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Clinico-morphological manifestations of atherosclerotic lesions of cerebral basilar arteries

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Abstract

Background: Endothelial dysfunction is an early sign of atherosclerosis, which favors the increase of vascular permeability, activation of mast cells and migration of leukocytes, lymphocytes, macrophages, platelet adhesion, smooth muscle cell proliferation and eventual vasospasm, which together determine a proinflammatory status. Angiogenesis is an important pathogenic element of atherosclerosis in stages of complicated plaques, along with mast cells and macrophages.

Histotopographical analysis of the distribution of newly-formed vessels as a feature of angiogenesis, expression of mast cell and macrophage in different types of plaques in different arterial vessels in patients with atherosclerosis and metabolic syndrome complicated by atherosclerosis.

Material and methods: We studied 34 patients with cerebral artery atherosclerosis. To determine the expression of mast cells in the affected vessels, we used anti-MCT. Macrophages were identified using specific marker CD-68 and newly-formed vessels respectively through the application of CD-105 (endoglin).

Results: Assessment of the results was based on the final determination of the density and intensity of reaction, as reflected in the quantitative ratio of the different areas of atheromatous plaques. Stained positive mast cells, macrophages and newly-formed vessels have been found in several types of atherosclerotic plaques, especially in adventitia and in the immediate vicinity of the plaques and subendothelial layers. We found a statistical correlation between the type of plaque and clinical data.

Conclusions: Immunohistochemical method is efficient for the determination of mast cells, macrophages and newly-formed vessels of atherosclerotic plaques, directly reflecting many important pathogenic factors of atherogenesis in patients with atherosclerosis of the brain arteries.

Key words: atherosclerosis, angiogenesis, mast cells, macrophages.

Introduction

One finds it hard to nominate another disease like atherosclerosis, on the pathogenesis of which there exists such a large number of theories, hypotheses, assumptions and even speculations. For understanding the pathogenesis of atherosclerosis, precise knowledge of ways and mechanisms that determine the penetration of atherogenic lipoproteins in the vascular wall is of major importance. An equally major role is assigned to the analysis of protection and adjustment reactions, which condition the metabolism of the vascular wall in normal circumstances and in atherosclerosis. For a deeper understanding of mechanisms that set off in vascular wall in atherosclerosis, it is necessary to conduct a detailed, structural-functional study of cellular elements of the artery wall, their participation in lipids uptake and synthesis of collagen and elastin. Knowing the vascular wall metabolism in atherosclerosis, defines, to a wide extent, the ways of preventing and treating this disease.

We believe that atherosclerosis is the primary cause for developing ischemic heart disease, stroke, arterial lesions of lower limbs and other organs. Atherosclerosis is a disease of the XXI century, which turns more into a burden or a cross that mankind carries as punishment for current civilization. One gets the impression that, by this disease, God has punished people for their compulsion towards social conflicts, limitation of active lifestyle, excess of food and sleep and many other bad habits.

The clinical features of atherosclerosis depend largely on the lesion position in the vascular ramification. The analysis of the sectioned sample shows that, sometimes, it is enough to have in the coronary heart artery or cerebral basilar artery an atherosclerotic plaque in a certain location to cause extensive transmural myocardial infarction or vascular changes in the brain (ischemic or hemorrhagic strokes) [1].

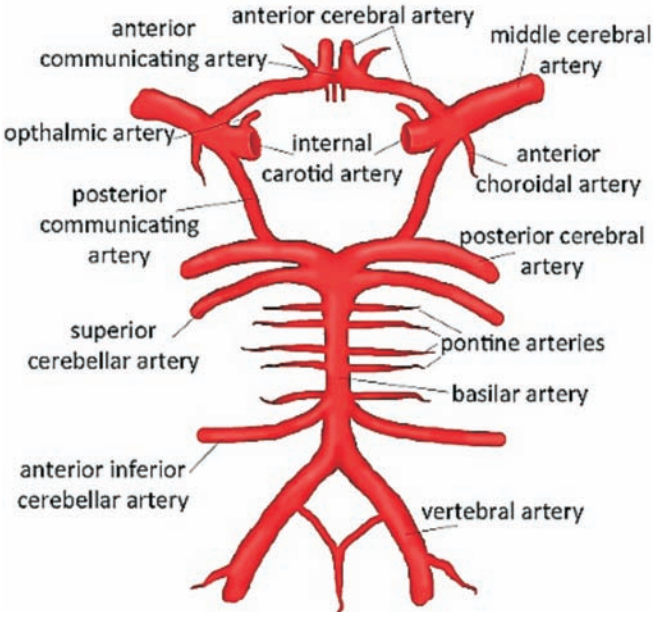

Fig. 1. Circle of Willis.

Atherosclerosis primarily affects elastic and muscular elastic arteries. Cerebral arteries lack external elastic lamina, and the internal elastic lamina is well developed. The arteries of the Circle of Willis (fig. 1) are muscular arteries, as their middle layer is well developed.

Strokes can occur at any age and in any gender. To a large extent, they reflect the degree of impairment of cerebral vessels (carotid, basilar and cerebral arteries). Figure 2 shows the normal structure of the inner layer of the basilar artery in scanning electron microscopy.

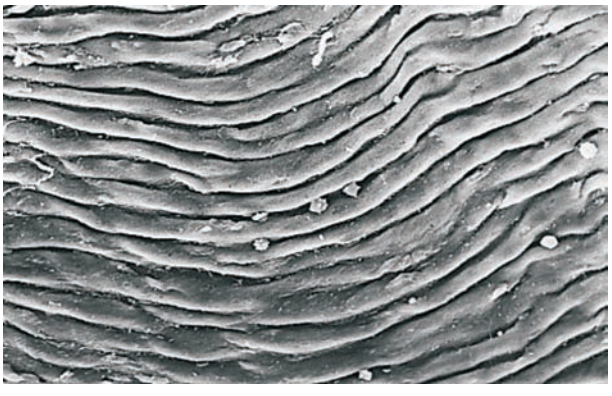

Fig. 2. Scanning electron microscopy of the surface of normal basilar artery (x 3200).

