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Selected biomarkers of atherosclerosis: clinical aspects

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Abstract

Atherosclerosis is a inflammatory-immunological-degenerative process. Cardiovascular diseases account for 42% of premature deaths among men and 52% of premature deaths among woman. Identification of classical biomarkers of atherosclerosis, such as LDL, HDL and triglycerides may not be helpful in patients with moderate or unusual cardiovascular risk. Non-classical indicators of atherosclerosis include markers of the inflammatory process, markers of atherosclerotic plaque injury, acute phase proteins, ischemic markers, markers of tissue necrosis, markers of myocardial dysfunction. The identification of CVD biomarkers enables the classification of patients to appropriate cardiovascular risk groups. Knowledge about the CVD risk group makes it possible to take rapid therapeutic intervention aimed at limiting this risk. Pharmacotherapy for cardiovascular diseases is primarily based on lowering cholesterol's level in the blood. Additional properties of statins (the most important lipid-lowering drugs) enable their pleiotropic effect by limiting the progression of atherosclerotic lesions by reducing the volume of atherosclerotic plaque. Further research on the pathogenesis of atherosclerosis will allow learning new risk factors and new biomarkers of this disease.

Key words: biomarkers, atherosclerosis, cardiovascular risk, cardiovascular diseases, statins

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Introduction

Atherosclerosis is a inflammatory-immunological-degenerative process involving small and medium-caliber arteries, which is responsible for the majority of CVD (cardiovascular diseases) [1]. Atherosclerotic lesions arise in childhood and even in fetal life [2]. Cardiovascular diseases account for 42% of premature deaths among men and 52% of premature deaths among woman [3]. Cardiovascular diseases are the most common cause of death in Poland. According to GUS (Central Statistical Office) data, in 2014 these diseases accounted for > 48% of premature deaths of Poles. Atherosclerosis is also responsible for peripheral arterial diseases (PAD), and what is more, it is the most important cause of amputation of legs [4]. Risk factors of progression of atherosclerotic lesions are divided into modifiable and unmodifiable, as well as, classical and non-classical [5] (Fig. 1). According to the National Health Service (NHS), the biggest life threats in developed countries are hypertension, smoking, hypercholesterolemia, obesity, dietary mistakes, lack of physical activity, excessive alcohol consumption, infections, non-traffic accidents, traffic accidents, illegal drug use, murders, medical complications, war, pregnancy and childbirth. The prevalence of the most important risk factors for cardiovascular disease in Poland was examined in the NATPOL 2011 Study (Fig. 2.). The most important cause of premature deaths in the world is hypertension [6]. The Cardiovascular Disease Prevention and Treatment Program (POLKARD 2017–2020), which aims to combat the classic risk factors for atherosclerosis, introduce modern effective diagnostic and therapeutic methods and act to level out disproportions arising in the country

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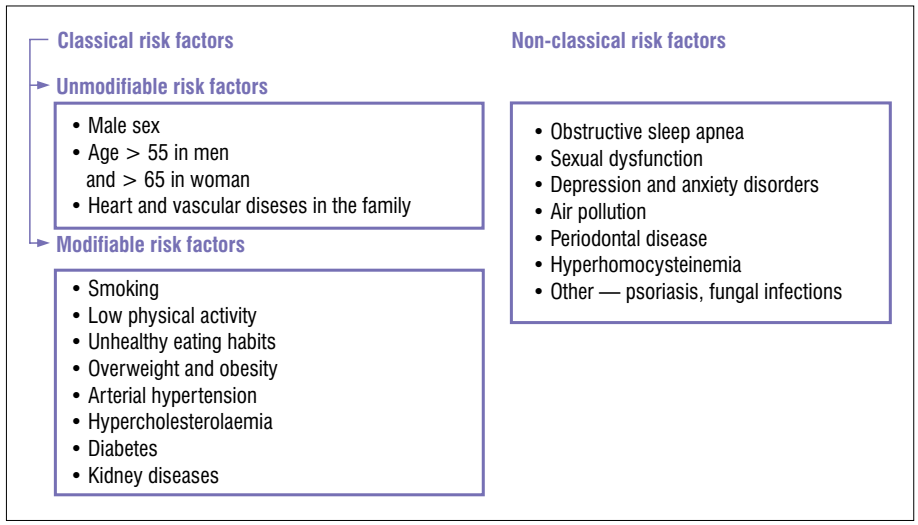

Figure 1. Risk factors for cardiovascular diseases [based on 5]

