



## PENTRAXIN 3 AS A NOVEL MARKER IN CARDIOVASCULAR DISEASES?

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### **ABSTRACT**

Pentraxin 3 (also known as TNFAIP5, TSG-14) belongs to the superfamily of proteins characterized by cyclic multimeric structure. Pentraxin 3 (PTX3) is synthesized locally at the inflammatory sites by endothelial and smooth muscle cells upon exposure to inflammatory signals such as IL-1 $\beta$ , TNF- $\alpha$  or ox-LDL, but not IL-6. Furthermore, PTX3 is highly expressed in vascular cells and myocardial cells in patients with cardiomyopathy. These data suggest that pentraxin 3 may be a useful biomarker for local vascular inflammation and cardiovascular system disorders.

### **INTRODUCTION**

Cardiovascular diseases (CVDs) are number one cause of death worldwide. An estimated 18 millions people die from CVDs, which is 30% of all global deaths. Around 8 millions are due to coronary heart disease and around 7 millions due to stroke. Most affected are low- and middle-income countries, almost 80% of CVDs deaths take place in those countries. Statistics say, that in 2030 around 24 millions of people will die from cardiovascular diseases (1).

Cardiovascular diseases are a group of disorders which include ischemic heart disease (IHD), congestive heart failure and stroke.

Ischemic heart disease is one of the main causes of deaths in the developed countries. There are two leading symptoms of IHD: acute myocardial infarction and angina. Acute myocardial infarction (AMI) manifests when the major coronary artery is blocked by the clot with a deficiency of nutrients and oxygen. Acute myocardial infarction diagnosis is based on the chest pain history, changes in electrocardiogram and by the elevated serum biomarkers (troponin T or I and/or CK-MB). Angina reveals as a chest pain of ischemic heart muscle. The cause of above-mentioned chest pain is ongoing atherosclerotic process which leads to the partial or total vessel occlusion. Unstable angina pectoris is diagnosed in the same way as AMI. The 30 days mortality after cardiac event is still high; around 33% of patients die before they reach the hospital Emergency Department. Even in the very well equipped, high level Coronary Care Units the mortality hits 7%. (2)

Congestive heart failure (CHF) is described as a disorder in which the pumping action of the heart is impaired. It manifests by the fatigue, retention of fluids in the tissues and organs or breathlessness (3). CHF is mostly the end-stage of many heart diseases (4).

Stroke occurs when the blood flow is interrupted to the part of brain. The lack of oxygen and nutrients leads to the necrosis of the brain cells. There are two types of stroke : ischemic stroke and hemorrhagic stroke. Ischemic stroke occurs as an result of occlusion within a blood vessels supplying blood to the brain. Hemorrhagic stroke occurs as the result of rupture of a blood vessel and bleeding to the surrounding brain tissue. There are two types of weakened blood vessels which usually cause the hemorrhagic stroke : arteriovenous malformation and aneurysms (5).

### INFLAMMATION AND CARDIOVASCULAR DISEASES.

Around thirty years ago we understood atherosclerosis as a “lipids storage disease”. Lipids were deposited within on the artery and grew up till they totally blocked the flow of blood. The result of those facts were cardiovascular disorders such as stroke or myocardial infarction (6).

Today we understand better the patomechanism of atherogenesis. We know also, that the inflammation plays a important role in this process and that atheromatous plaque develops within, not “on” but within the arterials walls. Most of the vascular events are caused by the rupture of unstable plaque , which result in the thrombus formation.

Inflammation participates in atherosclerosis process from the start till the end. There are many risk factors which trigger atherosclerosis and inflammation process such as high-fat diet, obesity, smoking, hypertension, insulin resistance, infection, ionizing radiation etc. These factors can initiate the expression of adhesion molecules (VCAM-1) by endothelial cells and attach the leukocytes and macrophages to the arterial wall (6). Oxidized lipids accumulated in the intima-media can induce the expression of VCAM-1 through the inflammatory pathways by mediators such as nuclear factor- $\kappa$ B, tumor necrosis factor (TNF- $\alpha$ ) and interleukin-1 $\beta$ . Figures 1 and 2 show the inflammation pathway and effects.

