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The formation of atherosclerotic plaque, its destabilisation and diagnostics

Tworzenie blaszki miażdżycowej, jej destabilizacja i diagnostyka

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Summary

According to the established medical knowledge, the atheromatous lesions occur in the arteries of large and medium diameter. Their presence in the aorta, arteries of extremities as well as extracerebral and coronal arteries is clinically relevant. The evolution of atherosclerotic plaques probably starts in the prenatal development, what may be proved by the presence of the fatty streaks in endothelium of coronal arteries in some newborns. Then it evolves through lipid accumulation, media inflammatory response, *vasa vasorum* proliferation, fibrination and calcification of plaques. Researches proved that the matter of atherosclerosis is exaggerated inflammatory proliferative reaction to the arterial wall damage. The oxidative stress phenomenon and infections with common pathogens play an undoubtful role in this process. Ultimately the direct damage is an effect of immune response cells infiltration and secretion of cytokines and proinflammatory factors. Among the cells of immune system responsible for formation and development of atheromatous plaque are considered: macrophages, dendritic cells, T and B lymphocytes, monocytes. Attention was also paid to the inflammatory mediators and growth factors. Scientist are interested in unstable atherosclerotic plaque and accompanying inflammatory process within the artery wall for a long time. Meanwhile, there are conducted researches on inflammation markers underlying the destabilisation of plaques. Revealing the role of these cells in evolution of atherosclerosis would enable more complex understanding of the mechanism of lesions development. Then it would facilitate an introduction of the new and upgraded methods of treatment and prevention. Also the progress of imaging examinations is meaningful for diagnostics and treatment. It is contributory to the choice of therapeutic strategy and assessment of surgical intervention urgency. In the clinical practice there are recognized standards of imaging the morphology of atheromatous plaque. Development of diagnostics aims the indirect assessment of possible dynamics of lesions progression. Targeting the complex plaque analysis is based on excellence of established standards such as ultrasound examination or computed tomography.

Key words: atherosclerosis, atheromatous/atherosclerotic plaque, inflammation, mediator, immune response cells, unstable plaque, diagnostics, imaging

Streszczenie

Według powszechnej wiedzy medycznej zmiany miażdżycowe dotyczą naczyń tętniczych dużego i średniego kalibru. Kluczowe kliniczne znaczenie ma ich powstawanie w aorcie i tętnicach kończyn dolnych, tętnicach domózgowych czy tętnicach wieńcowych. Ewolucja zmian miażdżycowych rozpoczyna się prawdopodobnie już w życiu płodowym, czego dowodem może być istnienie u niektórych noworodków pasm tłuszczowych (*fatty streaks*) w śródbłonku naczyń wieńcowych. Obejmuje ona kolejno etapy gromadzenia lipidów, odpowiedź immunologiczną błony środkowej, proliferację *vasa vasorum*, włóknienie oraz wapnienie blaszek. Badania naukowe wykazały, iż istotą miażdżycy jest nadmierna zapalno-proliferacyjna odpowiedź na uszkodzenie ściany tętnicy. Niekwestionowaną rolę w tym procesie odgrywają zjawisko stresu oksydacyjnego oraz infekcje

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powszechnie występującymi patogenami. Jednak bezpośrednie uszkodzenie jest efektem napływu komórek odpowiedzi immunologicznej oraz wydzielanych przez nie czynników zapalnych. Wśród komórek układu immunologicznego zaangażowanych w proces tworzenia i rozwoju blaszki miażdżycowej na szczególną uwagę zasługują m.in. makrofagi, komórki dendrytyczne, limfocyty T i B oraz monocyty. Zwrócono również uwagę na mediatory zapalne i czynniki wzrostu. Od dawna naukowcy zainteresowani są niestabilną blaszką miażdżycową i związanym z nią toczącym się procesem zapalnym w obrębie ściany naczynia. W chwili obecnej trwają poszukiwania markerów zapalnych podłoża destabilizacji blaszek miażdżycowych. Poznanie roli tych komórek w procesach rozwoju miażdżycy w przyszłości pozwoliłoby na szersze oraz dogłębne zrozumienie mechanizmu powstawania blaszek miażdżycowych. To z kolei daje możliwość szybkiego wprowadzenia nowych i udoskonalonych metod leczenia tej choroby lub spowalniania jej rozwoju. Nie bez znaczenia dla diagnostyki i leczenia pozostaje także rozwój badań obrazowych. Umożliwia on przede wszystkim wybór strategii terapeutycznej i ocenę pilności interwencji chirurgicznej. Dotychczas podstawową rolę odgrywały badania określające hemodynamiczną istotność zmian. W praktyce klinicznej funkcjonują ugruntowane standardy obrazowania morfologii blaszki miażdżycowej. Celem diagnostyki jest jednak pośrednie określanie możliwej dynamiki jej zmian. Dąży się do coraz bardziej wnikliwej analizy zmian, doskonaląc takie uznane metody, jak ultrasonografia czy tomografia komputerowa.