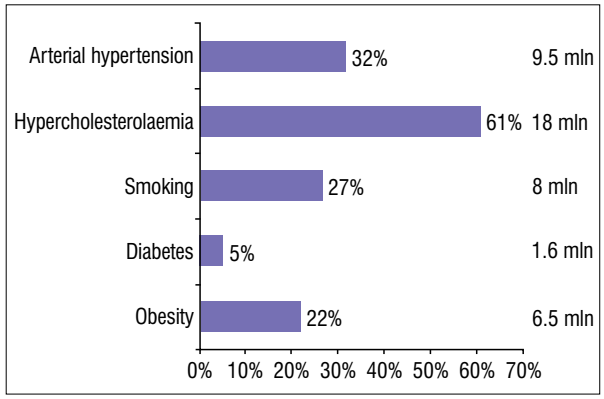

Figure 2. Prevalence of risk factors for cardiovascular diseases in Poland among patients aged 18–79 according to the results of the NATPOL 2011 Study

in accessing highly specialized health services in the field of cardiology, pediatric cardiology, cardiac surgery and neurology. Early detection of atherosclerotic lesions enables the introduction of appropriate prophylaxis aimed at reducing cardiovascular risk. Biomarkers can be used in the early detection of atherosclerosis. The main risk factors (classical risk factors) for CVD affecting cardiovascular risk are SBP (systolic blood pressure), total cholesterol, age, sex and smoking [5]. Cardiovascular risk can be presented as a risk of death (European systems: Euro-SCORE, Pol-SCORE) or as a risk of a cardiovascular event (American systems, for example, *Framingham Risk Score*). The influence of classical CVD risk factors on the incidence of CVD is shown in fig. 3. It is also worth remembering about other risk factors for CVD. An important risk factor of CVD is also diet. It has been reported that oxysterols increase inflammation, lipid markers levels and reflect accelerated endothelial dysfunction [7]. The number of CVD risk factors does not always correlate with the actual cardiovascular risk. In 1/5 of patients with diagnosed coronary artery disease, no classic risk factors are found, while 40% have only one risk factor [8, 9].

Pathogenesis of atherosclerosis

Atherosclerosis is a chronic disease with a multifactorial pathogenesis. It mainly concerns the aorta, coronary vessels, and in particular the branching of these vessels. Atherosclerotic lesions localize in the *tunica intima* and *tunica media* of vascular wall. The location of atherosclerotic lesions varies between men and women. Among women, atherosclerotic lesions are located in the carotid arteries — 24.1%; coronary arteries — 4.5%; abdominal aorta — 22.8% and ilio-lumbar arteries — 30.1%. Among men, the incidence of atherosclerosis in the above locations is as follows: 36.9%; 26.1%; 27.2% and 54.1% [10]. Atherosclerosis can be generalized — including > 3 vascular bearings; indirect — including 2-3 vascular bearings and local — including 1 vascular bearing [10]. Atherosclerosis gradually narrows the lumen of the vessel, which reduces the flow of blood to the tissues, leading to their ischemia. The scientific beginnings of research on atherosclerosis date back to the second half of the 19th century. In 1856 Rudolf Virchow showed that atherosclerotic lesions are composed of large amounts of lipid components (cholesterol and triglycerides). Another researcher dealing with ather-

