Multiple sclerosis (MS) is an inflammatory degenerative disease of central nervous system (CNS) in which damage of the myelin oligodendrocyte glycoprotein – a soluble, basic protein of the myelin sheath is the major pathology. MS is responsible for the majority of neurological disability among young adults. Data from the World Health Organization indicate that, its median prevalence is of 80 per 100.000 people in Europe. Histopathologically, MS is considered as a heterogenous neurological disease with a variable clinical course (MS is classified into relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS) subtypes) and several pathophysiological mechanisms, such as, focal inflammatory infiltrates, demyelination, gliosis, axonal/neuronal damage within the CNS and in some cases remyelination and repair mechanisms. MS predominantly affects young adults around 40 years old. Only about 10% of MS cases have a PP course, while most cases of RR MS is converted to SP, generally decade after clinical onset.

Blood platelets are multifunctional cells which have long been suspected in the pathophysiology of various neurodegenerative diseases. In MS the immune cells, mainly autoreactive T cells infiltrate the CNS, release proinflammatory cytokines that activate macrophages, lead to the inflammation of white matter and subsequent myelin destruction. Blood platelets possess a large variety of compounds stored in α-granules, a numerous membrane receptors, immunomodulatory mediators and cell adhesion molecules, by which they affect the permeability of blood-brain barrier and support the infiltration of autoreactive T-cells to form new neuroinflammatory lesions in CNS. There is quite a lot of work, together with our reports, confirming the platelet abnormalities in MS patients. Some papers demonstrated that blood platelets are significantly activated in MS and they are trapped in chronic active demyelinating lesion. However, the specific significance of platelets in the pathology of MS still needs to be clarified.

In our latest study of platelet proteome we have shown the increased level of prothrombotic proteins (mainly fibrinogen and apolipoprotein A-I) in blood platelets delivered from SPMS patients. Fibrinogen (Fg) is the principal protein of blood clotting and is also involved in platelet aggregation.

Fg presents in platelets may be synthesized in megakaryocytes (precursors of blood platelets) or in platelets (platelets contain part of the transcriptome received from megakaryocytes) or may originate from a pool of plasma Fg (synthesized in hepatocytes).

In the current project we plan analysis of sequence and expression of mRNA for Fg in platelets and megakaryocytes obtained from patients with SPMS. As well as we want to examine miRNA expression profiles since these molecules are responsible for regulation of mRNA translation process.

Finding the molecular basis of enhanced level of Fg in the blood platelets of patients with SPMS can provide a new perspective on the current knowledge of high activation and reactivity of platelets in SPMS, and especially their increased aggregation and adhesion.

The comparative analysis of sequence and expression for Fg mRNA and expression for miRNAs will be conducted both in megakaryocytes and platelets. In addition, using the ELISA assay, we compare the level of Fg in megakaryocytes derived from SPMS patients and in control group. So that we determine whether an increased synthesis of protein already occurs in the megakaryocytes in the course of MS.

In order to verify whether the increased amount of Fg could be absorbed by the platelets from the plasma, we plan to carry out a comparative analysis of Fg presents in platelets and in plasma of SPMS patients based on differences in post-translational modifications evaluated by mass spectrometry.

Platelet aggregation is dependent on Fg. To assess the significance of an increased amount of intra-platelet Fg in platelet aggregation a flow cytometric analysis will be used. After platelet isolation, and stimulation with the typical agonist such as ADP, the degree of platelet aggregation will be measured on a digital analytical flow cytometer. Moreover, we use a transmission electron microscope to assess the morphology of aggregates and also to determine a participation of platelet Fg.

Megakaryocytes for all analysis will be isolated from whole blood of patients and volunteers by using a cell sorter. Blood platelets will be isolated by BSA-Sepharose 2B gel filtration from plasma after removing of contaminants of erythrocytes/leukocytes by DynaBeads. Purity of platelet suspension will be assessed by using a flow cytometer.

The complexity of MS and its unknown etiology cause of the current pharmacological treatments are only directed towards modifying the course of the disease, but there is no effective cure for this pathology. Our results may provide a basis for the development of much more specialized neurological research in MS, not only in SP phase of MS. The understanding of molecular processes of platelet function underlying the SPMS might be beneficial and may improve more specific therapies of MS in the future.