Słowa kluczowe: miażdżyca, blaszka miażdżycowa, zapalenie, mediatory, komórki układu immunologicznego, niestabilna blaszka miażdżycowa, diagnostyka, obrazowanie

FORMATION OF ATHEROSCLEROTIC PLAQUE

Acute cardiovascular incidents, i.e. sudden cardiac death, myocardial infarction, ischaemic brain stroke and many others, develop as a result of the stenosis of atherosclerotically altered artery supplying the respective vascular bed[1]. Long-lasting studies indicate that only a small percentage of sudden cardiovascular incidents is triggered by such mechanism, and only 60% result from intraluminal changes associated with sudden rupture of atherosclerotic plaque or its ulceration[2–6]. It is of crucial importance to carefully recognise the mechanisms responsible for the formation of atherosclerotic plaque (fig. 1) and the cells involved in this process.

Previous studies demonstrated that it is a immune-inflammatory process that plays a key role in the development of atherosclerosis[7,8]. The basic role in the development of such changes is attributed to the endothelial dysfunction. The following factors are known to damage the endothelium: oxidative stress associated with arterial hypertension, diabetes mellitus and hypercholesterolemia, elevated concentration of free radicals or toxins released in patients smoking tobacco[9,10]. It is currently well established that bacterial and viral infections, caused most commonly by *Helicobacter pylori*, *Chlamydia pneumoniae*, herpesviridae, cytomegalovirus, mycobacteria, initiate the atherogenesis[9,11–15]. A very well understood role in the development of arterial atherosclerotic lesions is attributed to the lipid accumulation within the vascular endothelium, which results in the increased endothelial permeability. It leads to lipid modifications, migration and proliferation of myocytes and blood inflammatory cells (T lymphocytes, macrophages) as well as to the production of proinflammatory cytokines[9,16].

The structure of primary atherosclerotic lesions consists of lipids accumulated in the intima and phagocytic cells, i.e. peripheral blood macrophages and myocytes from the media. Activated myocytes synthesise the extracellular compounds of the connective tissue (i.e. collagen, proteoglycans, elastin), which subsequently accumulate within the plaque. It leads to the formation of lesions with connective tissue fibres dominating over lipid compounds, among which cholesterol crystallises. It results in the formation of fibrous cap, consisting of collagen type I and III. The central part of the atherosclerotic lesion is composed of lipid and destroyed-cells-containing elements (white, fatty-fibrous, fibrous plaques). The structure of such plaque is considered **stable**. They are formed in the period of 20–30 years. Such plaque grows into the lumen of the vessel, leading to its gradual stenosis and blood flow impairment[17].

Sudden circulatory dysfunction is most commonly associated with the phenomena resulting from **atherosclerotic plaque instability**. Such lesions are formed mainly due to the intensification of inflammatory process taking place within the vessel wall[17]. Characteristic features of such plaque include greater vascularisation, thinner and more rupture-prone fibrous cap with increased component of inflammatory cells (macrophages, T lymphocytes). The lipid core becomes spacious and contains more and more of liquid cholesterol esters[18–21]. The abnormal and in-growing vessels are the main source of haemorrhage into the plaque and its neighbourhood, which in consequence leads to plaque rupture[10]. The significant role in the plaque destabilisation is attributed to the IFN-γ, which deactivates myocytes, inhibit their proliferation and collagen synthesis. It concomitantly stimulates macrophages, which produce metalloproteinases (MMP), responsible for the degradation of connective tissue structures.

The fibrous cap becomes thinner and thinner[9,19]. The T lymphocytes infiltrating the plaque stimulate macrophages to produce MMP and lead to the formation of unstable atherosclerotic plaques. IFN-γ, secreted by activated lymphocytes, intermediates in these phenomena[10,22,23]. The experimental studies reveal that most of such lymphocytes is chronically activated[24].

The population of CD4+CD28$^-$ lymphocytes, which is found only within the unstable atherosclerotic plaques, is present in patients with instable coronary disease. These cells play a significant role in the **destabilisation** process. It is associated with the uncontrolled secretion of excessive IFN-γ, which stimulates the plaque-infiltrating macrophages. These cytotoxic cells directly damage the endothelial cells via secreted perforins. These are proteins, which inserted into the plasma membrane form the canals enabling the inflow of sodium ions (Na^+) and water particles into the cell and in the consequence lead to its destruction[25,26]. The presence of helper lymphocytes (CD4+) within the atherosclerotic plaque prove that the inflammatory process taking place in the vessel wall is not only the cause of endothelial damage, but also the result of immunological reaction being a response to a specific antigen[9,10]. It was demonstrated that the activated macrophages and myocytes present in the plaque demonstrate the increased expression of MHC-II proteins. They are capable of presenting antigens to the above-mentioned lymphocytes. The lymphocytes activation entails the secretion of proinflammatory cytokines and intensification of inflammatory response taking place in the vessel wall[27,28]. Apart from focal effect, the proinflammatory cytokines secreted in the atherosclerotic lesion play a systemic role as well. They stimulate the liver to produce inflammatory markers (CRP, fibrinogen, serum amyloid protein)[19]. Their concentration reflect the intensity of the inflammatory process[13,29].