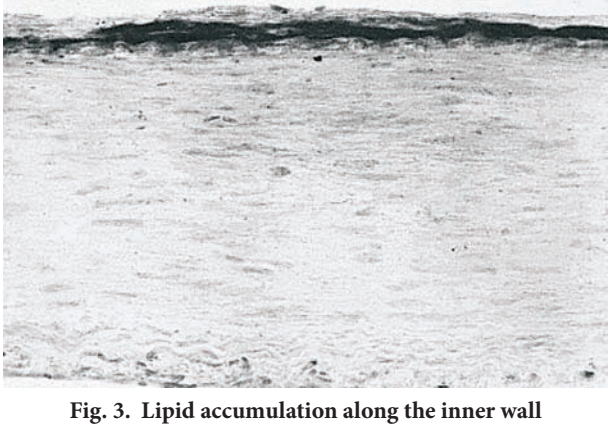

Fig. 3. Lipid accumulation along the inner wall of basilar artery elastic lamina, patient's age – 37 years old (Oil Red staining, x 460).

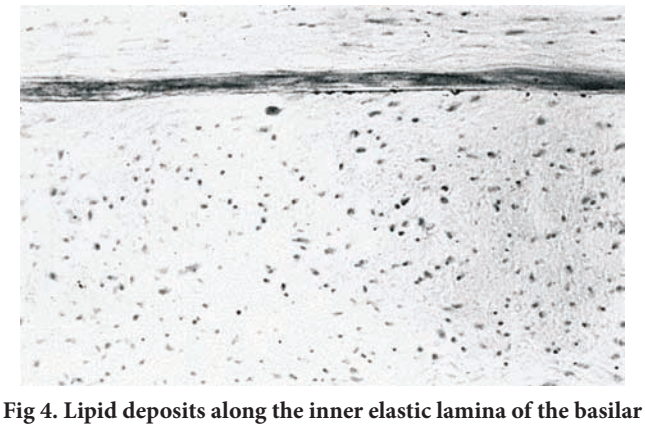

Fig 4. Lipid deposits along the inner elastic lamina of the basilar artery, patient's age – 67 years old (Oil Red staining, x 460).

In lipid spots from the basilar artery of the brain, lipid deposits in the ground substance are observed mainly in the surface layers of the internal tissue, in the internal elastic lamina (fig. 3, 4). At this stage of lipidosis, the elastic lamina keeps its tinctorial properties and is intensely fuchsin-stained by Weigert method.

In further development of lipidosis of the cerebral basilar artery, significant lipid depositions are observed not only in the internal elastic lamina, but also on its loose fibers. Sometimes, the internal tissue lipidosis is diffuse; lipids are deposited on the internal elastic lamina, on its plaques, which are separated and on tissue layers between them.

Without making a comparison with similar changes in other arteries, in incipient forms of atherosclerosis of cerebral basilar artery, one can get the impression of primary isolated adipose degeneration of the internal elastic lamina in atherosclerosis and only a secondary lipid deposition in the intima. The study of characteristics of lipid deposition in different arterial systems, as well as in various segments of the cerebral basilar artery, makes it possible to determine the stage of lipid deposition on the surface of elastic membranes or in cell elements [2].

Degenerative changes in the internal elastic lamina of the cerebral basilar artery differ from the changes of elastic elements of other arteries investigated by us, which can probably be explained by significant selective lipid deposition on the internal elastic lamina that becomes adipose and simultaneously thickened. In this case, one does not observe primarily the lysis, but the tumefaction and the cleavage of the membrane. On fuchsin staining, such a membrane has a dark violet color and loses its double refrangibility (refractive power) in polarized light.

At lipid deposition and subsequent impregnation in the aorta and coronary arteries of the heart, signs of degenerative changes are also found in intima elastic structures. These changes occur not only by changing their tinctorial properties, but also by adhesion, evening and fragmentation of some fibers. Contours and boundaries of elastic fibers become blurred; in some sites, thin elastic fibers are subject to lysis, as if dissolved in the surrounding lipid mass. At the same time, one often observes the alternation of areas of lysed elastic fibers with areas where their destruction is just beginning, while their tinctorial properties are maintained.

In progressive forms of atherosclerosis, the vascular wall metabolism is directed to the use of excessive amounts of emerged lipids and, thus, is connected to the discharge in lipidosis portions of various cellular elements from the changed sites of the arterial wall (predominantly smooth muscle cells) and mobile cellular elements (mononuclear monocytes) from the bloodstream, as well as to the activation of oxidative-regenerative and hydrolytic enzymes.

This process is accompanied by formation of new connective tissue, a phenomenon which inevitably leads to production by the mesenchymal cells in situ of sulfated fractions of glycosaminoglycans, in particular dermatan sulfate. Probably, in the early stages, this reaction can be viewed as a protective-adaptive one.

The foam cells accumulate a large number of cholesterol compounds and fatty acids. In the process of absorption of lipids by the cells and formation of foam cells, the latter, due to reactive changes, are surrounded by immature connective tissue, which, in maturation, forms layers that separate foam cells from their surface layer and the conjunctive tissue.

Alongside with lipid accumulation in foam cells and connective tissue growth, one observes marked degenerative

changes of elastic fibers that also comprise the internal elastic lamina. On accumulation of lipids in the basilar artery inner coat, basophilia and metachromacia of the internal elastic lamina are especially evident. Lamina „dissolves" gradually, which results in its penetration by the lipids (fig. 5).

Fig. 5. Internal elastic lamina lysis. Lipids penetration in basilar artery middle coat and atheroma formation in the media under internal elastic lamina defect (Oil Red staining, x 460).

