**Antithrombin activity as a modulator of plasma fibrin clot properties in patients with antithrombin deficiency: effects of glycation and oxidation**

Antithrombin (AT) is the main human endogenous anticoagulant that inactivates thrombin, and activated factor X. AT deficiency occurring in up to 0.17% of European population, mostly inherited in an autosomal dominant pattern, is associated with 14-times higher risk of venous thromboembolism (VTE). About 65% of patients with hereditary AT deficiency develop thrombosis. Thus, it is of major importance to search for pathomechanisms of prothrombotic tendencies associated with AT deficiency.

The incidence of thrombosis in AT-deficient patients increases in the presence of additional risk factors, such as mutations of particular clotting factors and can be induced by factors that do not usually cause thrombus formation, such as minor injury, surgery or pregnancy. Recently, the MEGA study has shown that even a mild AT deficiency is associated with increased risk of recurrent VTE. Hereditary AT deficiency can be divided into two types: type I presenting low blood level and activity of AT (about 50%) and type II AT deficiency with normal antigen level but reduced activity. There are available plasma-derived AT concentrates to replace AT in different clinical states, i.e. in AT-deficient patients before surgery. However, the appropriate use of AT replacement therapy remains unclear. Since, AT is a major thrombin inhibitor, it influences the structure of clots. It is known that clots formed at high thrombin concentrations are more compact and resistant to lysis due to limited access of fibrinolytic enzymes. Such prothrombotic clot phenotype is a known risk factor for thrombosis. We hypothesize that AT deficiency is related to unfavorably modified fibrin properties. Thus, a comprehensive assessment of fibrin clot features, clot proteomics, along with the thrombin generation potential and thromboelastography in subjects with diagnosed AT deficiency will be performed. Another goal of the current project is to explain whether *in vitro* normalization of AT activity using a purified protein added to AT-deficient plasma will improve fibrin clot properties. It is also not known whether subjects with reduced AT activity (type II AT deficiency) or both activity and antigen levels (type I AT deficiency) are characterized by similar clot properties. Additionally, this project is aimed to investigate an influence of posttranslational modifications of AT molecule, such as glycation and oxidation, on fibrin clot phenotype using both purified protein and plasma obtained from patients with type 2 diabetes mellitus and advanced atherosclerosis. The main goal of this proposal is to assess the effects of AT activity and AT modifications such as glycation and oxidation on plasma fibrin clot phenotype. Along with analysis of most important fibrin clot properties, including fibrin clot porosity and susceptibility to lysis, thrombin generation, scanning electron and confocal microscopy imaging, and additional parameters known to affect fibrin structure and function, i.e. plasma lipid profile, plasma levels of plasminogen or antiplasmin, plasma proteins oxidation or glycation, we will perform a battery of advanced *in vitro* assays, testing an influence of AT molecule modifications on fibrin clot features.

This project addresses an issue with major implications to human health. Besides limited number of case-reports, it is unknown whether prothrombotic clot phenotype is associated with AT deficiency or if normalization of plasma AT activity can return fibrin clot properties to values observed in controls. There is a lack of data regarding the influence of inherited AT deficiencies or known disease-related AT posttranslational modifications such as glycation and oxidation on fibrin clot structure and function. This project will help to understand pathomechanisms of prothrombotic tendencies associated with AT deficiency, which might have potential clinical implications, including appropriate use of AT concentrates.