The formation of atherosclerotic lesions is significantly influenced by heat shock proteins (Hsp). These proteins, which are produced by human and microorganism, are normally present inside the mitochondria. They are present in the endothelial cells of the blood vessels, whereas the endogenous Hsp were also found on the surface of the cells. The presence of *Chlamydia pneumoniae*-Hsps was additionally confirmed in the macrophages extracted from human atherosclerotic plaques[13,29].

DESTABILISATION OF THE ATHEROSCLEROTIC PLAQUE

Atherosclerosis is not limited to one organ or vascular bed only, as it affects entire arterial bed, i.e. large and medium-calibre vessels. The mechanism taking part in the plaque destabilisation process are similar in various regions[30–32]. The unstable atherosclerotic plaque is so-called rupture-prone. The rupture triggers the formation of mural thrombus, which occludes the arterial lumen. It leads to circulation deficiency in such place, which results in acute ischaemia of the tissue supplied by the respective artery[33,34]. Another cause for acute circulation deficiency is an embolus originating from the newly formed mural thrombus[35,36]. The increased MMP activity was demonstrated in the plasma of patients with unstable plaques in the carotid arteries. The evaluation of plasma activity of such enzymes could serve as an instability marker of atherosclerotic plaques[37].

Many studies revealed the association between the inflammation and plaque destabilisation in the extracranial arteries. The close correlation was found between the elevated level of inflammatory markers, such as CRP, α1-antitrypsin, fibrinogen, orosomucoid, and the risk of acute vascular incidents, including ischaemic brain strokes[38,39]. The isolated LDL-cholesterol elevation does not pose a risk for brain stroke, yet it is significantly increased by the concomitant presence of hypercholesterolemia and elevated concentration of inflammatory markers[40]. High concentration of serum homocysteine was observed to be one of the independent risk factors for ischaemic stroke, promotes the formation of unstable lesions due to increased secretion and activation of MMP[41].

There are currently many **methods** available that enable the visualisation and evaluation of atherosclerotic plaques in the extracranial vessels. The Doppler ultrasound, new diagnostic modalities, such as optical coherent tomography or magnetic resonance tomography with modern contrast containing iron oxide (ultrasmall superparamagnetic iron oxide, UPSIO), tend to become a reliable imaging modalities of atherosclerosis within the arterial wall. Such methods allow for the detailed evaluation of plaque morphology and inflammatory process as well[42,43].

Despite the fact that many studies were carried out concerning the features of the structure of atherosclerotic

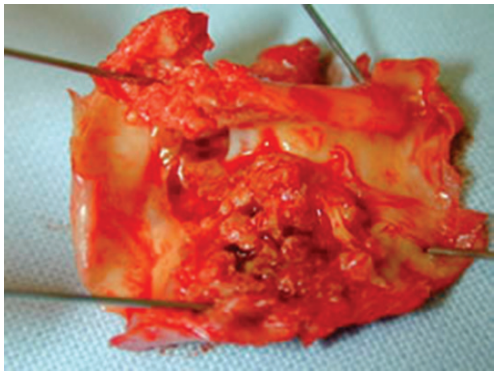

Fig. 1. The unstable atherosclerotic plaque removed from the internal carotid artery. Department of Vascular, General and Oncologic Surgery, Copernicus Memorial Hospital, Lodz, Poland

plaque, which influence their instability, we still do not know, which plaques demonstrate higher risk of causing ischaemic lesions and neurologic symptoms. The recognition of the role the inflammatory process plays in the pathogenesis of atherosclerosis and destabilisation of atherosclerotic lesions as well as the determination of the serum inflammatory markers of plaque destabilisation would pose a significant completion of imaging modalities in the diagnostic of unstable plaques. It seems that the CRP is the best known marker[38]. Other proteins, such as P-selectin, IL-6, IL-12, MMP and lipoprotein A are being subject of clinical studies[44].

MORPHOLOGIC STRUCTURE OF THE ATHEROSCLEROTIC PLAQUES INCLUDING ITS DIVISION ACCORDING TO TYPE

The fully formed atherosclerotic plaque consists of connective tissue elements, i.e. elastin, collagen, proteoglycans, which are produced mostly by smooth muscle cells and form a plaque framework[45]. Cellular composition and plaque morphology are the most important factors influencing the patient's clinical status, more significant than the stenosis grade itself[46].

