Non-vitamin K antagonist oral anticoagulants (NOACs) as modulators of valvular calcification and inflammation in aortic stenosis in humans

Aortic stenosis (AS) is the most common cause of acquired valvular heart failure in adults, with no available pharmacological treatment to inhibit the disease progression. AS shares a few similarities with atherosclerosis, at least in terms of risk factors and crucial underlying pathomechanisms. Aortic valve replacement is the only option for AS treatment, resulting in the all-cause mortality up to 39%. Thus, it is of major importance to search for long-term therapeutic strategies aimed to retard disease development in subjects with mild-to-moderate AS.

It is known that thrombin and factor (F)Xa, as key coagulation factors, are modulators of the atherosclerotic plaque phenotype and that they act as specific signaling molecules activating protease activated receptors (PARs) present, among others, on valve interstitial cells (VICs). Signal transmission via PAR enhances inflammation, endothelial cells activation, and leukocytes migration. Non-vitamin K antagonist oral anticoagulants (NOACs), such as rivaroxaban, apixaban, and dabigatran are direct thrombin or FXa inhibitors, but to date it has not been shown whether they can affect the activity of pathways involved in valvular calcification. In mice treated with NOACs, decreased expression of FXa and thrombin within atherosclerotic plaques led to their stabilization. The role of the coagulation proteins in the valvular calcification during AS progression has not been clarified yet, however taking into account the similarity between pathogenesis of atherosclerosis and AS, it seems reasonable that NOACs can modulate processes involved in AS progression through reduced PARs activation. In addition, NOACs may affect coagulation activation on phospholipids- and metalloproteinase-rich microvesicles (Mv) released by VICs during calcification. Rivaroxaban can inhibit the expression of nuclear factor (NF)-κB pathway, a master regulator of inflammatory responses, and in turn, the expression of tissue factor, which initiates coagulation cascade. Rivaroxaban can also inhibit the metalloproteinase-9 through the NF-κB-mediated signaling. Importantly, the COMPASS trial has shown that the rate of cardiovascular events was lower in patients taking low dose of rivaroxaban and aspirin as secondary prevention. Increased hemodynamic forces in AS patients may induce the release of neutrophil extracellular traps, called NETs. NETs play an important role in activation of coagulation system and are mediators of inflammation. Moreover, a relationship between the intensity of NETosis and the severity of atherosclerosis was shown. To our knowledge, the presence of NETs in stenotic valves has not been studied yet. We suppose that NETosis may exacerbate inflammation and enhance prothrombotic state within stenotic valves.

The main goal of this proposal is to assess the effects of NOACs on the mechanisms, particularly those associated with the PAR signaling pathway, leading to valve calcification in patients with AS. Along with Raman microscopy and the micro-computed-tomography (micro-CT), we will perform a battery of advanced *in vitro* and *ex vivo* assays testing valvular calcification biology upon anticoagulation treatment.

This project addresses an issue with major implications to human health and it may clarify whether in AS patients therapy with NOACs can retard disease progression, at least in patients with mild-to-moderate AS, and improve the benefit-to-risk profile of anticoagulant therapy and indicate NOACs as potential drugs to inhibit valvular calcification in AS patients.