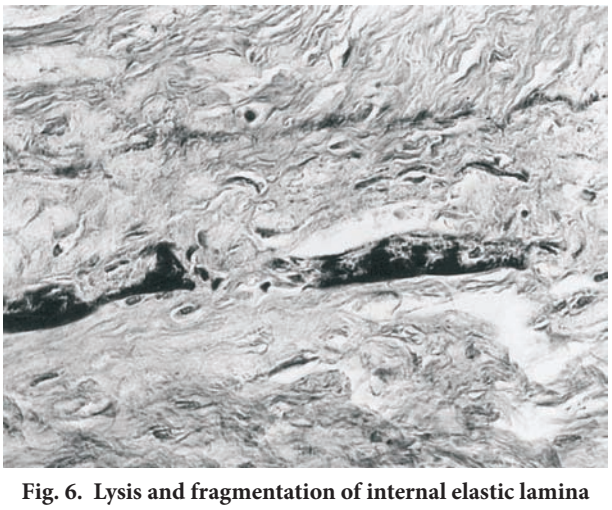

Fig. 6. Lysis and fragmentation of internal elastic lamina in the site of atherosclerotic plaque in the basilar artery (Weigert-Hart's elastic stain, x 840).

In such cases, the internal elastic lamina edge of the intima, i.e. the edge with distinguished deposited lipids is the one that is mostly changed, with a scalloped form (fig. 6). Meanwhile, the lower edge of the elastic plaque, adjacent to the middle coat, registers no visible changes almost to full lysis of internal elastic lamina, which serves as confirmation of the secondary character of dystrophic changes in the elastic membrane in response to lipid deposition. In more advanced stages of internal elastic lamina decomposition, the latter is missing at a considerable distance or exists as fragments and lumps [3].

The intensive destruction of elastic structures of internal artery tissue is a reflection of general metabolic changes that take place in the vascular wall, including tissue hypoxia, release of hydrolytic lysosomal enzymes, tissue acidosis, deposition of immunoglobulin with formation of an autoimmune complex, intima imbibition with plasma proteins, penetration of elastase and blood plasma.

In the early stages of atherosclerosis, one may observe the formation of cholesterol crystals in foam cells and in the extracellular space. Although a relatively inert part of cholesterol, however, cholesterol crystals can cause mechanical tissue damage, exacerbating the atherosclerotic process. Unlike cholesterol esters, its crystals are not absorbed by the cell, so, even in regression, they stay in the arterial wall.

The formation of cholesterol crystals between atheromatous masses is related to the decay of foam cells and release of their content into the intercellular space, including membrane-lacking lipid vacuole, consisting of cholesterol esters (fig. 7).

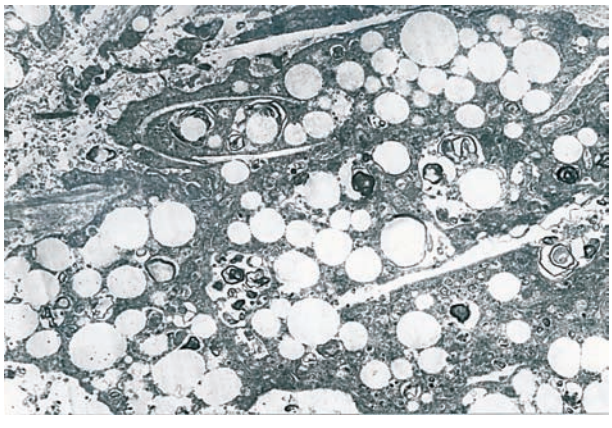

Fig. 7. Atherosclerotic plaque of basilar artery. Formation of cholesterol crystals in the site of decayed foam cells of smooth muscle origin, with lipid vacuole in cytoplasm (x 60000).

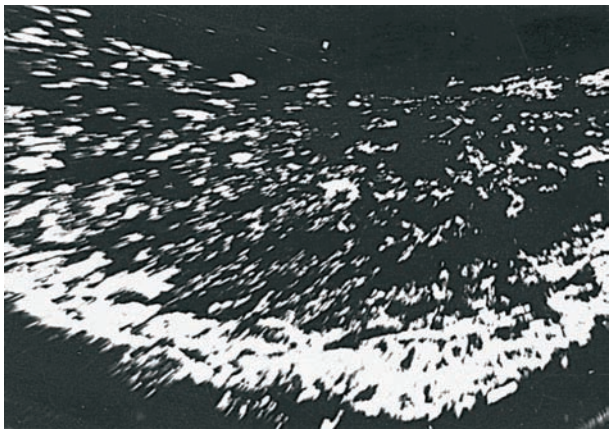

Fig. 8. Polarizing microscopy shows that the cholesterol crystals are deposited along the internal elastic lamina of the basilar artery (x 460).

The accumulation of cholesterol crystals is mainly concentrated along the internal elastic lamina (Fig. 8), which can also cause elastic damage and lysis.

On destruction of the elastic membrane and penetration of lipids into the middle tissue, intima foam cells can be formed in situ, i.e. in tunica intima and in the sites surrounding it. Meanwhile, smooth muscle cells of the subintimal layer penetrate intima through affected elastic areas, contributing to further disintegration of internal elastic lamina. Smooth muscle cells participate in the formation of connective tissue components, including collagen and elastic fibers (fig. 9-12). On lipid penetration into tunica media, in the sector under

adventitia, one observes an accumulation of macrophages and lymphocytes that create infiltrates. The infiltration of macrophages through the membrane in lipid deposition sites is also possible.

Fig. 9. Lipids penetration in the media through internal elastic lamina defect (Oil Red staining, x 460).