Human atherosclerotic plaques were classified by American Heart Association – Committee on Vascular Lesions in 1994 and 1995 according to the characteristic morphologic, structural, histochemical, histological and ultrastructural features[47,48]. This classification was revised in 2000[49].

First three types of lesions are asymptomatic, which can initiate the formation of more advanced atherosclerotic lesions as disease progresses. At first they are of focal nature, cause the damage to the intima and media. Their common feature is the abnormal accumulation of cholesterol esters and lipoproteins as well as the increase in the number of cells, mainly lipid-containing macrophages. From the histological point of view, the atherosclerotic lesions as type IV, V, VI, VII and VIII are characterise by lipid, cell and mineral accumulation, which leads to structure disorganisation and initiate reparative processes and thickening of the intima.

The formation of the plaque was divided into the following phases: I – primary, early lesions, which can undergo fibrosis (phase II); III – the formation of thrombus or haematoma; IV – the forming thrombus leads to the vessel occlusion, which results in acute coronary incident; phase III and IV plaques can undergo fibrosis, reaching phase V, resulting in the vessel stenosis or occlusion. Type I and II lesions are observed in infants and children. Type III lesions appear during the puberty. Type IV plaques are found in patients in their third decade of life, whereas type V and VI are observed in mid-aged adults and elderly[48].

ATHEROSCLEROTIC PLAQUE MINERALISATION

The calcification is the characteristic feature of atherosclerotic plaque, considered until recently as a passive, incurable and unavoidable process, associated with the advanced age. The studies of the recent years demonstrated, however, that calcification is an active phenomenon in fact, regulated and organised[50,51], in which the calcium phosphate (hydroxyapatite), accumulating in the vessel wall, is observed in the phases of formation and remodelling of the bones as well[52]. Electron microscopy revealed the presence of so-called matrix vesicles in the atherosclerotic plaque, which form the nucleus of hydroxyapatite crystals[53,54], as well as proteins of bone matrix, such as Gla protein, type I collagen, osteonectin, osteopontin and osteocalcin[55–59]. It should be emphasised that the detailed role of each of these protein in the above-mentioned process is being permanently under investigation.

The mineralisation of the atherosclerotic plaque has two morphological variants[60,61]. The first one consists of point-shaped calcifications within the inflammatory plaque consisting of lipids and macrophages[47]. In the second variant, the area of calcification within the atherosclerotic plaque reflects the area of connective tissue formation by SMC (structural maintenance of chromosomes)[62,63]. Calcium concernments bind together into the larger formations, which makes them visible in the radiological examinations and leads to vessel stenosis[64]. Arterial wall calcifications are observed as focal infiltration localised within the atherosclerotic plaques. Both the acute, and the chronic inflammation intensify the arterial calcification.

IMMUNE SYSTEM CELLS INVOLVED IN ATHEROSCLEROSIS

Immune system (IS) cells, that are present in the atherosclerotic plaques and secrete growth factors and cytokines, are responsible for the formation of the plaque. Recent studies indicate that the following IS cells play the significant role in the development of atherosclerosis: monocytes, macrophages, mast cells, lymphocytes T and B, dendritic cells and progenitor cells. The latter features the ability to differentiate into various cell types[65,66]. As the latest studies emphasise the formation of atherosclerotic lesions may be attributed to the leukocyte populations, which accumulate in the various phases of plaque development[66]. Arterial sclerosis is in fact the chronic inflammation. Therefore a critical issue is to recognise the contribution each cell type makes into the course of atherosclerosis[65].

PROGENITOR CELLS AND ATHEROSCLEROSIS

Circulating progenitor cells contribute to the development of atherosclerosis[67]. Two types of progenitor

cells are distinguished in the peripheral blood of human and mice: endothelial progenitor cells (EPC) and smooth muscle progenitor cells (SPC)[67,68]. More studies were focused on EPC, identifiable in the bone marrow, blood and adventitia[69,70]. The following factors trigger the secretion of EPC from the bone marrow: erythropoietin, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF)[69,71,72].

SPC stemming from the bone marrow as well, contribute to the formation of the intima and stabilise the atherosclerotic plaque. SPCs migrating to the atherosclerotic plaque prevent the development of their instability and rupture of the fibrous cap. The number of circulating SPCs increases in the patients with acute coronary incident, therefore such cells play a protective role during the formation of atherosclerotic plaques[73–76].

