclerosis. Pro-inflammatory monocytes are the first to enter the process, transforming into macrophages, that have the possibility to proliferate and change into foam cells, after lipid uptake. On the other side, the significance of adaptive immune cells, such as T helper - 1 and B1 lymphocytes has been also recognized (9). Manifestation of the inflammation of atherosclerosis is reflected by the correlation of the level of inflammatory markers in the blood, especially C-reactive protein (CRP) with the onset and progression of atherosclerosis (12).

Current research on the role of inflammation in atherosclerosis has identified several groups of factors that can initiate and lead to progression of lesions. These factors include (but are not limited to) exogenous and endogenous factors, such as environmental (noise, air pollution, high fat diet), stress, insomnia, diabetes, smoking, but also some hematological disorders (polycythemia, anemia, chronic hypoxia), as well as chronic infection and inflammation (endotoxinaemia, paradontosis). All of these factors act through two basic patterns, Pathogen-Associated Molecular Patterns - PAMP (such as chronic endotoxinemia, viral ribonucleic acid), and Damage-Associated Molecular Patterns - DAMP (oxidized LDL, glycated proteins, neutrophil extracellular traps). There are five classes of pattern recognition receptors on the surface (Toll-like receptors) or in the cytoplasm (NOD-like receptors - NLR) of macrophages, neutrophils and other inflammatory cells. The NLR molecules (inflammasomes) when activated, trigger the production of pro inflammatory cytokines, proliferation of macrophages, Th1 and Th17 lymphocytes. Activated NLRP3 inflammasome was correlated with the

level of coronary atherosclerosis, as with the presence of major adverse cardiovascular events (MACE). Also, it is shown that statins and colchicine as anti-inflammatory drugs reduce the expression of NLRP3 in subjects with coronary atherosclerosis, leading to better outcomes (13).

The initial step in atherosclerosis could be the disturbance in blood flow (turbulence at the bifurcation of arteries, for example), leading to dysfunction at the level of endothelial cells forming the inner layer of intima. This dysfunction could allow higher uptake of low-density lipoproteins and triglycerides from plasma into intima. In the second step, endothelial cells become activated in response to oxidative modification of LDL, with the expression of VCAM - 1, intercellular adhesion molecule 1 (ICAM - 1) and other factors promoting the adhesion of monocytes, and other white blood cells. Lipid hydroperoxides, lysophospholipids, carbonyl compounds have been found in the lipid fraction of atheroma (14). Oxidized LDL acts through DAMP leading to activation of inflammatory pathway. The adhesion and entering of the monocytes in the intimal layer are followed by their differentiation into macrophages. There are two main categories of macrophages, M1 with pro-inflammatory, and M2 with atheroprotective effects. The progression of atherosclerotic lesions is related to accumulation and proliferation of M1 macrophages. Although there is some evidence of direct lipid oxidation in macrophages and in the intestine, contributing the inflammatory process in atherosclerosis, it is suggested that most of the macrophages in the plaque uptake already oxidized LDL through phagocytosis. So-called foam cells seen in the lesions are mostly macrophages overfilled with cholesterol, that undergo the process of apoptosis or necrosis, creating a plaque core. Vascular smooth muscle cells migrate from the media into the intima, undergoing changes in their phenotype, so, besides the contractile features, they acquire pro-inflammatory macrophage-like, fibro myocyte, osteogenic or adipocyte features. These changes in smooth muscle cells behavior stimulate the collagen fiber synthesis, but, on the other hand, induce further macrophage proliferation, foam cell production and accelerate inflammation. A sensitive marker of smooth muscle cell transformation is a cytoskeleton associated protein, labeled as smooth muscle 22 alpha (SM22α) (15, 16).

Initiation and progression of atherosclerotic lesions is correlated with the activity of T and B lymphocytes. Plaque growth and instability is strongly influenced by T-helper 1 lymphocytes secreting interferon gamma, while T-helper 2 cells produce interleukins 5 and 13, that have anti-inflammatory effects. Similarly, some subpopulations of B-lymphocytes can stimulate atherosclerosis, such as ones producing antibodies to LDL, oxidized LDL, apolipoprotein B, cytomegalovirus, or IgE immunoglobulins (17).

Diagnosis of atherosclerosis

Atherosclerosis is the foundation of several cardiovascular diseases with high morbidity and mortality, such as coronary artery disease (CAD), myocardial infarction (MI), aortic aneurysm, peripheral vascular disease, or stroke. Given the fact that subjects with atherosclerosis usually go asymptomatic, and therefore, undiagnosed for a prolonged period of time, there is a strong need for timely diagnosis and treatment of atherosclerosis in preclinical phases of the disease.