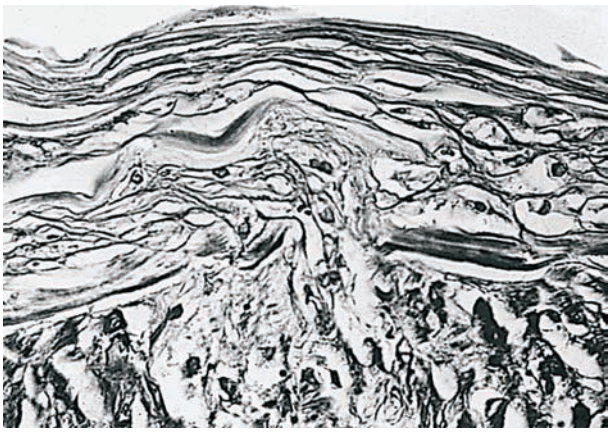

Fig. 10. Migration of smooth muscle cells through internal elastic lamina defect (Weigert's stain method, x 460)

Fig. 11. Migration of mononuclear cells through internal elastic lamina defects in the atherosclerotic plaque, with formation of infiltrates in the deep layers of the plaque (Weigert's stain method, x 460).

Alongside with the decay of vascular wall fibrous structures, a formation of new structures occurs. Yet, the elastic fiber-forming processes are manifested much weaker than the appearance of collagen fibers. There are many reasons for this phenomenon. Essentially, all factors that lead to lipid deposition in intima and metabolic disorders of the vascular wall are reasons that contribute to the development of connective tissue and atherosclerotic fibrous plaque formation.

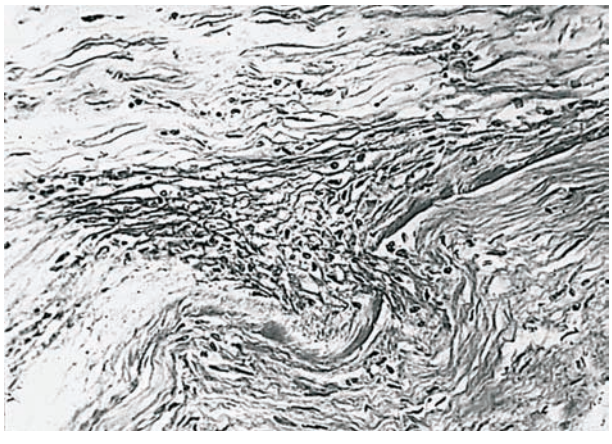

Fig. 12. Formation of precollagen fibers that cover internal elastic lamina defects (Weigert–Hart's stain method, x 460).

To some extent, the newly-formed elastic fibers cover the fiber defects, especially over the damaged internal elastic lamina. Thin elastic fibers are located in the muscular elastic layer between acid glycosaminoglycans. Particularly noteworthy is the genesis of new elastic fibers in the cerebral basilar artery, where they are formed even before complete lysis of the internal elastic lamina. The newly-formed elastic fibers, in turn, are subject to dystrophic changes. They stick together, are intensely stained with fuchsin, mix; in some places, they are subject to lysis and gradually dissolve into the surrounding mass of lipids and proteins (fig. 13).

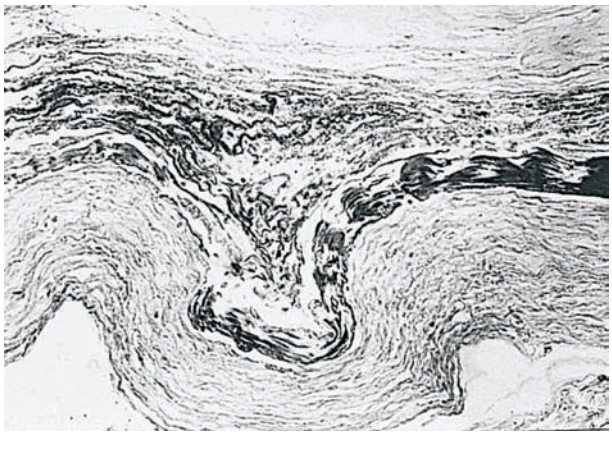

Fig. 13. Homogenization, lysis and fragmentation of newly-formed fibers in sites of internal elastic lamina rupture, which partially cover the lamina defect in the subendothelial layer of foam cells (Weigert–Hart's stain method, x 840).

Together with lipids deposition on elastic fibers, they also accumulate in intima argyrophilic structures and on collagen fibers on the layer connecting the plaques. As to their appearance, lipid deposits on argyrophilic structures and collagen fibers are somewhat different from lipid deposits on elastic

membranes. Changes taking place in argyrophilic structures are quite difficult to follow, as they are very closely attached to elastic fibers, weaving them around. This is the reason why, for example, in the cerebral basilar artery, in sites of internal elastic lamina lysis, one can observe the formation of only a few elastic fibers covering the lysate lamina defect, while the formation of argyrophilic fibers takes place throughout the thickness of atherosclerotic plaques (Fig. 14).

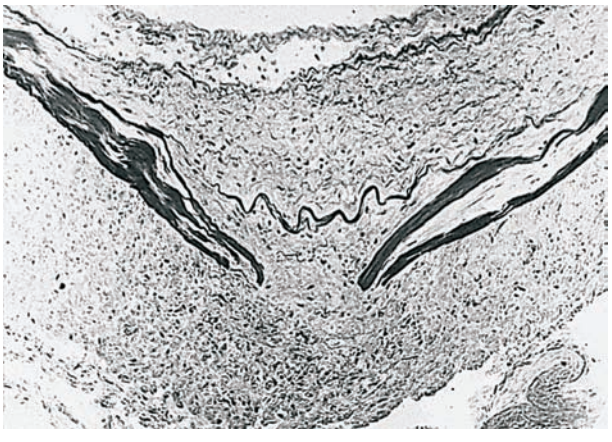

Fig. 14. Tumefaction, fragmentation, lysis and disintegration of internal elastic lamina; partial defect "covering" with newly-formed elastic fibers. Foam cells accumulation in the subendothelial layer (Weigert–Hart's stain method, x 840).