GRANULOCYTES AND ATHEROSCLEROSIS

Granulocytes migrate to the arteries affected by the chronic inflammation and secrete proinflammatory mediators, which stimulates the growth of atherosclerotic plaques. It was demonstrated that the products secreted by the neutrophil granulocytes contribute to the attraction of macrophages to the atherosclerotic plaque[77]. The role of eosinophil granulocytes and basophil granulocytes in the course of atherosclerosis remains ambiguous. The identification of eosinophil granulocytes within the atherosclerotic plaque is difficult due to their limited half-life and early apoptosis[77]. Basophil granulocytes constitute the low percentage of immune system cells present in the atherosclerotic plaque and no sufficient data is available to establish their role in the pathogenesis of atherosclerosis[66,78].

DENDRITIC CELLS AND ATHEROSCLEROSIS

The number of conventional dendritic cells (cDC) gradually increases with the growth of atherosclerotic plaque. They are found clustering with T lymphocytes[79,80]. cDC of the adventitia stimulate T lymphocytes to colonise this layer, secrete the IFN-γ and consequently initiate the inflammation[81]. Plasmacytoid DC (pDC) were identified in the atherosclerotic plaques obtained from the carotid arteries[82]. T lymphocytes are stimulated weaker by pCD than by cDC[82,83].

MAST CELLS AND ATHEROSCLEROSIS

Mast cells that are found in the atherosclerotic plaques from the carotid and coronary arteries are present in the areas of erosion, plaque rupture or the blood should the plaque haemorrhage have taken place. These cells and their products promote the progression of atherosclerotic plaque, accumulation of lipids, stimulate the degradation of high-density lipoproteins (HDL), whereas the latter have the protective features against atherosclerosis[84–87]. The secreted products, i.e. cytokines, proteases, autacoids, being the mediators of inflammation, alter the vessel permeability and lead to their remodelling. It was shown that the secreted TNF and IL-6 contribute to the formation of atherosclerotic plaques[88].

LYMPHOCYTES T AND B AND ATHEROSCLEROSIS

The macrophages present in the atherosclerotic plaque outnumber the T lymphocytes. Th2 lymphocytes, which produce IL-4, IL-5 and IL-10, are present in the great number in the atherosclerotic plaques. They stimulate the synthesis of immunoglobulins G and M by lymphocytes B. These cells inhibit the process of atherosclerotic plaque formation on a murine experimental model (hyperlipidaemia)[89–91]. Th1 lymphocytes produce IFN-γ, which stimulates the expression of MHC-II molecules on APC. Regulatory T lymphocytes (Treg) are also associated with atherosclerosis as they produce the transforming growth factor beta (TGF-β), which in turn has a decisive influence on their anti-inflammatory and anti-atherosclerotic activity[91]. A specific type of Tregs, i.e. type 1, is characterised by the capability to produce TGF-β and large amounts of IL-10. The latter plays an extremely important role in the elimination of vessel inflammation and atherosclerosis[92]. Murine aortic adventitia is an area, where local adaptive immunological reaction takes place during the atherosclerotic plaque formation[93]. The splenectomy in mice increases their susceptibility to atherosclerosis, which proves the protective role of the spleen in the pathogenesis of the disease[94].

MACROPHAGES/MONOCYTES AND ATHEROSCLEROSIS

Monocytes and macrophages play a significant role in the early stages of atherosclerotic plaque formation. Monocytes habitually migrate to the plaque during their growth. The number of these cells reflects the size of the lesion[95]. Recent *in vivo* studies in mice revealed the presence of two functionally different subpopulation of such cells, i.e. inflammatory, short-living and permanently colonising the non-inflammatory tissues[96]. Both populations may *in vivo* transform into antigen presenting cell (APC), e.g. macrophages[95]. The migration of monocytes into the atherosclerotic plaques is observed on every stage of atherosclerosis, which definitely indicates their significant role in this process[24,95–98].

REGULATORY T CELLS AND ATHEROSCLEROSIS

It should be emphasised that most studies concerning the participation of Tregs in atherosclerosis was performed on animal (murine) model, whereas atherosclerotic lesions develop in human for many years (even few dozen). It is not currently possible to reproduce such conditions in mice. Such limitations do not, however, deprecate the attractiveness of such concept and the capability of studying the role of Tregs in the atherosclerosis pathogenesis in human. The concept of the therapy of pathological inflammation within the vessel wall with Tregs may be realised in near future. The mechanism of Treg-induced suppression of atherosclerotic lesions remains unexplained, i.e. we still do not know whether it depends on the cytokine production or cell-to-cell interaction, or both. Where does the regulation take place – in the lymphoid tissue or in the arterial wall. The introduction of Treg-therapy should be preceded by detailed explanation of these mechanisms.