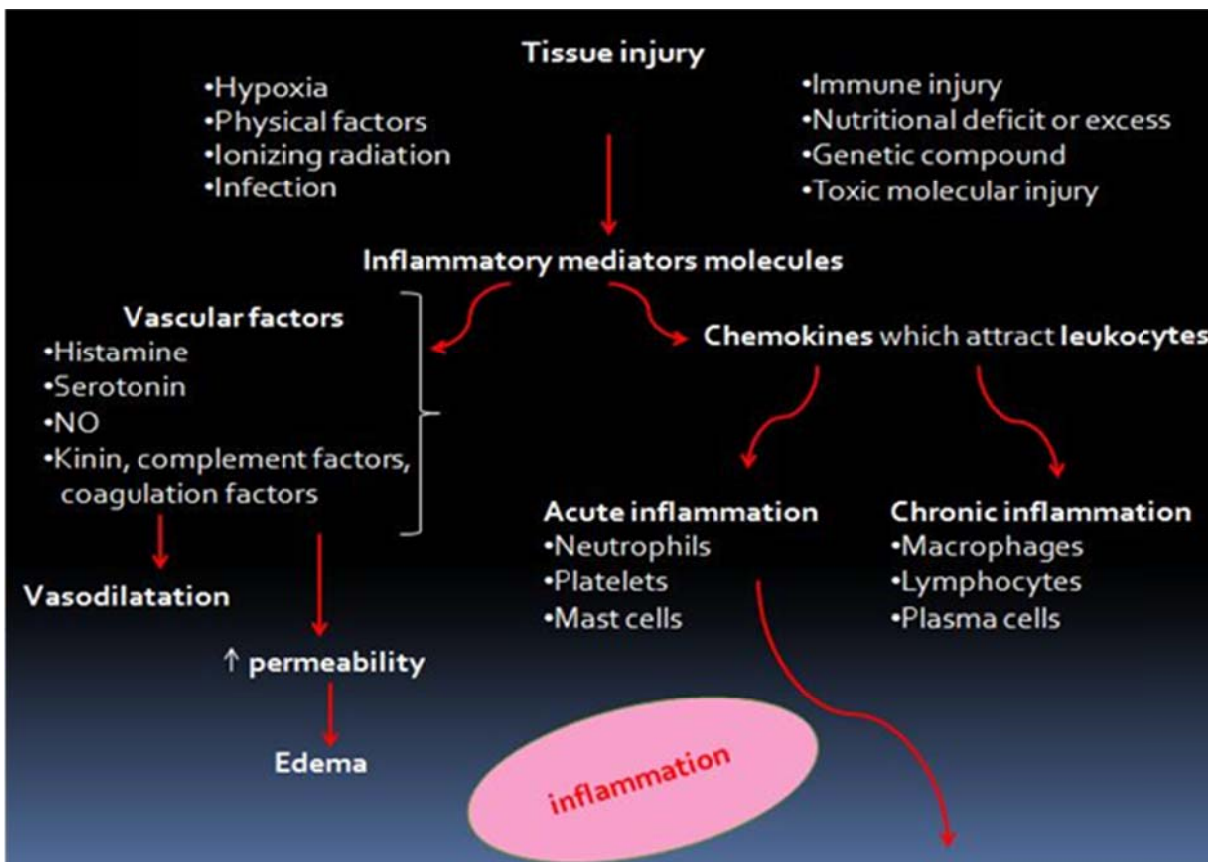


Fig.1 Inflammation pathways (Authors Fulgheri G.,Malinowski B. “The role of IL-33 in the inflammation process of asthma and atherosclerosis”)