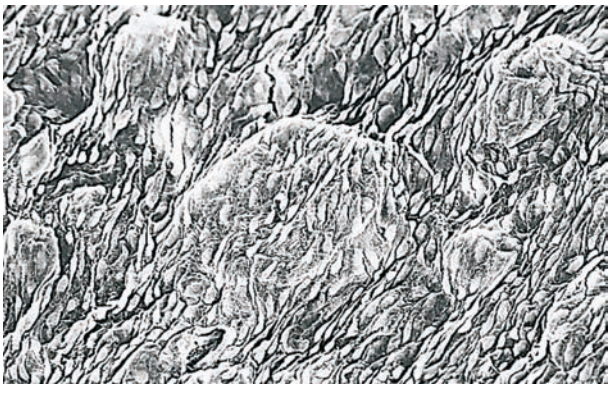

Fig. 15. Scanning electron microscopy. Swollen endothelium focus above the site of lipid deposition in intima (x 3200).

Concurrently, an increased content of collagen fibers is observed in such areas. The argyrophilic fibers newly formed in atherosclerotic plaques lose their characteristic tortuosity, stick together, as well as to elastic fibers, and impregnate intensely with silver.

Increased production of collagen fibers results in a thickening of the inner layer. Intima and subintimal layer, even in normal conditions, especially in the elderly, are constantly at anoxia limit. In anoxia, elements of connective tissue are intensively formed, leading to thickening and deformation of artery walls.

On analysis of basilar artery surface with the scanning electron microscope, above the lipid spots and plaques, one observes a characteristic feature of atherogenesis – the endothelial monolayer change. The endothelium, on the surface of edema and lipid deposition in focus in intima, takes the form of a cobblestone pavement, with deposits of erythrocytes in the folds of prominent cells (fig. 15).

Two types of endothelial cells can be distinguished above the emerging atherosclerotic plaques [4]. The first type consists of functionally activated endothelial cells; in their cytoplasm, one observes an increase in the number of hypertrophic mitochondria, concentrated at nucleus poles; expansion of rough (granular) endoplasmic reticulum cisternae; a large number of ribosomes and unlimited endocytic vesicles; Weibel-Palade corpuscles are prominent. The second type consists of endothelial cells with irreversible dystrophic and degenerative changes, with a background of formation of large lipid vacuoles in the rough endoplasmic reticulum.

Along with destructive processes in the basic elements of the vascular wall - destruction of elastic, collagen fibers and cellular elements: endothelium, SMCs, monocytes, macrophages, fibroblasts, there also take place compensation, adaptive-regenerative processes, accompanied by formation of new blood vessels - neoangiogenesis (neovascularization). We studied this process by means of special research methods.

The intima of newly-formed vessels, associated with atherosclerotic plaques, was firstly studied in 1876 by Koester. In atherosclerotic plaques, angiogenesis allows for the formation of new microvessels, in order to maintain the required level of oxygen and nutrients in the vascular wall.

CD105 is a homodimeric membrane glycoprotein, complete, consisting of 90-95 kDa subunits, bisulphite-bound, and is part of the transforming growth factor beta receptor TGF-β. CD105 is manifested in angiogenic endothelial cells [5]. Thus, CD105 is a sensitive marker for identifying newly-formed blood vessels [6]. Concurrently, CD105 is a more specific and sensitive marker for the evaluation of newly- formed blood vessels in atherosclerotic plaques than CD31 or TGF-β1 [7, 8].

Determining the level of circulating soluble CD-105 - sensitive antigens - allows us to determine exactly the presence of unstable plaques or their disruptions [9].

Neovascularization occurs in sites of atherosclerotic lesions undergoing constant change, reconstruction and prone to rupture. Some studies show that the formation of new blood vessels contributes to the growth of atherosclerotic lesions and is a key factor leading to destabilization and disintegration of the plaque.

Some of the newly-formed blood vessels are immature, similar to those observed in the neovascularization of solid tumors and, therefore, can lead to tearing and bleeding in the plaque site, and subsequently - to its instability.

Mast cells, macrophages and T-lymphocytes, which interact together, usually form inflammatory cell infiltrations of atherosclerotic plaques and intercellular substance [10].

Material and methods

Using immunohistochemical methods, we studied a number of different brain vessels in 34 patients with cerebrovascular disease (ischemic and hemorrhagic stroke). The patients included representatives of all age groups, but one

Table 1

Immunohistochemical methods used in the study

Antibody	Source	Clone	Dilution	Detection system	Antigen retrieval	Primary antibody incubation
Alpha Smooth Muscle Actin	Novocastra, Newcastle upon Tyne, UK	Human Alpha Smooth Muscle Actin, ɑsm-1 clone	RTU	NovoLink Max Polymer Detection System (Novocastra Newcastle upon Tyne, UK)	Microwaves, 5 minutes, pH 6	30 minutes, room temperature
CD68	Dako Glostrup Denmark	Monoclonal mouse anti-human, QBEnd 10	1:25	NovoLink Max Polymer Detection System	Microwaves, 5 minutes, pH 6	30 minutes, room temperature
Endoglin	Dako Glostrup Denmark	Monoclonal mouse anti- human, clone SN6h	1: 10	NovoLink Max Polymer Detection System	Proteinase K, 10 minutes	30 minutes, room temperature
CD34	Dako Glostrup Denmark	Monoclonal mouse anti-human, 1A4	RTU	NovoLink Max Polymer Detection System	Microwaves, 30 minutes, pH 6	30 minutes, room temperature
Mast cell tryptase	NeoMarkers, Fremont, CA	Mouse Monoclonal Antibody, AA1clone)	RTU	NovoLink Max Polymer Detection System	Microwaves, 30 minutes, pH 6	30 minutes, room temperature

could notice that the extent of damage increases past the age of 40. The age range was between 44 and 83 years (mean age – 62, 8 years). Women accounted for 41, 2%, men – 58, 8%. During autopsy, fragments of cerebral arteries were taken for study.