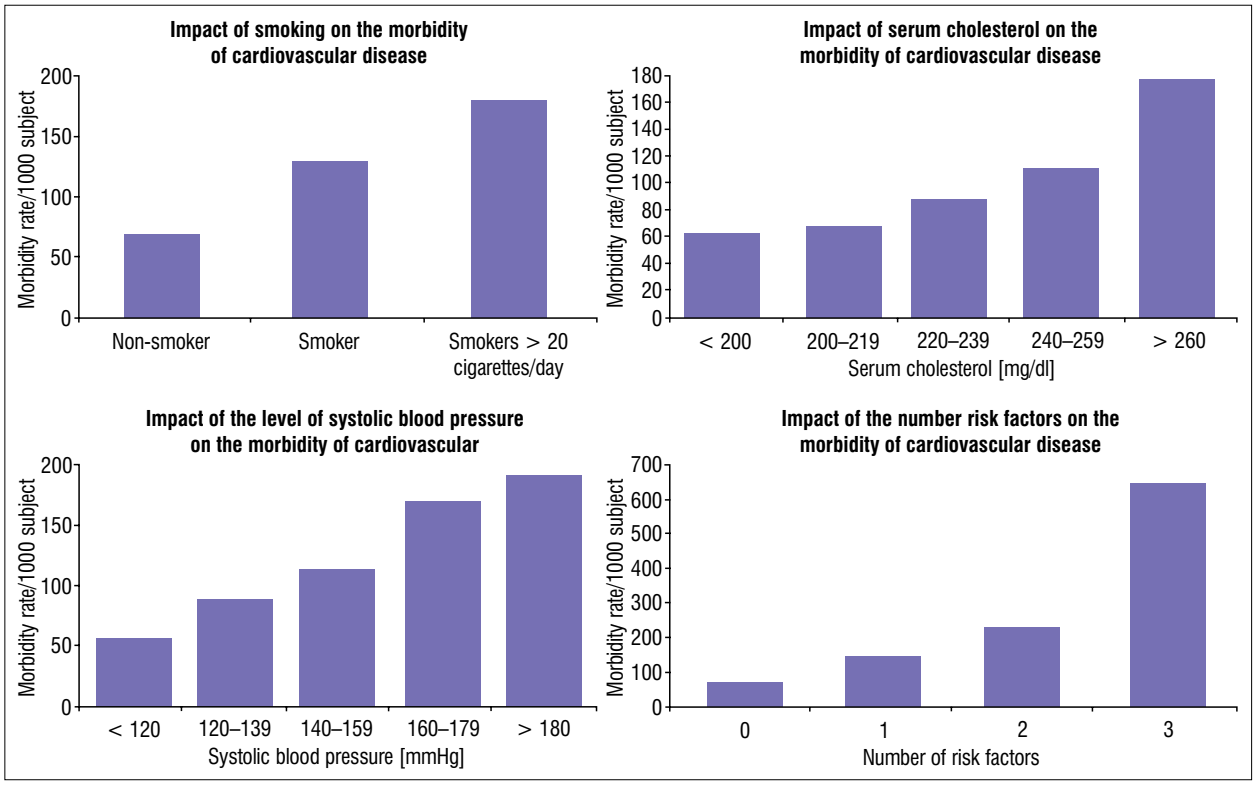

Figure 3. Morbidity of ischemic heart disease depending on the number of cigarettes smoked, serum cholesterol, level of systolic blood pressure and the number of risk factors — *Framingham Heart Study*

osclerosis was Nikolai Aniczkov. In 1913, he showed that the use of a cholesterol-rich diet in a rabbit for a dozen or so weeks leads to atherosclerotic lesions. In 1874, Joseph Goldstein and Michael Brown described the mechanism of cholesterol uptake by the cells at the participation by the $apoB_{100}$/apoE receptor. Another important event in the history of atherosclerosis research was Russell Ross's demonstration that the key to atherosclerosis is the interaction of monocytes/macrophages with vascular endothelium (modified theory of uniform response to injury). Macrophages under the vascular endothelium remove cholesterol, transforming into foam cells. Foam cells produce, among others, extracellular matrix metalloproteinases (MMPs) that damage the vascular endothelium [11]. Atherosclerotic lesions are classified according to AHA (American Heart Association) recommendations [12]. There are three types of atherosclerotic lesions that constitute the early phase of the atherosclerotic process. Type I is characterized by the presence of cholesterol-containing foam cells. The II type is characterized by an increase in the number of foam cells and the appearance of pathologically altered smooth muscle cells. Type III is characterized by extracellular lipid accumulation and the onset of fibrosis. Atherosclerotic changes begin in early childhood and even fetal life. It should be mentioned that the stages I–III are reversible [13]. Further accumulation of lipids in the vascular wall leads to the formation of atherosclerotic plaques. Lipids constitute 10–70% of the volume of atherosclerotic plaque. The construction of atherosclerotic plaque can be varied. In type IV, the surrounding enamel-sheath core consists only of the inner membrane. In type V, the atherosclerotic plaque is surrounded by collagen and smooth muscle cells. In addition, type V is divided into subtypes. The Va subtype is characterized by a pronounced lipid core, the Vb subtype is characterized by calcifications, while the Vc subtype is characterized by a lack of the core and a large amount of connective tissue. Atherosclerotic plaques type IV and V were found in 20% of men aged 30–34 [14]. Type VI is an unstable atherosclerotic plaque (damage of inner membrane) or atherosclerotic plaque that has burst. Types V and VI atherosclerotic plaque are irreversible [15]. Gradually enlarging atherosclerotic plaque does not cause clinical symptoms until the narrowing of light does not exceed 70–80% of the diameter of the vessel, which significantly reduces blood flow, eg to the myocardium — a stable example of angina pectoris. In contrast, atherosclerotic plaque rupture, most often at less than 50% of the vessel lumen, is responsible for rapidly occurring symptoms such as acute coronary syndrome or stroke [16]. In 79.5% of

patients with coronary artery disease, PAD is diagnosed. Among patients with PAD, atherosclerosis occurred in 52.2%, while atherosclerosis of the lower limbs in 68.5% of them [17]. The severity of the progression of atherosclerotic lesions depends on the number of risk factors. With age, thickening of the inner membrane occurs, which is caused by forces acting on the vessel (mainly shear stress). The dishes become thicker and have a higher average. Thickening of the inner membrane promotes the loss of vascular endothelial cell integrity which leads to increased migration of LDL and monocyte lipoproteins into the inner wall layer of the vessel. The accumulation of lipids and cells of the immune system in the vascular wall promotes the development of atherosclerosis [18, 19].