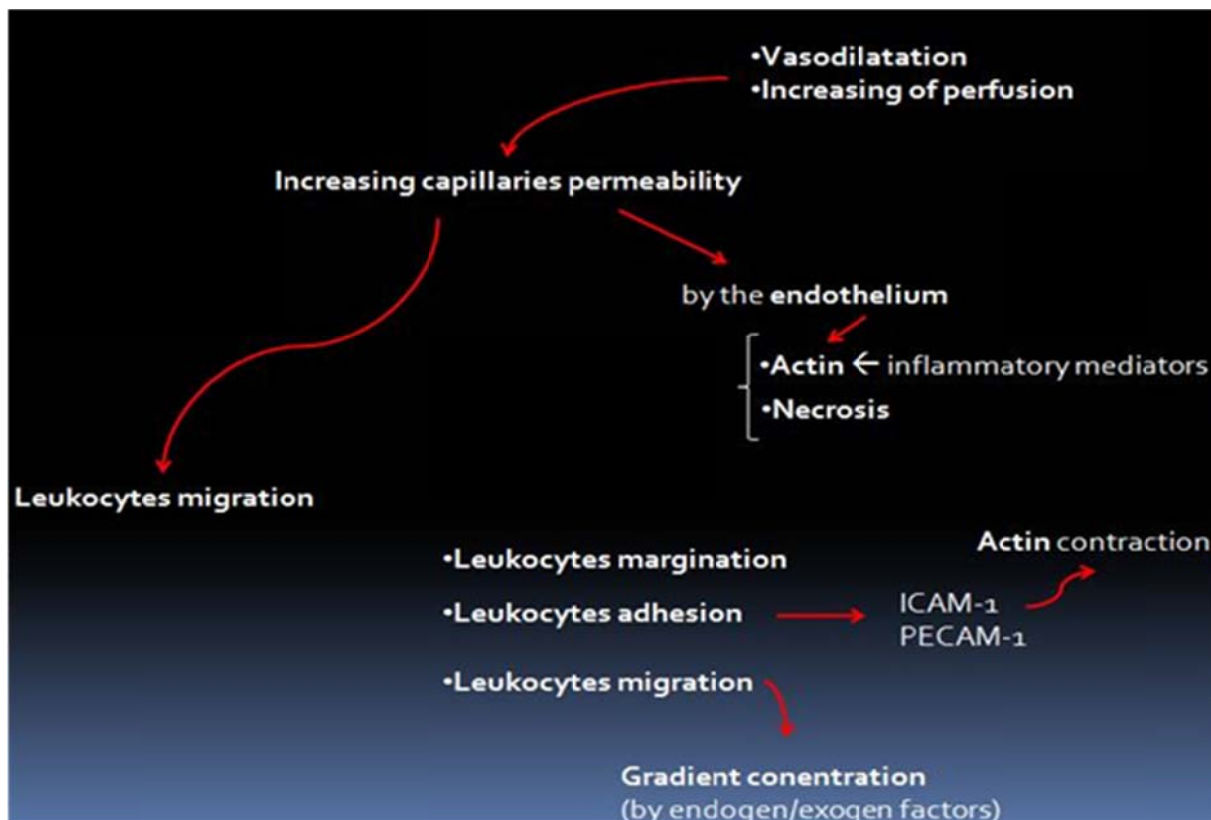


Fig.2 Inflammation effects (Authors Fulgheri G., Malinowski B. "The role of IL-33 in the inflammation process of asthma and atherosclerosis").