The concept of using regulatory T cells in autoimmune diseases is based on increasing their number or on stimulation of their function. In that way the researchers would like to control the inflammatory reaction accompanying self-destruction. Most authors agree that the depletion of Tregs, either genetically induced or with the use antibodies leads to the intensification of atherosclerotic lesions, whereas Treg transfer reduces their size[99]. Atherosclerotic studies are most commonly performed basing on the experimental model with apoE-deficient mice (ApoE-KO). In the experiments, basic for the development of this field of science, a regulatory T cells were generated and then transferred to the mice in the experimental atherosclerosis model (apoE/-)[100]. The authors demonstrated the reduction of atherosclerotic lesions in aorta, no change in the cholesterol concentration and the reduction of vessel wall destruction inflicted by macrophages and T lymphocytes in animals receiving Treg-infusions. The presence of IL-10 was confirmed in aortic lesions of animals treated with such immune therapy. It was one of the first evidence of immune modulation in atherosclerosis. Mor *et al.* have similarly demonstrated the reduction in the number of Tregs in atherosclerotic mice in comparison to the control group of animals[101]. By subsequent infusion of Tregs they obtained a significant reduction of such lesions. Therefore, it can be assumed that Tregs play a protective role in the development of atherosclerotic lesions, at least in murine model.

Other approach to the therapy of autoimmune diseases includes the administration of monoclonal antibodies against CD3-antigen, leading to the depletion of T cells[102]. Such therapy resulted in the reduction of atherosclerotic lesions, production of proinflammatory cytokines, such as IFN-γ and TNF-α, and the stimulation of TGF-β secretion. Despite the fact, that no increase in the percentage of CD4+CD25+ lymphocytes was observed, the expression of transcription factor FoxP3 was elevated both in the spleen and peripheral blood. In the most recent experiments, apoE-deficient mice were orally given an anti-T-cell antibody (anti-CD3), which led to the increase in the regulatory T cell population, anti-inflammatory cytokine production, the reduction of proinflammatory cytokines secretion, and above all the reduction of atherosclerotic lesion formation and macrophages infiltration[103]. Among medicines with other mechanism of action, the administration of pioglitazone (agonist of the peroxisome proliferator-activated receptor gamma – PPAR-γ) inhibited the development of atherosclerosis, due to the influence on the balance between the effectors and regulatory cells in favour of the latter[104].

DIAGNOSTIC MODALITIES OF ATHEROSCLEROTIC PLAQUES

Currently there is no sole, fully reliable diagnostic method, which would evaluate the atherosclerotic plaque in respect of such criteria as: the presence of active inflammation, thin fibrous cap with large lipid core, endothelial erosion with mural plaque aggregation, the presence of the fissure, plaque damage and critical vessel stenosis.

Smaller (accessory) criteria are being used very often, which comprise: superficially localised calcium concernments, yellow colour (assessed in angioscopy), haemorrhage into the plaque, endothelial dysfunction and positive vessel remodelling in the area of the plaque (eccentric – positive remodelling).

Concomitant use of several currently used diagnostic modalities significantly increases the possibility of detecting "instable" lesion.

Coronary catheterisation still remains the irreplaceable tool for evaluating lesions stenosing the lumen of the vessel. Basing on such angiographic features as ulceration, presence of separated fragment of intima, irregular lumen contour, aneurysm or mural thrombus one can conclude on the presence of already ruptured atherosclerotic plaque. The study itself does not, however, provide enough information on the vessel wall structure, which would prospectively enable the detection of instable plaque.

Intravascular ultrasound – intravascular ultrasound imaging (IVUS) allows for *in vivo* classification of atherosclerotic plaques into three categories[105]:

- I – soft plaques (hypoechoic), which corresponds to plaques with high lipid content in histological examination;
- II – fibrous plaques with intermediate echo;
- III – calcified plaques with strong wave reflection and acoustic shadow.

Basing on their group of 106 patients undergoing IVUS and subsequent clinical observation, Yamagishi *et al.*[106] prospectively proved that the eccentric, soft plaques are associated with higher risk of future cardiovascular incident. The ultrasound image provide additional information, helpful in precise evaluation of the size of the atherosclerotic plaque, the presence of possible ruptures and ulcerations and positive eccentric vessel remodelling in the place of the plaque[107]. Ruptured plaque presents in the IVUS as: plaque ulceration and the presence of fragments of the torn fibrous cap. The presence of the atherosclerotic plaque fissure that does not communicate with the vessel lumen is not considered as ruptured plaque.

Plaque instability and its thrombogenicity is directly confirmed by the presence of mural thrombus. It should be emphasised, however, that in many cases soft, and newly formed thrombi are characterised by echogenicity similar to this of the blood and are not visible in IVUS[108]. Main limitation of the IVUS is its imaging resolution, which is ca. 150 nm, which does not allow for the visualisation of the thin (<65 nm) fibrous cap and subjective nature of the examination itself.