Vascular fragments were processed by standard method (fixing in 10% buffered formalin solution, embedding in paraffin blocks; 4-5 micrometers thick sections were obtained). The determination of the type and stage of plaques was based on AHA (American Heart Association, 1995) morphological classification, considering the macroscopic and histological image of sections stained with hematoxylin-eosin, as well as the histochemical techniques - silver and orcein impregnation.

Additional sections of paraffin blocks were processed immunohistochemically; they were deparaffinized, hydrated, subject to reaction for detection of antigen in the PT Link module (Dako Cytomation Denmark). The next step was the primary antibody incubation, using NovoLink Max Polymer Detection System, and, to visualize the final reaction, we used 3, 3'-diaminobenzidine dihydrochloride as brown chromogen.

CD105 interpretation: we quantified vascular structures with lumen, positive for CD105, brown-colored at cytoplasmic level in endothelial cells. The microvascular density was determined using the hot spot method [11, 12].

Concomitantly, we also considered the positive signals for CD105-positive endothelial cells, capillaries and plexi of CD105 positive endothelial cells, as well as in periplaque site and in the plaque itself.

The histopathological analysis revealed the existence of three major (conditional) types of atherosclerotic lesions in two study groups: intermediate injury (II), fibrous (formed) plaque (FP), calcified (and/or complicated) fibrous plaque (CFP) in all the studied vessels, which, in turn, were stained with hematoxylin-eosin, orcein and were impregnated with silver.

Score of antibody expression in arterial vessel walls and atherosclerotic plaques

Score	Immunolabeling	Positive cells (%)	Intensity
0	-	<1%	-
1	+	1-25%	Low
2	++	26-50%	Moderate
3	+++	>50%	High

Results

Table 3

Biochemical laboratory data of the patients included in the study

Age	63,4 (average)
Gender	21/13 (m/f)
Glycemia	4,74 (mmol/l)
Total cholesterol	6,90 (mmol/l)
Triglycerides	0,86 (mmol/l)
High-density lipoproteins	1,003 (mmol/l)
Low-density lipoproteins	1,99 (mmol/l)
Leukocytes	8,42 (×109/l)
Lymphocytes	24, 5 (%)
Monocytes	7, 57 (%)
Prothrombin	73, 6 (%)
Fibrinogen	3,3 (g/l)
Erythrocyte sedimentation rate	17,5 (mm/h)

These images, studied in optical microscopy of the basilar

Table 2

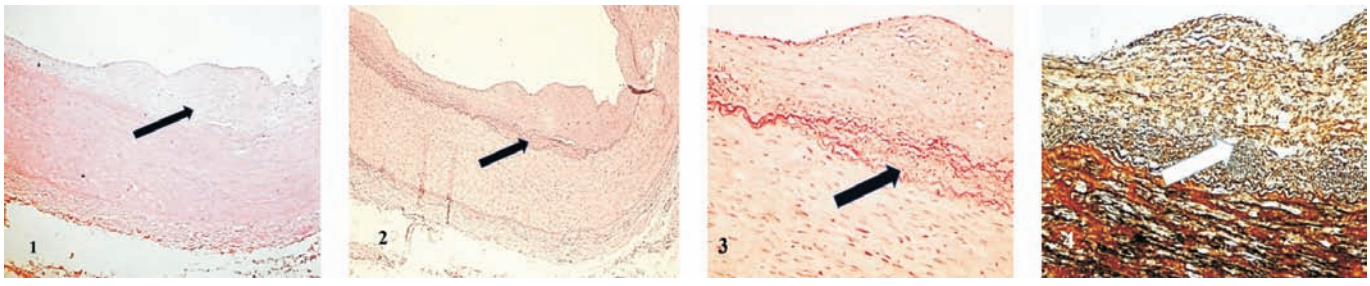

Fig. 16. Fibrous atherosclerotic plaque of basilar artery.
1. The fibrous plaque (H-E x 20); 2, 3. The disintegration of elastic lamina (Orcein, x 20, x 40);
4. Elastic and reticular fibers (Silver Impregnation, x 40).

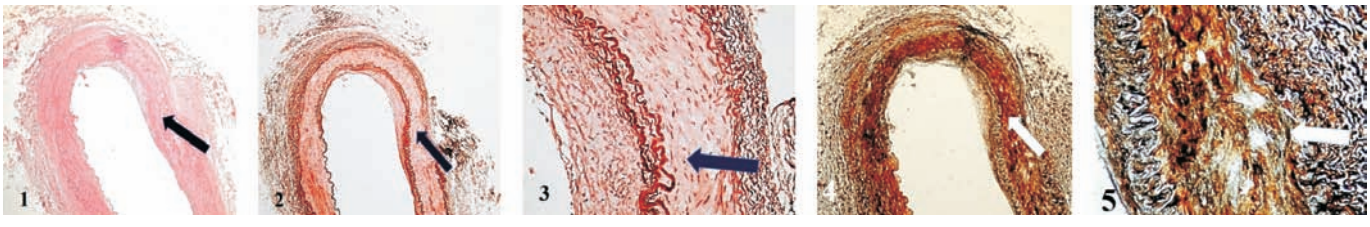

Fig. 17. Fibrous atherosclerotic plaque of basilar artery.
1. The fibrous plaque (H-E x 10); 2, 3. The disintegration of elastic lamina (Orcein, x 10, x 20);
4, 5. Elastic and reticular fibers (Silver Impregnation, x 10, x 40).

Immunohistochemical methods demonstrate the existence of the anti-MCT, CD68 and CD105 (endoglin) differential expression in different types of atherosclerotic lesions and various types of vessels in combination with histotopographic distribution.