### THE PENTRAXIN SUPERFAMILY

The first described pentraxin was a C-reactive protein (in 1930), that is an acute phase protein produced in the liver under inflammatory conditions (7). Serum amyloid P was found subsequently that possessed in 51% a similar amino acid sequence to CRP. The name "pentraxins" comes from their appearance in electron microscopy (disc-like structure with pentameric symmetry)(8). In the early 1990s a new pentraxin was identified as an IL-1-inducible gene in endothelial cells (PTX3)(8). Pentraxin 3 (also known as TNFAIP5, TSG-14) belongs to the superfamily of proteins characterized by cyclic multimeric structure. Acute phase proteins: C-reactive protein (CRP) and serum amyloid P (SAP) are the classic short pentraxins. They are produced in the liver in response to the inflammation process (9). The prototypic long pentraxin PTX3 gene is localized on human chromosome 3 band q25 which include three exons, separated by two introns. First two exons encode specific N-terminal domain which is unique for PTX3 (9).

### PTX3 AND INFLAMMATION

The normal concentration of PTX3 in the blood is low (in the rat approx. 25 ng/ml and in the humans approx. 2 ng/ml) but under inflammatory conditions increases rapidly up to 200-800 ng/ml. Garlanda et al proved in vivo the role of pentraxin 3 in inflammation using PTX3 transgenic mice model. This investigation showed increased resistance to LPS toxicity and higher levels of IL-10 and macrophages were primed for increased nitric oxide production in response to INF- $\gamma$  and TNF (10).

Pentraxin 3 is synthesized locally at the inflammatory sites by endothelial and smooth muscle cells upon exposure to inflammatory signals such as IL-1 $\beta$ , TNF- $\alpha$  or ox-LDL, but not IL-6. Agonists of toll-like receptor (TLR) family stimulates PTX3 production (Fig.3) (10).

