1. Research project objectives.

The global incidence of aneurysmal subarachnoid haemorrhage (aSAH) is 6.67 per 100,000 persons: nearly half a million individuals suffer from aSAH each year. Of these, approximately 15% will die before reaching hospital, 25% die within 24 hours, and 45% die within 30 days. A major contributor to death and disability in the survivors of aSAH is Delay Cerebral Ischemia (DCI). DCI mechanisms are poorly understood; no effective pharmaceutical treatment options are available. Unfortunately, most experimental drugs fail to elicit significant improvement in the outcome of patients with aSAH, with only oral nimodipine demonstrating significant effects. It does not counteract DCI, but does improve neurological outcome**.** Therefore, to identify novel therapeutic targets, a new approach to investing in the pathophysiology of DCI at the molecular and cellular levels is required. It is known that the presence of subarachnoid hemoglobin induces the release of reactive oxygen species (ROS), resulting in peroxidation of membrane lipids of endothelial cells and proliferation of smooth muscle cells. In the brain compartment, enzymatic roles protecting against free radical production are played by superoxide dismutase (SOD), glutathione system (including glutathione peroxidase (GPx), and reductase (GR) and thioredoxin system (including thioredoxin peroxidase (TPx) and reductase (TR)) all of these need nicotinamide adenine dinucleotide phosphate (NADPH), which main source is glucose-6-phosphate dehydrogenase (G6PD). Following aSAH, these enzymatic systems become downregulated or modulated in a way that reduces their antioxidant capabilities. Additionally, secondary messengers (miRNA) may also play an important role in this process; in addition, general vascular reactivity may be controlled by specific miRNA, which expression may correlate with the risk of DCI. Therefore, we hypothesize, that the risk of DCI depends on the activity and concentration of SOD, GPx, GR, TPx, TR, G6PD, concentration of NADPH and expression of miRNA regulating antioxidant and vascular homeostasis.

2. Basic research in the project.

The analyzed group will consist of about 132 patients with aSAH and 44 volunteers (patients with nerve entrapment syndrome). From each patient, a serial blood samples will be collected.

To identify metabolites associated with DCI, liquid chromatography with tandem mass spectrometry (LC-MS/MS) will be used in 704 patients. This will allow for the identification of DCI-associated differences in metabolites related to oxidative stress and antioxidant mechanisms. Using ELISA (Immunoenzymatic assay), we aim to assess concentration and activity of the key enzymes of antioxidant mechanisms, namely: SOD, GPx, GR, TPx, TR, G6PD and NADPH concentration in all aSAH patients.

The analysis will be performed with ELISA in 132 plasma samples in 5 time points and once in the control samples. Statistically significant differences between groups will be established (with DCI and without). It will be determined, which antioxidant enzyme works impropriety in DCI patients.

Then the miRNA analysis will be performed. We will profile nearly all microRNAs detectable in human plasma, in 20 patients after aSAH (10 with DCI and 10 without) at 2 time points. Those miRNAs which will be assessed as statistical significantly according to DCI status, will be validated (its expression will be checked) in the full group consisting of 132 aSAH patients and in the controls. It will be confirmed whether miRNA expression has changed with DCI and whether the miRNAs that differentiate patients according to DCI status are also sensitive to surgery (i.e. whether their expression changes in response to surgery).

Then, we will determine whether the established miRNA signature can be used as a monitoring marker in the serum of the DCI patients. We hope that these analyzes will bring us closer to clarification of DCI pathophysiology.

3. Justification of the research subject.

DCI after aneurismal subarachnoid haemorrhage is a serious condition with high morbidity as well as a substantial mortality rate resulting from permanent ischemic neurological deficit. As little is known about its pathophysiology and consequently, therapeutic options are scanty, this condition remains a challenge both for physicians and molecular biologists. The main purpose of this project is to answer the question about crosstalk between metabolomics, antioxidant mechanisms and miRNA expression in relation to DCI pathogenesis. In perspective, this project may bring clinically useful information to help prognosis of individual risk of DCI occurrence and monitoring. We expect, that this project will provide information useful in planning new targeted therapies aiming to prevent development and progression of the DCI.