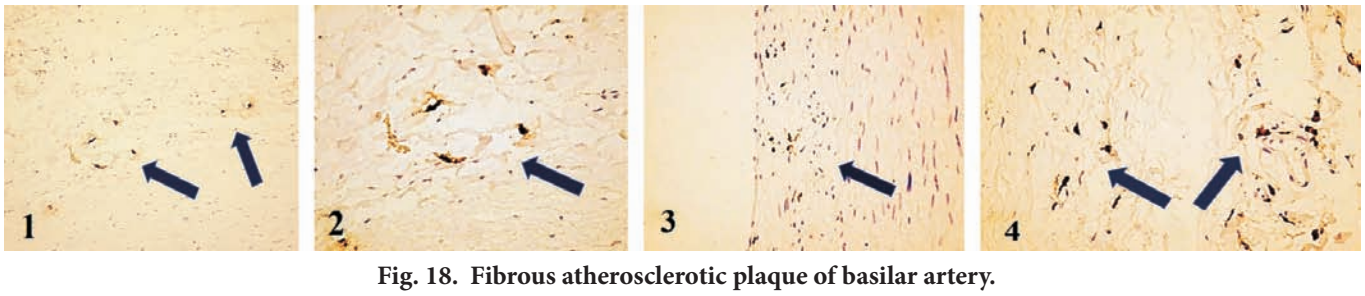

Fig. 18. Fibrous atherosclerotic plaque of basilar artery.
1, 2. The fibrous plaque (anti-MCT, x 20, x 40); 3, 4. The fibrous plaque (anti-CD 68, x 20, x 40).

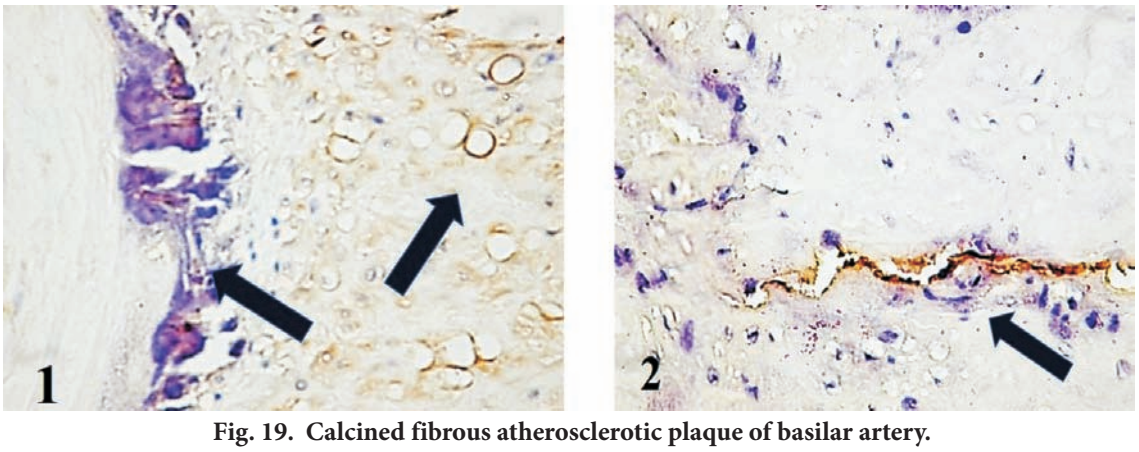

Fig. 19. Calcined fibrous atherosclerotic plaque of basilar artery.
1. 2. The calcined fibrous plaque and microvessels (anti-CD 105, x 40).

artery and applying histochemical staining methods, reflect structural changes of the vascular wall components, characteristic to fibrous plaque stages. The most representative are the disruption of the endothelial layer, with its significant thickening, atherogenic masses storage at the subendothelial level, endotheliocytes hyperplasia (Fig. 16.1 and 17.1, hematoxylin-eosin-stained x10, x20). On applying orcein staining with x20, x40 magnification on these sections, in Fig. 16.2, 16.3, 17.2 and 17.3 one clearly notices the changes at the level of lamina propria, accompanied by its thickening, deformation and splitting, something that is not characteristic for intact blood vessels. The basal membrane, with endotheliocytes on it, is split at the level of fibrous plaque and one also observes here atheromatous masses, fibrous tissue, thus explaining the specific deformation of fibrous plaques in atherosclerotic damage. Silver impregnation with x10 and x40 magnification in Fig. 16.4, 17.4 and 17.5 allows highlighting elastic and reticular fibers at the plaque level, with complex disruption of extracellular matrix. The observed optically blank areas are areas of lipids accumulation, surrounded by thick and deformed fibers. In some complicated plaques, they have a chaotic layout and can be combined with calcium deposits, hemorrhagic imbibition and inflammatory cellular components in unstable plaques, which, in turn, can be complicated by rupture, with clinical manifestations of fatal acute hemorrhagic strokes.

On analysis of immunohistochemical expression of cellular components involved differently and dependent on the evolution stage of atherosclerotic plaques, a diverse participation of inflammatory cells in different compartments of the vessel wall is defined. In Fig. 18.1 and 18.2 anti MCT x20, x40 magnification, one observes mast cells positively brown stained with the specific marker for these anti-MCT (tryptase mast cells). The distribution of mast cells is varied and proportional to the evolution stage of atherogenic process in outbreaks of inflammatory reactions. More frequently, mast cells are located in the subendothelial site and around the inflammatory process, as well as in the plaque, if the inflammatory process extends therein.

On the other hand, in various evolution stages, along with mast cells, one can also observe macrophages, which are highlighted with the marker selectable for them - CD68 (macrophage cells are positively brown stained) in Fig. 18.3 and Fig. 18.4, x20, x40 magnification. Like mast cells, macrophages also have a varied distribution in the plaque, depending on the immunoreactive condition and the evolution stage of the atherogenic process. The number of mast cells and macrophages is varied, but some features were observed in the laws of their expression - mast cell growth is characteristic for acute inflammatory reactions, while macrophages growth occurs at the decrease of acute inflammation stages. Their strictly defined functions explain their important role in inflammatory processes in general and in the development of atherosclerotic plaques, in particular.