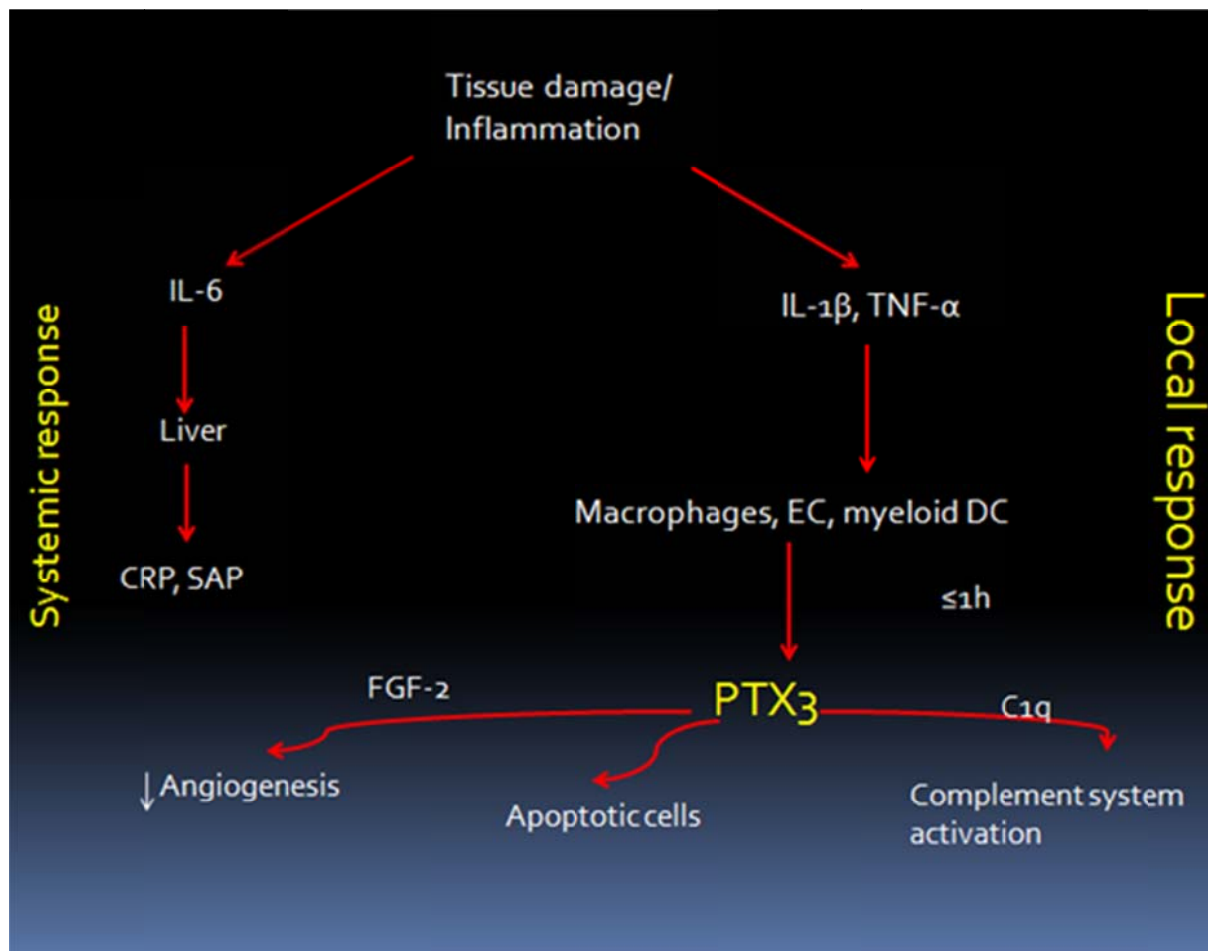


Fig.3 Systemic and local inflammatory response.

Furthermore, PTX3 is highly produced in vascular cells and myocardial cells after cardiomyopathy (12). It has been discovered, that plasma PTX3 is significantly higher in patients with acute myocardial infarction and congestive heart failure (11,12). It suggests that pentraxin 3 may be a useful biomarker for local vascular inflammation and cardiovascular system disorders (13, 14).

### LIGANDS OF PTX3

The first ligand of PTX3 is complement component C1q. The interaction between PTX3 and C1q is calcium-independent and leads to the activation of classic complement pathway (15). Similar to CRP and SAP, PTX3 makes a complex with apoptotic cells and inhibits their recognition by dendritic cells (DCs). Bottazzi et al. showed that PTX3 incubated with apoptotic cells enhanced C1q binding and C3 deposition on the cell surface. It suggests, that PTX3 is involved in the clearance of apoptotic cells (16).

PTX3 has also an ability to bind fibroblast growth factor 2 (FGF-2). This complex prevents the binding of FGF2 to its cognate tyrosine kinase receptors, leading to inhibition of the angiogenic activity of the growth factor. It has been suggested that PTX3 may inhibit FGF2-mediated tumor angiogenesis and growth (17).

### PTX3 AND CARDIOVASCULAR DISEASE.

The homology between PTX3 and CRP suggests, that PTX3 can be a valuable diagnostic and prognostic tool. The facts such as increased level of PTX3 in the heart during local inflammation process, the expression by vascular cells in the response to inflammation and the occurrence in atherosclerotic lesions led to the investigation of the role of PTX3 in cardiovascular disease. Peri et al showed, that PTX3 peaked in plasma 6-8h after cardiac event, and no correlation with CRP was observed (18).

In 2009, Jenny et al demonstrated the association of circulating PTX3 and CVD risk factors in healthy patients free of clinical CVDs. In this study PTX3 was associated with CRP but not with serum amyloid P (SAP) and with cardiovascular risk factors such as age, insulin resistance and fasting glucose (19).

### **CONGESTIVE HEART FAILURE**

Elevated concentrations of PTX 3 were found in patients with heart failure (HF) by Suzuki et al and Kootoka et al. Interestingly, pentraxin 3 was an independent predictor of outcome in these patients. These findings have been extended by two studies conducted by Ishino et al and Kootoka et al. They have found that the best prediction value was reached by the combination of three biomarkers such as heart fatty acid binding protein (H-FABP), BNP and PTX3 (12, 13).