Biomarkers of atherosclerosis

Identification of classical biomarkers of atherosclerosis, such as LDL (low-density lipoprotein), HDL (high-density lipoproteins), and triglycerides may not be helpful in patients with moderate or unusual cardiovascular risk. For more accurate management, non-classical atherosclerosis biomarkers may be helpful in these patient groups. Biomarker (indicator) of high clinical value should be characterized by high sensitivity, high repeatability of results and the possibility of application in clinical practice (low cost of laboratory mark) [7]. Biomarkers of atherosclerosis are characteristic of its particular stages. These include markers of the inflammatory process, markers of atherosclerotic plaque injury, acute phase proteins, ischemic markers, markers of tissue necrosis and markers of myocardial dysfunction.

Biomarkers of the inflammatory proces

Interleukin 6 (IL-6)

One of the most important and most multi-functional interleukins. Together with TNF-α (tumour necrosis factor α), it belongs to pro-inflammatory cytokines. IL-6 is mainly produced by monocytes and macrophages. IL-6 levels increase with age and are associated with increased mortality in nondisabled persons over age 65 from both cardiovascular and non-cardiovascular events [20]. IL-6 is elevated in coronary heart disease patients and may be a biomarker of inflammation related to cardiovascular risk. IL-6 is involved in the formation of atherosclerotic plaques [21–23].

Myeloperoxidase (MPO)

Myeloperoxidase is a biomarker of both the inflammatory process and the destabilization of atherosclerotic plaque. MPO is produced by neutrophils and macrophages. Participates in the antimicrobial and antiviral reactions through the production of hypochlorous acid (HOCl). Myeloperoxidase also participates in the oxidation of LDL lipoproteins (oxLDL). Oxidized LDL are highly atherogenic. Higher MPO activity in the blood of patients with ischemic heart disease was demonstrated in comparison to healthy people. In addition, plasma MPO levels have been shown to be higher in patients with acute coronary syndrome [24].

Tumour necrosis factor α (TNF-α)

Tumour necrosis factor α is the most important cytokine that mediates effector pathways in both inflammatory disease target tissues and in atherosclerotic vessels. In addition, TNF-α is involved in the formation and enlargement of atherosclerotic plaques. TNF is an inhibitor of eNOS (endothelial nitric oxide synthase), increases the production of reactive oxygen species and reduces the effect of EDHF (endothelium-derived hyperpolarizing factor). These mechanisms lead to impairment of vasodilatation and damage to the vascular endothelium due to the severity of inflammation [25]. Is a biomarker of increased cardiovascular risk [26]. It has been shown that the concentration of TNF-α in the blood increases with age. Elderly people who had higher levels of TNF-α in the blood more often had clinically diagnosed atherosclerosis [27].

Matrix metalloproteinase 9 (MMP-9)

Metalloproteinases are a very large family of calcium-dependent, zinc-containing endopeptidases. MMP-9 is associated with inflammation and destabilization of atherosclerotic plaque. MMP-9 increases the infiltration of monocytes under the vascular endothelium. In addition, there is a positive correlation between the concentration of MMP-9 in the blood and the size of the lipid core and the risk of atherosclerotic plaque rupture [28]. Patients with unstable angina and NSTEMI (non-ST-elevation myocardial infarction) have increased levels of MMP-9 in the blood [29]. A positive correlation was found between the increased concentration of MMP-9 in the blood and the occurrence of myocardial infarction or stroke. However, it was not found that the elevated concentration of this metalloproteinase was a strong and independent cardiovascular risk factor [30].

Intercellular adhesion molecule 1 (ICAM-1; CD54) and vascular cell adhesion protein 1 (VCAM-1; CD106)

The most important for the development of atherosclerosis are ICAM-1 and VCAM-1. The presence of these adhesive molecules has been demonstrated in vessels predisposed to the development of atherosclerosis and within existing atherosclerotic lesions [31]. The ligand for ICAM-1 is LFA-1 (lymphocyte function-associated

antigen 1), which occurs on all types of leukocytes. The ligand for VCAM-1 is VLA-4 (very late antigen-4), found on monocytes and lymphocytes. Adhesion molecules are involved in the first stage of leukocyte penetration into the vascular endothelium - rolling. The interaction between ICAM-1 and LFA-1 and VCAM-1 and VLA-4 is supported by PECAM-1 (platelet endothelial cell adhesion molecule 1). PECAM-1 is located on the surface of vascular endothelial cells at sites affected by the inflammatory process, therefore the migration of leukocytes under the vascular endothelium is intensified in the vascular regions where the process of inflammation occurs [32]. Increased expression of ICAM-1 and VCAM-1 has been shown to be an indicator of subclinical atherosclerosis [33].