Another important pathogenetic link is atherosclerotic angiogenesis, shown in Fig. 19. The atherosclerotic angiogenesis, like the tumoral one, has some similar evolutionary features. The specific marker CD105 (endoglin) highlights in brown the vessel endothelium newly formed around atherosclerotic plaques and, in some cases, inside them. The expression of positively stained endothelium and the newly-formed capillaries is varied and dependent on many factors. In Fig 19.1 and 19.2 x40 magnification, one observes a complicated atherosclerotic plaque, with calcinosis focus, with multiple newly-formed vessels, which can form clumps of endothelial cells, plexuses and capillaries with prominent lumens.

Conclusions

The information obtained confirms the evolution theories and the pathogenic mechanisms of atherogenesis. Despite these assertions, at present, there are a lot of unresolved scientific and clinical issues.

For comparison, one may bring as example the angiogenesis of tumoral processes, in which mast cells and macrophages, similar as in the processes of formation of atherosclerotic plaques, are involved in neovascularization (only when discussing mechanisms, general functions and pathophysiological links).

Anti-MCT and CD68 are selective markers for mast cells, macrophages, which are important components of immune processes, in the initiation, proliferation and differentiation of cells in atherosclerotic lesions. Along with T-lymphocytes and macrophages, other immune effector cells are involved in atherosclerotic lesions, while lymphocytes and macrophages prevail over mast cells, which play an important role in the development of atherosclerotic plaque in different vessels. This fact can be explained by the generation of large quantities of proteases, including those produced by macrophages, with their accumulation in the necrotic nucleus site of the plate.

The factors produced by mast cells and macrophages can cause the destruction of intercellular matrix and a further change of low-density lipoproteins. Most examined vessels were positively stained by anti-MCT and CD68 at the level of endothelium, atherosclerotic plaque, in the site of the media and the adventitia, as well as vasa vasorum.

Endothelial cells, mast cells, macrophages and lymphocytes are, obviously, effector cells involved in atherogenesis, with development of atherosclerotic plaques in patients with atherosclerosis of cerebral vessels. Mast cells regulate the conduct of smooth muscle cells (SMCs), probably through their secreted mediators. Collagen fibers produced by smooth muscle cells can prevent rupture of atherosclerotic plaques. Yet, the chymase inhibits mast cell proliferation and collagen synthesis by SMC, thus reducing the stability of the plaque.

The action of proinflammatory cytokines, such as TNF-α, induces the expression of SMC protease. TNF-α-positive mast cells, MMP-cysteine, cathepsin-positive SMC proteases, together with macrophages, suggest a regulatory role in the expression of cellular mediators, mast cell proteases in SMC activation in sites of atherosclerotic plaque rupture. The position of mast cells and macrophages in the vascular wall, especially perivascularly and at intima level assumes an important role in the pathogenesis of atherosclerosis and is probably the main cause of acute cardiovascular diseases

(especially myocardial infarction and stroke).

The role of angiogenesis in the development of atherosclerosis is a complex one and depends on the stage of the pathologic process. Microvessel development in atheromatous plaques is the outcome of neovascularization; these newly-formed capillaries are fragile and prone to rupture with bleeding. Fibrin deposition in plaque, hemosiderin formation and onset of immune inflammation constitute bleeding evidence in atherosclerotic lesions. The role of angiogenesis in atherosclerotic plaque destabilization and destruction remains an open question, but some recent judgments about the primary causes of plaque instability can lead to a promising new interpretation of atherogenesis in general.

The laws of atherosclerotic plaque development (stability and instability) depend largely on the angiogenesis of the atherosclerotic process. Our results show that the comparative immunohistochemical method using vascular markers demonstrates significant pathogenetic aspects in atherosclerotic plaque formation. Macrophages, mast cells and other immune cells play an important role in the development of atherosclerotic plaques and, not the least, in the process of angiogenesis. The question arises: can the angiogenesis inhibition be a therapeutic target in atherosclerosis or how can it be used in metabolic syndrome?

Available data suggest that anti-angiogenic therapy may have a potential impact on the development of neointima in atherosclerotic lesions, and the side effects and harmful factors are likely to inhibit the function of the endothelium and its regeneration. These statements are supported by scientific evidence of a large number of laboratories showing that VEGF has a protective effect on the endothelium of arteries. Recent clinical studies of VEGF inhibitor antibodies, at the administration of bevacizumab, avastin in malignant tumors, indicate that up to 5% of patients treated with avastin may be at increased risk of thromboembolic events, including acute strokes, myocardial infarction and phlebothrombosis. These data allow us to assume that endogenous VEGF may play a certain atheroprotective role in vascularization. The multiple significant biological functions of VEGF and the integrity of vascular endothelium functions are solid arguments that currently limit any anti-angiogenic approach for the treatment of cardiovascular diseases.

In conclusion, we mention that there is a close relationship between plaque morphology and clinical manifestations in patients with atherosclerosis of cerebral arteries. Positive remodeling and a larger plaque site can be found in the mobile plaque, while negative remodeling and smaller plaque site can be found in the stable plaque. The degree of CD105 solubility is linked to the characteristics of the plaque and can serve as a predictor for the soluble plaque. Large sample studies are needed to prove whether soluble CD105 can predict atherosclerotic plaque rupture.

CD105 is a valuable marker of angiogenesis of atherosclerotic plaques, intimal arteries and adventitial vessels, an indicator of the difference degree in the pathological development of atherosclerosis, all these factors that may be of great importance to the introduction of modern methods of research, diagnosis, treatment and prognosis of these diseases.

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