Inoe et al assessed the value of plasma PTX3 in patients with CVDs. In this study several biomarkers were investigated : NT-proBNP, CRP, myeloperoxidase (MPO) and creatine phosphokinase (CK). Final diagnoses included AMI in approximately 66%, angina pectoris 3%, aortic dissection 2,5% and atypical chest pain in 9% of patients. Multiple logistic regression analysis showed that PTX3 and NT-proBNP significantly and independently predicted CHF or cardiac death (20).

### **UNSTABLE ANGINA PECTORIS.**

In the current year, Inoe et al, reported that pitavastatin suppress PTX3 gene expression in the human endothelial cells, among more than 6000 other human genes. They suggest PTX3 to be a new biomarker for inflammatory vascular disease. Recently, Inoe et al developed a new ELISA method for measurement of human plasma PTX3. By using this method, the authors showed that PTX3 may serve as a predictor of unstable angina pectoris in patients suspected of acute coronary syndrome. However, it still remains unclear why the level of PTX3 is increased in patients with ACS (21).

### **STABLE ANGINA PECTORIS.**

Koga et al, performed the optical coherence tomography (OCT) in patients before percutaneous coronary intervention (PCI). The aim of the study was to assess whether plasma PTX3 can be a predictor of thin-cap fibroatheroma (TCFA) in patients with stable angina. The TCFA was defined as a plaque with lipid contents of >90% and with the thinnest site of the fibrous cap measuring <70µm. Three biomarkers such as PTX3, hsCRP as an inflammatory markers and plasma 8-isoprostane as an oxidative stress marker were measured in the group of patients. Multiple logistic regression analysis showed that PTX3 level was the significant and independent factor that correlated with TCFA presence. These results suggest that plasma PTX3 may be a useful biomarker reflecting plaque vulnerability in vivo (22).

### **LEFT VENTRICULAR DYSFUNCTION.**

Suzuki et al assessed the influence of PTX on cardiac hypertrophy and left ventricular dysfunction (LV). Expression of PTX3 was induced in the heart by pressure overload caused by transverse aortic contraction operation (TAC). To investigate the mechanism of influence of PTX3 on LV dysfunction, Suzuki used two different genotypes of mice – PTX3 systemic knock-out (PTX3-KO) mice and PTX3 cardiac specific overexpression (PTX3-TG) mice. Both types of mice underwent TAC operation. The ratio of heart weight after the operation was significantly increased in PTX3-TG . Remodeling and interstitial fibrosis demonstrated by echocardiography and microscopic analysis were suppressed in PTX3-KO mice and were higher in PTX3-TG. It suggests that a local inflammatory marker has an accelerated effect on hypertrophy and LV dysfunction by increased overload (23).

### **ATRIAL FIBRILLATION.**

Increasing evidence indicates that inflammation participates in the pathogenesis of atrial fibrillation (AF), but the exact mechanism is still unknown. Soeki et al, performed the experiment to demonstrate whether local level of PTX might be a specific marker for the local inflammatory process of AF. Blood samples were taken from patients with atrial fibrillation undergoing pulmonary veins isolation. The concentration of CRP, IL-6, TNF-α, and PTX3 were measured in the plasma of periphery and left atrial appendage (LAA). Interestingly, PTX3 concentration in the periphery and LAA

was significantly higher in patients with AF but not in the control group. Other biomarkers have not shown differences in concentrations in periphery and LAA between patients with AF and the control group. It suggests that PTX3 may be superior to other biomarkers which are reported to be elevated in AF (24).

## PERSPECTIVES

Traditional pentraxins such as CRP and SAP are expressed in the liver in the response to inflammatory mediators, most prominently IL-6. PTX3 is structurally related but distinct from classic pentraxins with different ligand's specificity. In this perspective, it is interesting that PTX3 levels are not significantly correlated with CRP and SAP. Therefore, PTX3 may be an independent contributor among inflammatory components in cardiovascular events being produced and released locally (18).

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