Evaluation of prognostic value of platelet-derived extracellular microvesicles and microRNA in patients witch ischemic stroke of undetermined source.

Stroke is the second single-most common cause of death in Europe, accounting for almost 1.1 million deaths each year. In 2014, the clinical construct of "embolic stroke of undetermined source" (ESUS) was introduced to identify patients with cryptogenic ischemic stroke (IS), representing approximately 20% of IS. ESUS is defined as IS that is not associated with proximal arterial stenosis or a recognized cardioembolic source, such as atrial fibrillation of left ventricular thrombus, and that is not lacunar. Currently about one in five patients with IS meet the criteria for ESUS, but this may be underestimated because in many patients the diagnostic evaluation is not complete. The relatively young patients with ESUS have a substantial risk of recurrent stroke, estimated for 4.5% per year during long term follow-up. The pathophysiology of IS is related with platelet activation, inflammation and blood coagulation. In course of thromboembolic stroke, activated platelets trigger a thrombo-inflammatory cascade by promoting thrombus formation and growth, activating leukocytes and potentiating cerebral endothelium injury. Given that, one cannot exclude that activation of platelets contributes to the pathogenesis of ESUS as well. From this reason, biomarkers of platelet activation, increased inflammation and coagulation processes might become the first reliable biomarkers of diagnostic/prognostic value in patients with ESUS.

In our study we will evaluate the utility of new particles as a potential biomarker of platelet reactivity in population of patients with ESUS, namely 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 IS. 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 the utility of PEVs, EV-associated miRNAs and total plasma miRNAs as a prognostic marker in a population of patients with ESUS, in which platelet activation likely is of prognostic value. Moreover, no study in the field of cardiovascular disease has sought to analyze both total plasma and EV-associated miRNAs to compare their prognostic and diagnostic utility.
In our study, we will use previously prepared samples along with both clinical and biochemical data from selected patients that were participating in the NAVIGATE ESUS Biomarker substudy and we will perform a complex analysis of both PEVs and miRNAs using state-of-the-art techniques according to latest guidelines on this topic, which allow comparing the inter-laboratory results of experiments and trials on PEVs and platelet-derived miRNAs as novel biomarkers in diagnosis and risk stratification of ESUS.