Virtual histology is a diagnostic method which was developed basing on the current IVUS technique. The addition of spectral analysis of the frequency domain allows for colour-coding of four basic tissue types within the atherosclerotic plaque. The following tissue types are distinguished: fibrous tissue, fibro-fatty tissue, necrotic core, calcifications. Criteria to recognise a lesion with plaque morphology with thin connective tissue cap according to this imaging modality include:

- well organised necrotic core;
- necrotic component covering over 10% of plaque surface area;
- direct contact of the necrotic core with vessel lumen;
- no ultrasound features of connective tissue strip between the necrotic core and vessel lumen.

The multicentre PROSPECT Study[109] is currently being carried out, in which proximal segments of 3 main coronary arteries are imaged in patient with ACS using virtual histology and palpography. The preliminary identification of atherosclerotic plaques will be attributed to the results of prospective clinical observation and angiographic evaluation[109].

Angioscopy allows for *in vivo* visualisation of the internal surface of coronary arteries. It allows for the evaluation of such parameters as: colour, presence of glossy surface, surface regularity. Uchida *et al.*[110] proved that the angioscopically observed yellow, glossy atherosclerotic plaques is associated with increased risk of ACS. The modality itself has some specific limitation, including: need to occlude the vessel, subjective interpretation of the found lesions and possibility to visualise only large-calibre vessels.

Thermography, basing on the elevated temperature, may conclude about the inflammatory activity. The small intravascular thermodetector maps areas of differing temperature. The prospective study carried out by Stefanadis *et al.*[111] on 86 patients proved the significantly higher risk of ACS in patients with increased intracoronary thermal heterogenicity.

Angiography is an imaging modality, allowing for the visualisation of blood vessel lumen using the X-rays. The examination requires an administration of the contrast medium. It is most commonly performed once the vessel (arterial, venous) stenosis is suspected (fig. 2). The examination is performed to visualise the vessel course, and its lumen. It allows for a possible detection of stenoses, and changes in the shape of arteries and veins. The examination may concomitantly be followed by the intravascular treatment procedure of arterial angioplasty.

The Duplex ultrasound is a method of choice in the examination of extra- and intracranial segments of the carotid arteries. It enables concomitant imaging of tissues and blood flow. Its essential advantage consists of the assessment of arterial wall, i.e. pathological processes taking place within it, such as atherosclerosis. The use of high-frequency transducers allows for the highly sensitive evaluation of plaque character, its size, surface area and changes within its structure, such as ruptures, bleeding, calcifications, ulcerations and mural thrombi. Due to this fact, it is widely and commonly utilised to determine its stability according to the certain criteria and validated classifications. The addition of colour-coded flow imaging increases the sensitivity and specificity of this vascular imaging modality, especially when it concerns the identification of hypoechoic lesions, such as mural thrombi.

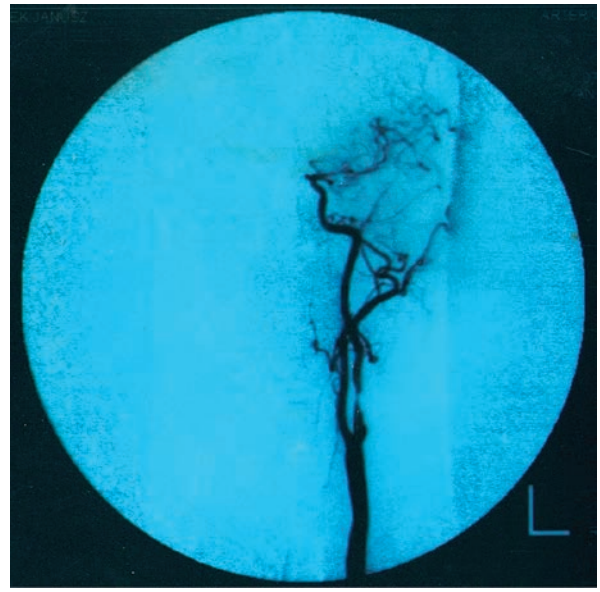

Fig. 2. Angiographic image of the extracranial segment of the left carotid arteries (arrow indicates the critical stenosis of the left internal carotid artery). Department of Vascular, General and Oncologic Surgery, Copernicus Memorial Hospital, Lodz, Poland