C-reactive protein (CRP)

C-reactive protein is an acute phase marker. CRP is a biomarker of inflammation and destabilization of atherosclerotic plaque. CRP is produced mainly in hepatocytes as a result of IL-6 stimulation. It belongs to the pentraxin family. It can be used for the early identification of high cardiovascular risk patients. The FDA (Food and Drug Administration) recommends the determination of CRP concentration in the blood using the high sensitivity method (hsCRP, high-sensivity CRP). An increase in CRP concentration in blood above 10 mg/l indicates an inflammatory proces [34]. The increase in CRP in the blood leads to: activation of the complement system, LDL oxidation, increased LDL to macrophages, reduction of NO (nitric oxide) production, stimulation of TF (tissue factor) secretion by macrophages, increase in the concentration of adhesion molecules, increase of blood clotting due to the increase of PAI gene expression -1 (plasminogen activator inhibitor-1). CRP also stimulates the production of IL-12 and INFγ (interferon γ) [34]. CRP is an important indicator of the risk of acute coronary syndrome without ST segment elevation [35].

Growth/differentiation factor 15 (GDF15)

Growth factor produced by macrophages, cardiomyocytes and endothelial cells in response to the inflammatory process. It can be a potential biomarker in the stratification of cardiovascular risk. The GDF-15 concentration below 1200 ng/l corresponds to the low cardiovascular risk (upper limit of reference values for healthy people), 1200-1800 ng/l — moderate (average) CVD risk, and exceeding 1800 ng/l — high CVD risk [36]. The increase in GDF-15 levels is accompanied by atherosclerosis, atrial fibrillation, heart failure, pulmonary embolism, acute inflammation, renal failure and also some cancers [37]. The increased concentration of GDF-15 in response to inflammation aims to reduce the inflammation in the myocardium, its pathological remodelling and apoptosis of cardiomyocytes [38].

Fibrinogen

Fibrinogen is an acute-phase protein produced by the liver. It can be used to assess cardiovascular risk in patients with atypical cardiovascular profile [39]. It has been shown that fibrinogen concentration is higher in people with cardiovascular system diseases compared to healthy people. In addition, it has been found that determining fibrinogen levels in the blood may be useful in assessing the risk of thrombosis. Fibrinogen increases plasma viscosity, increases blood coagulation (through increased fibrin and increased aggregation of platelets), increases inflammation [40].

Uric acid

Uric acid is the final metabolite of purine bases. Numerous epidemiological studies have shown that increased uric acid levels in the blood are an independent risk factor for cardiovascular disease [41]. Other studies suggest that hyperuricemia is not a risk factor of CVD but is a complication of CVD (obesity, the use of diuretics, hypertension, insulin resistance) [42]. Further clinical trials are needed to assess the clinical usefulness of the determination of uric acid levels in the blood.

Lipoprotein (a) — Lp (a)

Lipoprotein (a) is a modified LDL lipoprotein by attaching a specific apolipoprotein (a) to apoB100 [43]. Increased plasma Lp (a) is a genetically determined, independent, causative risk factor for cardiovascular disease. The physiological functions of Lp (a) include wound healing, promoting tissue repair and vascular remodelling. Like other lipoproteins, Lp (a) is also susceptible to oxidative changes, leading to extensive formation of proinflammatory and proatherogenic oxidized phospholipids, oxysterols, oxidized lipid-protein adducts in Lp (a) molecules that consolidate the progression of atherosclerotic lesions and intimal thickening by induction of M1 macrophages, inflammation, autoimmunity and apoptosis [44]. In a prospective cohort study by Zhang et al. showed that elevated plasma Lp (a) concentration was a factor increasing the risk of stroke in adult Chinese [45]. Sadkowska et al. [46] on a group of 142 men conducted a study to determine the usefulness of measuring Lp (a) and homocysteine in plasma. The subjects were divided into 4 groups depending on the number of pathological changes in the coronary vessels. Both Lp (a) and homocysteine have been shown to be elevated in patients with coronary artery disease. In their conclusions, the authors state that routine determination of homocysteine in patients with signs of coronary heart disease and determination of lipoprotein (a) in people

with a positive family history of cardiac disease may be diagnostically and clinically useful.

