Evaluation of platelet-derived extracellular vesicles and selected platelets-derived miRNAs in relation to platelet reactivity and their changes over time in patients with acute ischemic stroke.

Stroke is the second single-most common cause of death in Europe, accounting for almost 1.1 million deaths each year. Acute ischemic stroke (AIS) is characterized by the sudden loss of blood circulation to an area of the brain, resulting in irreversible brain injury and subsequent neurologic deficits. There are two major pathophydiological mechanisms of AIS: thrombotic and embolic. Whereas thrombotic AIS occurs when cerebral artery is blocked by blood clots that form within the brain, embolic AIS occurs when cerebral artery is blocked by blood clots that form somewhere else in the body and travel to the brain. Thrombotic AIS represents more than 50% of all AIS cases. It is increasingly evident that atherosclerosis is one of the most important factors associated with AIS. Platelets play a significant role in the development of cerebral ischemia through their participation in the generation of thromboemboli, which may initiate stroke symptoms. Once activated, platelets particularly contribute to the large-vessel subtype of ischemic stroke, in which artery occlusion is caused by atherosclerotic plaque ruptures. In addition, several antiplatelet agents significantly reduce the incidence of ischemic stroke after initial transient ischemic attacks. Therefore, assessments of platelet function and reactivity is of special importance for monitoring the onset and progression of AIS. From this reason, biomarkers of platelet activation might become the first reliable biomarkers of diagnostic and prognostic value in patients with AIS related to atherosclerosis.

In our study we will evaluate platelet reactivity using laboratory methods in different time points in order to asses changes over time. Moreover, in order to analyze the utility of new particles as a potential biomarker of platelet reactivity in population of patients with acute stroke we will also evaluate both **platelet-derived extracellular vesicles** (PEVs) and selected **microRNA** (miRNA). Activated platelets release fragments of their outer cell membrane, called PEV. PEVs are spherical nanoparticles surrounded by a phospholipid membrane, which retain cytoplasmic components such as proteins, lipids, second messengers, and genetic information, and expose specific proteins derived from the parent platelet. MicroRNAs (miRNAs) are small, noncoding RNA molecules with the ability to post-transcriptionally regulation of gene expression. Recent studies have shown that platelets contain and express high levels of miRNAs, whose abnormal expression in human platelets has been observed in inflammation, progression of atherosclerosis and other processes related to platelets reactivity. Platelet secreting miRNA has become a great attraction for scientists searching novel biomarkers associated with various pathologic conditions.

Altogether, miRNAs and PEV are key players in intercellular communication thereby contributing to inflammation, cell activation, cell survival and apoptosis, endothelial function, vascular remodeling and angiogenesis, which accelerate the progression of cardiovascular disease. Consequently, platelet-derived circulating miRNAs and PEVs are currently emerging as potential biomarkers for diagnosis, risk assessment and monitoring of therapy in patients with cardiovascular disease, including ischemic stroke. Although there have been attempts to use PEVs and circulating miRNAs as biomarkers in patients with stroke, hitherto studies do not allow to draw firm conclusions on the clinical utility of PEVs and circulating miRNAs in this population. To our best knowledge, **there is no study that aimed to assess changes over time of both PEVs and platelet -derived miRNAs in relation to platelet reactivity in a population of patients with ischemic stroke, in which platelet activation likely is of prognostic value**. Moreover, this study aims to conduct a complex analysis of both PEVs and miRNAs using state-of-the-art techniques, which allow comparing the inter-laboratory results of experiments and trials on PEVs and PEV-associated miRNAs as novel biomarkers in cardiovascular diagnosis and risk stratification.