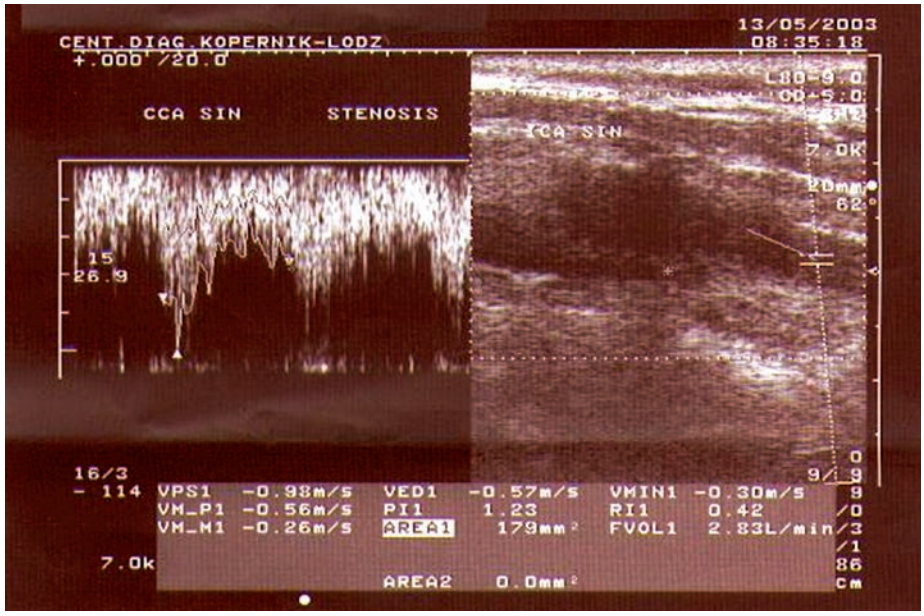

Fig. 3. Duplex ultrasound of the left carotid artery. A critical stenosis in the ostium of the internal carotid artery. The Doppler-spectrum chart indicates the abnormal, turbulent flow through the stenosed arterial segment. Department of Vascular, General and Oncologic Surgery, Copernicus Memorial Hospital, Lodz, Poland

Basing on the blood flow velocity assessment and the evaluation of its spectrum one can assess the arterial stenosis grade or diagnose its occlusion with great accuracy. **Magnetic resonance angiography (MRA)** increases its significance in the diagnostic of carotid arteries, as its sensitivity and specificity are comparable to those of duplex ultrasound.

Due to its perfect technical potency the **computed tomography angiography (CTA)** poses a completion of carotid arteries diagnostic. The findings obtained with CTA correlate strictly with those provided by intravascular ultrasound, in many cases eliminating the need to perform arteriography (fig. 4).

CONCLUSION

Due to the current trend towards aging society, the atherosclerosis becomes pathology of increasing significance. Many significant issues concerning the pathogenesis of the disease still remain to be explained. Much has recently been discussed about the role of the immune system cells in the development of atherosclerosis. A mutual interactions between various subpopulations of immunological cells contributing to the atherosclerosis development was observed. It should be emphasised that most of the observed interactions between the immune system cells and atherosclerotic plaques were observed mainly in experimental models. It seems that some of such results cannot be clearly related to the human pathology, which requires further studies in this field.

It seems that the described lesions and above-mentioned complications may pose a part of processes permanently taking place within the plaque. Their course does not have to indivertibly lead to the degradation of the plaque structure. Healing in the form of vascular remodelling takes place concomitant to the progression of the lesions within the plaque. The arbitrariness of these processes was presented in fig. 2. It should be judged that the morphology of the atherosclerotic plaque will become one

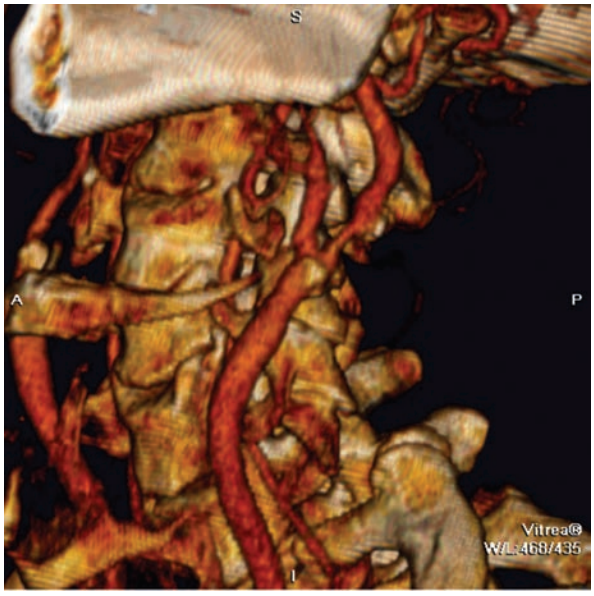

Fig. 4. Computed tomography angiography of the carotid arteries (arrow indicates the critical stenosis of the left internal carotid artery. Department of Vascular, General and Oncologic Surgery, Copernicus Memorial Hospital, Lodz, Poland

of the basic criteria in the process of establishing indications to carotid artery endarterectomy. The final aim of the studies concerning the plaque instability will most probably be the determination of the criteria of the morphological evaluation of such lesions that would require immediate or urgent surgery, and such that could be operated in the elective manner. Most of the currently available modalities of evaluating atherosclerotic plaque instability were coronary catheterisation and grey-scale IVUS. In the nearest future the virtual histology (due to the automated and repeated evaluation) and optic coherent tomography (due to its high resolution) will probably become most perspective in respect to common usage in the determination of plaque instability.

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