Biomarkers of destabilization of atherosclerotic plaque

Oxidized low-density lipoprotein — (oxLDL) and anti oxidized low-density lipoprotein antibody — anti oxLDL antibody

Oxidized low-density lipoprotein (oxLDL) is of key importance in the pathogenesis of atherosclerosis and the pathophysiology of major cardiovascular and brain events [47]. The oxLDL molecule acts as an antigen leading to the production of anti oxLDL antibodies [48]. Autopsy studies have shown that increased levels of oxLDL increase the risk of atherosclerotic plaque rupture [49]. In addition, many studies have shown that an increase in blood oxLDL levels positively correlates with the risk of developing coronary artery disease and worsens the prognosis in such patients [50]. LDL oxidation can affect different components of its molecule, which is why different anti-oxLDL antibodies can be formed. IgM anti-oxLDL antibodies have been shown to reduce the risk of severe coronary artery disease. In the case of IgG anti-oxLDL class, this compound is more complex and requires further research [50].

Soluble CD40 ligand (sCD40L)

Protein — CD40 receptor-ligand. It is mainly produced by cells of the immune system, vascular endothelial cells, vascular walls and epithelium. The CD40 receptor is found on B lymphocytes, macrophages, vascular endothelial cells and myocytes. Separation of sCD40L leads to the production of MMPs and destabilization of the atherosclerotic plaque. Increased sCD40L concentration was demonstrated in patients with myocardial infarction and unstable ischemic heart disease [51]. OPUS-TIM116 Study showed higher blood sCD40L concentration compared to the control group (0.78 μg/l *versus* 0.52 μg/l) [52]. In addition, it has been shown that increased sCD40L concentration in the blood occurs in patients with psoriasis [53]. The role of a CVD biomarker is unclear [7].

Placental growth factor (PlGF)

PlGF is a growth factor that belongs to the family of EGF (endothelial growth factor). It plays an important role in the pathogenesis of atherosclerosis by stimulating angiogenesis and increasing the migration of monocytes and macrophages into the vascular endothelium. As a biomarker, it can be important in assessing cardiovascular risk among overweight children, obesity or metabolic syndrome. It has been shown that children with excessively increased body weight and with the metabolic syndrome have higher PlGF levels in the blood compared to healthy children. In addition, a positive correlation was found between PlGF concentration in the blood and troponin concentration in the blood [54].

Pregnancy-associated plasma protein A; pappalysin 1 (PAPP-A)

The protein produced by the placenta. It is a metalloproteinase. Probably PAPP-A is involved in the destruction of the fibrous cap of the plaque leading to its destabilization. Increased levels of PAPP-A have been demonstrated in patients with myocardial infarction, unstable ischemic heart disease and in patients who died suddenly due to CVD [55]. The CAPTURE Study showed that the increase in blood levels of PAPP-A in patients with acute coronary syndromes is of unfavourable prognostic importance. As of today, there are technical problems in the performance of PAPP-A assays, because it occurs in the heterotetrameric form (in pregnant women) and homodimeric (in patients with acute coronary syndromes). PAPP-A may be used as a marker of death risk in acute coronary syndromes and identification of atherosclerotic plaques susceptible to rupture [56].

MicroRNA (miRNA)

MicroRNAs are short segments of RNA containing 18–25 nucleotides. They arise in the process of transcription of intron (non-coding) sequences and exon (encoding) sequences. Probably the expression of 1/3 of human genes is regulated by miRNA. miRNAs are involved in the pathogenesis of many diseases [57]. Selected miRNAs involved in the development of the atherosclerotic process are presented in the Table 1.

The biomarkers of athersclerotic plaque destabilization also include the previously described MPO, MMP-9 and CRP

Interestingly, an increase in atherosclerotic destabilization has been observed in patients with active ankylosing spondylitis. Increased blood levels of sCD40L and PlGF were observed in these patients. Importantly, these patients were not burdened with classic cardiovascular risk factors [59]. Whole-body cryotherapy decreases the levels of Inflammatory, oxidative stress, and atherosclerosis plaque markers in male patients with active phase ankylosing spondylitis in the absence of classical cardiovascular risk factors [60]. Chronic inflammatory state in these patients cause increased cardiovascular and cerebrovascular mortality [61].

Table 1. Characteristics of selected microRNA involved in the development of atherosclerotic process [based on 58]

MicroRNA (miRNA)	Role in the atherosclerotic process
miRNA-126	Inhibition of VCAM-1
miRNA-155, -222, -424, -503, -9, -17, -20a, -106a	Regulation of monocyte differentiation into macrophages in atherosclerotic plaque
miRNA-147, -155, -342-5p	Activation of M1 macrophages, increase secretion of TNF-α and IL-6
miRNA-125a, -146a, -33, -155	Inhibition of lipid accumulation in atherosclerotic plaque
miRNA-15a, -16	Modulation of macrophage apoptosis
miRNA-21, miRNA-34a	Production of MMP-9, VSMC proliferation
miRNA-210	Tubulogenesis and stimulation of macrophage migration
miRNA-146a	The formation of the Th1 mononuclear phenotype
miRNA-29	Inhibition of elastin expression
miRNA-221/222	Stimulation of cell proliferation or apoptosis
miRNA-365	Stimulation of endothelial cells apoptosis
miRNA-100, -127, -145, -133a, -133b	High expression in symptomatic atherosclerotic plaques in carotid arteries

