Review

BIOMARKERS FOR UNSTABLE ATHEROSCLEROTIC PLAQUES IN CAROTID ARTERIES

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ABSTRACT

Carotid atherosclerosis is one of the main cerebro-vascular risk factors. It is a complex inflammatory disease resulting in lipid accumulation in the artery wall. The efforts are directed to find reliable biomarkers that can predict the atherosclerotic burden, plaque instability, plaque transformation and the risk for the ischaemic stroke. The modification of the inflammatory factors will help in finding new promising treatment options. Future treatment strategies are directed to stratified medicine – better risk assessment for each patient and optimization of personal management.

Key words: unstable carotid plaque, atherosclerosis, biomarkers, vascular risk

INTRODUCTION

Atherosclerosis is a chronic and systemic disease characterized with the formation of atherosclerotic plaques, affecting mainly medium and large size arteries (1-3). Carotid atherosclerosis is a focal expression of the disease and is one of the leading cause for cerebro-vascular accidents (4).

The French pathologist Jean Lobstein first used the term “arteriosclerosis” (5). Lately Virchow suggests that immune mechanisms are involved in the processes of atherosclerosis. In the past this process was mainly characterized with lipid accumulation in the arterial wall. Nowadays it is accepted as a complex inflammatory disease (6).

The pathological mechanisms of carotid atherosclerosis (Figure 1)

Atherosclerosis is a slowly progressive process, occurring at predilection sites of the arterial walls with disturbed laminar flow (7, 8). Carotid atherosclerosis is mainly at the bifurcation of the common carotid artery and

into the initial part of internal and external carotid arteries (9-11). Endothelial dysfunction and structural alterations permit subendothelial accumulation of LDLs (low-density lipoproteins) (12, 13). A complex cascade of immune mediated mechanisms are involved in the formation of atherosclerotic plaque. The dysfunctional endothelial cells express adhesion molecules (ICAM-1, VCAM-1, and p-selectin) allowing the adherence of monocytes, T-lymphocytes and platelets to the endothelium. Lately they activate pro-inflammatory and pro-thrombotic factors (6, 14). Monocytes migrate into the endothelium mediated by monocyte chemo attractant protein-1(MCP-1). In response of macrophage colony-stimulating factor (M-CSF), they differentiate into macrophages. They are highly phagocytic modified lipids. By receptor-mediated phagocytosis, the accumulation of lipids within intimal cells leads to the formation of foam cells. This culminates in the appearance of fatty streaks in the arteries, altered intima and formation of a lipid core (15). The lipid necrotic core becomes separated from the arterial lumen by a fibrous cap (16). The cap is formed mainly by smooth muscle cells that migrate and proliferate from adventitia to intima. As the process goes the plaque became unstable with

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lipid and calcium deposits, new vessels, cellular debris and active immune cells. The fibrous cap is thinning and tearing. The connective tissue in the cap can be weakened and modified by the matrix metalloproteinases (MMPs) (14, 17). The new vessels from the adventitial vasa vasorum and the inflammation lead to intraplaque hemorrhages. The plaque's surface becomes vulnerable to rupture and

reveals the necrotic core prone to formation of thrombi (18). All these processes are mediated by different pro-inflammatory and anti-inflammatory cytokines and mediators.

Ischaemic stroke mechanisms of extracranial carotid atherosclerosis are artery-to-artery embolism, hypoperfusion and the combination of them.

Figure 1. Pathological mechanism of atherosclerosis.

Carotid plaque biomarkers (Table 1)

Table 1. Biomarkers and their role in atherosclerosis

Biomarkers	Role in atherosclerosis
low-density lipoproteins	Formation of plaque lipid core
acute phase protein (hs-CRP, PTX3)	predictors for the presence of carotid plaque and role in plaque activity
cell adhesion molecules (ICAM-1 and VCAM-1)	mediate leukocyte migration toward vascular wall tissue and subendothelial adhesion
Matrix Metalloproteinases	role in matrix degradation and plaque destabilization
Fibrinogen	role in the coagulation cascade and thrombosis
proinflammatory cytokines(IL-6,IL-18,IL-23,IL-27)	Plaque destabilization and instability

One of the most important risk factors for atherosclerosis is the plasma level of low-density lipoprotein cholesterol. In 2016 European Society of Cardiology and European Atherosclerosis Society published the new guidelines for the management of dyslipidemia (19). The Task force created the SCORE chart to estimate the 10 years fatal cerebrovascular risk and the treatment targets. The increased level of LDL-C, triglyceride-rich lipoproteins and some lipoprotein subfractions influence also vascular inflammation. They produce pro-inflammatory mediators that activate the mononuclear cells, disable the

endothelial cells and impair functionally HDL-C (17, 20, 21).