When looking at risk factors for atherosclerosis, they can be divided into modifiable and non-modifiable. Some of the latest research has shown that in addition to the standard risk factors, the most prominent of which are: dyslipidemia, hypertension, cigarette consumption and diabetes, there are also unconventional risk factors such as: increased oxidative stress, endothelial dysfunction and inflammation (18). Identification and elimination of modifiable risk factors is an important step in diagnosing and treating atherosclerosis. In a similar manner, identification of early type of atherosclerotic lesions (I - III) through different imaging techniques (Invasive coronary angiography - ICA; computed tomography coronary angiography - CCTA; intravascular ultrasound - IVUS; Positron emission tomography - PET) can help in slowing the progression of the disease (19, 20).

Standard biomarkers of atherosclerosis, such as serum triglycerides, LDL and HDL cholesterol are adequate only for patients already identified as high risk.

Biomarkers of inflammatory component of atherosclerosis, besides high sensitive CRP, include myeloperoxidase (MPO), matrix metalloproteinase 9 (MMP-9), intercellular and vascular cell adhesion molecules, as well as interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) whose level is correlated with cardiovascular risk and atherosclerotic plaque formation.

Other biomarkers of atherosclerosis presence and progression include midkine (MK), a heparin-binding growth factor, microRNAs (miRNAs), pentraxin 3 (PTX3), endothelial-cell-derived extracellular vesicles, as well as previously mentioned NLR3P inflammasome (21). In recent years, gut microbiome metabolites, such as carnitine metabolite TMAO, the phenylacetyl glutamine, and the lipid metabolite bile acids, have been suggested as potential biomarkers of atherosclerosis (22).

Treatment of atherosclerosis

Taking into consideration the complexity of atherosclerosis pathophysiology, as well as the factors that influence initiation and progression of atherosclerotic lesions, it of outmost importance to introduce preventive and timely treatment strategies. Potential targets include control of the external factors (exposure to noise, air pollution), regulation of endogenous factors (sympathetic activity) and corrections of lipid metabolism, inflammatory components, chronic infection and gut microbiota.

A critical cause of atherosclerosis is certainly dyslipidemia. Many therapeutic measures can moderate and reduce lipid risk factors for atherosclerosis. Statins, by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, lower the LDL-cholesterol levels. Other

lipid lowering drugs that have shown positive effects on atherosclerosis include PCSK9 inhibitors, ANGPTL3 inhibitors, ATP citrate lyase inhibitors etc. Glucose lowering drugs (GLP-1 receptor agonists, SGLT-2 inhibitors, etc.) have also shown positive effects in patients with type II diabetes and atherosclerosis (16).

Novel treatment strategies focus on the reduction of the inflammatory elements. The use of interleukin inhibitors (canakinumab, tocilizumab, sarilumab, etc.) has already shown reduction of MACE. The inflammasome inhibitors NLRP3, such as colchicine (COLCOT trial) can lead to significant reduction of adverse cardiovascular outcomes (myocardial infarction, need for urgent revascularization) (23).

The following tests are used to diagnose atherosclerosis:

- Angiography - this test uses special X-rays to locate and measure blockages;
- Brachial index - this test compares the blood pressure in the wrist with the pressure in the hand, thus measuring the blood flow in the limbs;
- Chest test - it is done with the help of a chest X-ray, which produces images of the interior of the chest pictures of the inside of the chest;
- CT scan - this scan captures images of the inside of the body and can show any hardening or narrowing of the large arteries;
- Echocardiogram (echo) - an echocardiogram is used to assess the structure and function of the heart;
- Electrocardiogram (ECG) - an ECG measures the electrical activity, speed and rhythm of the heart;
- Exercise stress test - this test measures heart function during physical activity;
- Carotid ultrasound - with the help of this test, ultrasound images of the arteries in the neck (carotid arteries) are made. It can detect hardening or narrowing of these arteries as blood flows to the brain; and
- Abdominal ultrasound - plays an important role in evaluating the abdominal aorta. It is commonly used to detect conditions such as abdominal aortic aneurysm, by measuring the size and assessing the structure of the aorta.

Some of the medical operations or procedures are suggested for severe atherosclerosis:

- Percutaneous coronary intervention (PCI);
- Fibrinolytic therapy;
- Coronary artery bypass grafting (CABG) is a type of surgery; and
- Carotid endarterectomy.

Conclusion

Atherosclerosis is a process that is specific for certain changes in the biology of arteries due to hemodynamic changes, and the cause is the growth and disruption of plaque. Dysfunctional vascular biology related to atherosclerosis and its risk factors include vasomotor dysfunction and plaque inflammation, as well as the prothrombin/antifibrinolytic state. If there is no adequate intervention, atherosclerosis will be clinically proven as coronary artery and cerebrovascular disease, both of which are among the leading causes of death in the world. Several studies suggest the main role of inflammation in atherosclerotic plaque progression, vulnerability and thrombogenicity. Certain clinical trials have demonstrated and documented the benefit of lipid-lowering therapy for primary and secondary prevention of cardiovascular events. Recent scientific findings on the role and impact of inflammation in atherosclerosis show that inflammation plays a major role in atherosclerosis. Although this hypothesis has many followers, there are also those who are not in that group, and on the other hand, they advocate that the role of inflammation can have different effects, thus reducing its importance in the development of atherosclerosis.

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