An influence of lipoproteins on fibrinolysis and pleiotropic effect of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors in aortic stenosis - links with inflammation and hemostasis

Aortic stenosis (AS) is the most common cause of acquired valvular heart disease among adults older than 65 years, with no available pharmacological treatment to inhibit the disease progression. Unfortunately, due to old age of patients, the all-cause mortality is very high. AS is considered as an active inflammatory process that occurs in response to endothelial damage through the high shear stress. Both inflammation and coagulation have been documented to be involved in AS development and progression. Our research indicates that progression of AS is associated with expression of tissue factor (TF). The highest expression of TF was found in the area of fatty-calcium deposits accompanied by abundant macrophages infiltration. Moreover, we found a positive correlation between the TF valvular expression and the level of low-density lipoprotein (LDL) cholesterol and TF in the blood, which supports the hypothesis that hypercholesterolemia leads to the activation of inflammatory processes and the activation of the coagulation system. We have also showed that impaired fibrinolysis in patients with AS was associated with increased expression of fibrin and the major fibrinolysis inhibitor (PAI-1) as well as with the degree of valve calcification and disease progression. However, the role of coagulation and fibrinolysis in calcification of aortic valves has not been fully elucidated yet. Moreover, growing evidence indicates that the level of lipoproteins and their oxidized forms are associated with the degree of inflammation and tissue remodeling in AS. Despite, there are few studies regarding the associations between lipoprotein(a) [Lp(a)] and AS development and progression, the mechanisms of Lp(a) action in promoting leaflets mineralization are still poorly understood. Lp(a) has a prothrombotic and proatherogenic properties and its structure is highly homologous with plasminogen – the main fibrinolytic protein. Competition between apo(a) and plasminogen for binding sites suggests that Lp(a) is able to inhibit fibrinolysis and promote coagulation. It has been demonstrated that lipid lowering therapy with statins does not reduce the rate of AS progression. However, clinical trials for proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have demonstrated that such treatment markedly reduced LDL cholesterol level, as well as Lp(a) and increased HDL cholesterol concentration in patients with hypercholesterolemia. Interestingly, recent analysis of the *FOURIER* randomized clinical trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) revealed that long term treatment with evolocumab reduced incidence of AS events compared with placebo. Moreover, data from *in loco* study demonstrated that valvular expression of PCSK9 was evidenced in both calcified and non-calcified aortic valve leaflets, while the calcified ones were characterized by significantly higher PCSK9 levels. Recently, it has been suggested that PCSK9 inhibitors, beyond lipid-lowering action, might present pleiotropic effects on hemostasis and inflammation. We hypothesize that high levels of lipids lead not only to enhanced valvular inflammation and activation of coagulation but also impair fibrinolysis which together drive the tissue remodeling. Moreover, we presume that PCSK9 inhibition might be associated with attenuated pro-inflammatory factors release and decreased activation of coagulation cascade in AS.

The goal of the presented project is a multidirectional assessment of the Lp(a) and LDL influence on inflammation and activation of the coagulation system and fibrinolysis (1) in AS patients' blood, (2) within stenotic valves and in (3) *in vitro* cultures of valve interstitial cells (VICs) together with mechanistic study of PCSK9 inhibitors to explain their potential benefits and pleiotropic effects. Postulated research hypothesis will be verified using various methods such as clot lysis time, immunofluorescence, *in vitro* cultures, computed micro-tomography, proteomic and genetic analysis, and ELISA tests. A total of 100 consecutive patients (50 AS patients with hypercholesterolemia and 50 AS patients with normal lipid profile) with isolated AS undergoing aortic valve replacement will be recruited. Stenotic valves obtained during the surgery will serve as a source of cells for *in vitro* cultures including mechanistic experiments. Healthy valves obtained from autopsy donors will serve as control for evaluation of valvular markers.

In our opinion, verification of postulated objectives is important for human health and might allow to understand the pathobiology of AS and thus provide rationale for the implementation of PCSK9 inhibitors in retarding valvular calcification in AS patients with high levels of circulating lipids.