Biomarkers of thrombocyte activation

Lipoprotein-associated phospholipase A2 (Lp-PLA2) and secretory phospholipase A2 (sPLA2)

Lp-PLA2 is a protein also known as platelet-activating factor acetylhydrolase (PAF-AH). It is mainly produced by monocytes and macrophages. Lp-PLA2 has pro-inflammatory and atherogenic effects. Takes part in the oxidation of LDL lipoproteins. Increased Lp-PLA2 has been shown to increase cardiovascular risk. Inhibition of Lp-PLA2 (by the Darapladib) has a positive effect on the risk of CVD, therefore Lp-PLA2 may become a future target for pharmacotherapy [62]. sPLA2 is the phospholipase A2 isozyme. It is produced by inflammation cells in the atherosclerotic plaque and by ischemic cardiomyocytes. The relationship between sPLA2 concentration and CVD risk was demonstrated [63].

The biomarkers of thrombocyte activation are also described sCD40L.

Biomarkers of neurohormonal activation

Copeptin

Copeptin is the C-terminal peptide of pre-provasopressin. It is produced by brain cells. After transport from the hypothalamus to the pituitary gland and pre-provasopressin cleavage, the copeptin is released into the circulation in stoichiometric amounts along with vasopressin. Both neuropeptides are mainly secreted in response to hemodynamic or osmotic changes. Copeptin is more stable in blood than vasopressin, which is why it has been used in laboratory diagnostics. It has been shown that the increased concentration of copeptin in blood positively correlates with the risk of developing coronary heart disease and the risk of death due to CVD [64].

Midregional proadrenomedullin (MR-proADM)

It is produced by the adrenal medulla, the heart and vascular endothelial cells. It is a promising biomarker of the risk of developing coronary heart disease and heart failure. In addition, MR-proADM may be a prognostic biomarker after STEMI (ST-Elevation Myocardial Infarction) [65].

Biomarkers of shear stress in the vascular endothelium

Shear stress biomarkers include various miRNAs. miRNA-143 and miRNA-145 change the phenotype of vascular smooth muscle cells to contractile. miRNA-126-5p limits the proliferation of vascular endothelial cells, whereas miRNA-92a enhances the development of inflammatory processes in the vascular wall [58].

Biomarkers of blood vessel microcalcification

Microcalcification of the atherosclerotic plaque increases the risk of its rupture. miRNA-125b is involved in the differentiation of vascular smooth muscle cells into osteoblasts that build up calcium in the atherosclerotic plaque [58].

Changes in the structure and function of blood vessels with age

The structure and function of blood vessels change with age. These changes include a reduction in the number of elastic elements, such as collagen and elastin [66]. The blood vessels are becoming more rigid, which results in an increase in the speed of the pulse wave and its amplitude. The reflected pulse wave from the arterioles increases the afterload of the left ventricle, which leads to increased systolic blood pressure. The increase in afterload results in the enlargement of the left ventricle and decrease in coronary flow leading to heart failure. The lack of return of the pulse wave during diastole leads to a decrease DBP (diastolic blood pressure). An increase in SBP and a decrease in DBP leads to an increase in pulse pressure. The pulse pressure (SBP – DBP) increases after 50–60 years due to the structural changes described above. A positive correlation was found between pulse pressure and cardiovascular risk. An increase pulse pressure > 63 mm Hg dramatically increases the risk of CVD. It is worth noting that the increase in heart rate with age can be prevented through regular physical activity [67, 68]. With age, there is a reduction in nitric oxide production while increasing the production of endothelin-1. Endothelin-1 has a strong vasoconstrictive, proinflammatory and prooxidative activity. In addition, during the aging of the body, the production of proteins and pro-inflammatory cytokines, such as CRP, IL-6, IL-8, TNF-α, increases. All these changes lead to an increase in the progression of atherosclerotic lesions (Fig. 4.) [69, 70].