Several investigations study the inflammatory mechanisms of atherosclerosis. The identification of proper immunological biomarker and its correlation with the cerebrovascular risk in patients with carotid atherosclerosis will give better diagnostic and treatment options. It is known that there are circulating biomarkers that reliably predict the risk of ischaemic stroke.

Large epidemiological studies as Physicians' Health Study (22), Women's Health Study (23), Framingham Heart Study task (24) find

that high sensitive C-reactive protein (hs-CRP) in serum has a predictive role for ischaemic stroke. Studies indicate that the high serum level of hs-CRP can suppose the presence of atherosclerotic plaque in carotid arteries but without a correlation with the degree of the stenosis (25, 26). Studies on various inflammatory factors and the onset of a new cerebro-vascular accident, again find a connection with the high serum hs-CRP (24). It is also an independent predictor of the early carotid atherosclerosis as it participates in plaque activation (27).

Other acute phase protein that is connected with atherosclerosis is pentraxin-3 (PTX3). Some data show that its plasma levels correlate with the presence of plaques (28). Immunohistochemical study of paraffin and frozen sections indicate that macrophages, mainly foam cells and neutrophils, expressed PTX3 in advanced atherosclerotic lesions (29). But its role as a predictor of cerebro-vascular accidents is still not investigated.

Of interest are several types of immunoglobulin-like cell adhesion molecules (CAM), whose function is to support, organize and mediate leukocyte migration toward vascular wall tissue, with subsequent stable adhesion and migration therein. Representatives of this group are the biomarkers ICAM -1 and VCAM-1. They have a proven role in the endothelial dysfunction and in the processes of atherosclerosis. VCAM-1 promotes adhesion of lymphocytes and monocytes by binding beta 1 integrins (30). ICAM -1 is a major endothelial ligand for LFA-1, which mediates strong adhesion of cells to damaged endothelium at the site of inflammation (31). The expression of ICAM -1 and VCAM-1 on the endothelial cells is after the stimulation of pro-inflammatory mediators. After the adhesion of the leucocytes to the damaged endothelium, the cell adhesion molecules (ICAM-1 and VCAM-1) are eliminated by endothelial cells. They are released in the serum and circulate freely as variant structures that have no transmembrane and cytoplasmic divisions. Therefore, they become soluble CAM (sICAM -1 and sVCAM-1). This makes it easy to study them in the peripheral circulation. Some studies find that sVCAM-1 is higher in patients with carotid stenosis compared with healthy controls and has a positive correlation with the plaque instability (26, 32).

Matrix Metalloproteinases (MMP) are a group of proteases involved in matrix degradation and plaque destabilization (33, 34). They are a group of zinc-dependent endopeptidases that destroy proteins in the extracellular matrix. MMPs could also influence endothelial cell function and vascular tissue remodeling during various biological processes (35). Some studies try to find connections between the MMP-9 activity and the histological type of the carotid plaque (36). But still it does not mean a reliable serum marker. Some authors prove a connection between the histological features of the plaque and the serum levels of MMP-1, MMP-7 and tissue inhibitor of matrix protease (TIMP-1) (33). Recent study finds connection between the serum level of MMP-2 and MMP-3 and the ultrasound echogenicity of the plaque (37). Studying the role of MMPs will help not only for finding a good serum marker for plaque destabilization but will help in pharmacotherapy with the use of the MMPs inhibitors.

Fibrinogen plays an important role in the coagulation cascade and its plasma level can easily be measured. Some studies find that fibrinogen is higher in patients with carotid stenosis and even in symptomatic stenosis (38, 39). It can be used as nonspecific indicator of inflammation but not as an independent predictor of atherosclerosis progression (40).

Some pro-inflammatory cytokines are strongly related to the processes of atherosclerosis. IL-6 is associated with presence of carotid plaques and plaque instability (26, 38, 41). There are some hypothesis that IL-23 and IL-27 are also increased in carotid atherosclerosis (42, 43) and IL-18 is connected with plaque destabilization (44). Still their role and action in the inflammation and plaque development is not clear.

CONCLUSION

Different studies are trying to find reliable biomarkers that can correspond to the mechanism of carotid plaque formation and destabilization. The efforts are directed to find the proper biomarkers that will indicate the presence of atherosclerosis, the degree of the stenosis, plaque instability and the onset of the cerebro-vascular event. It is one step towards stratified and personalized medicine.

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