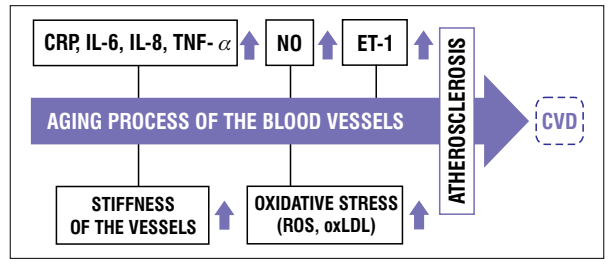

Figure 4. Structural and functional changes of blood vessels with age [on based 19, 66–70]. CRP: C-reactive protein; IL-6: interleukin 6; IL-8: interleukin 8; TNF-α: tumor necrosis factor α; NO: nitric oxide; ET-1: endothelin 1; ROS: reactive oxygen species; oxLDL: oxidazed low-density lipoprotein; CVD: cardiovascular diseases

The influence of statins and PCSK-9 inhibitors on atherosclerotic plaque

Statins reduce cardiovascular risk by reducing LDL cholesterol and pleiotropic effects. The most effective are the so-called strong statins (rosuvastatin, atorvastatin) [71]. Pleiotropic properties of statins include anti-inflammatory activity (decreased production of CRP, serum amyloid A, IL-6, IL-8, ICAM-1), lipid-lowering effect (reduction of endogenous cholesterol biosynthesis, increase in LDL receptors — $apoB_{100}$/apoE), anticoagulant (increase in NO production, increase in fibrinolytic activity, decrease in ET-1 production) and antioxidant activity (reduction of reactive oxygen species production, reduction of glutathione reduction, reduction of lipoprotein oxidation, reduction of NADPH oxidase activation, increased eNOS activity) [72]. Numerous studies have demonstrated the beneficial effect of statin therapy on reducing the progression of atherosclerosis. In the Kumbhani et al. study, it was concluded that statin therapy reduced the exacerbation of ischemia, the need for revascularization, the need for amputation. Statins restricted progression of PAD [73]. In addition, intensive statin therapy has been shown to reduce the volume of atherosclerotic plaque (by 7.69 mm^3) and increase the lumen of the vessel by 0.81 mm^3. Rosuvastatin more effectively reduces the volume of atherosclerotic plaque compared to atorvastatin [74]. Auscher et al. [75] showed that the use of statins leads to stabilization of the atherosclerotic plaque, and thus reduce the risk of its rupture.

Proprotein convertase of subtilisin/kexin type 9 (PCSK-9) was discovered in 2003. PCSK-9 is an inhibitor of the LDL lipoprotein receptor (LDL-R). PCSK 9 binds with LDL-R on the surface of liver cells and promotes degradation of LDL(R) in the liver cells by the lysozyme pathway and prevents its recycling back to the liver cell surface [76]. PCSK-9 inhibitors are human monoclonal antibodies (evolocumab, alirocumab). Phase I and II studies have shown that evolocumab reduces LDL levels by 40 to 80%, apolipoprotein B100 levels by 30–59% and apolipoprotein A by 18–36% [77–79]. In the GAUSS2 study, evolocumab showed a greater reduction in LDL compared to ezetimibe (56.1% versus 36.9%) [79]. The study of Stein et al. showed that the use of alicocumab in patients (n = 62) with hypercholesterolemia who had an initial LDL concentration of 140–170 mg/dl led to a reduction in LDL concentration by 29–68% [80]. PCSK 9 inhibitors have excellent lipid-lowering properties and beneficial effect on CV outcome [76]. According to the FDA (Food and Drug Administration), the indications for the use of PCSK-9 inhibitors are 1) patients suffering from homozygous familial hypercholesterolemia (HOFH) and heterozygous familial hypercholesterolemia (HeFH); 2) non-familial hypercholesterolemia patients who are severely statin intolerant (secondary prevention) and 3) as a research tool [76].

Table 2. Biomarkers of atherosclerosis — *resume*

Biomarkers of atherosclerosis			
Biomarkers of the inflammatory process	**Biomarkers of destabilization of atherosclerotic plaque**	**Biomarkers of thrombocyte activation**	**Biomarkers of neurohormonal activation**
IL-6	oxLDL	Lp-PLA2	Copeptin
MPO	antibody anti oxLDL	sPLA2	MR-proADM
TNF-α	sCD40L	sCD40L	
MMP-9	PIGF		
ICAM-1	PAPP-A		
VCAM-1	miRNA		
CRP	CRP		
GDF-15	MMP-9		
Fibrinogen	MPO		
Uric acid			
Lipoprotein (a)			
Biomarkers of shear stress in the vascular endothelium	**Biomarkers of blood vessel microcalcification**		
miRNA	miRNA		

Summary

The pathogenesis of atherosclerosis is very complex. Biomarkers of atherosclerotic lesions are often risk factors for its occurrence. These are proteins, enzymes, microRNAs and others (Fig. 5). There are many modern biomarkers that can become routine in the laboratory diagnosis of CVD in the future. Modern pharmacotherapy allows limiting the progression of the atherosclerotic process by affecting all its stages — from increasing the LDL cholesterol to the atherosclerotic plaque rupture. Further research will provide further information on the pathogenesis of atherosclerosis, and as a result, new risk factors and biomarkers will be recognized.

Conflict of interest:

None.

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