In the proposed project, our main objective is to explore the expression of the components of the NLRP3 inflammasome complex, its activity, and mechanisms of regulation dependent on selected miRNA molecules within one year from the diagnosis of multiple sclerosis (MS). The longitudinal analysis aims to monitor the anti-inflammatory potential of selected anti-CD20 therapies in the early course of MS (immediately after diagnosis). The comparative study will be performed at three time points: (1) immediately after diagnosis and patients recruitment (before start of therapy), (2) six months after beginning treatment, and (3) one year after beginning therapy. This will allow us to determine the baseline values of tested parameters before therapy and their changes in response to applied medications. A longer analysis is limited by the time frame of the project. However, the key is to determine the early monitoring of the anti-inflammatory potential of therapies, because the initially diagnosed form of MS is the relapsing-remitting (RR) phase, characterized by periods of inflammatory exacerbations and remissions. While further progression of the disease correlates with the frequency and intensity of relapses. Acute and chronic inflammation in the central nervous system (CNS) leads to demyelination and degeneration of axons, disturbing the conduction of nerve impulses. The molecular processes leading to neuronal damage are not completely understood in MS. However, inflamed CNS microglia are believed to be an excellent environment for the functioning of antigen-presenting cells that can regulate the expression of the immune response. Various symptomatic forms of therapy are used to treat MS, including the most innovative therapy using anti-CD20 antibodies. The exact molecular mechanism of action of these drugs in MS is not fully elucidated, but it is assumed that it involves immunomodulation by selectively reducing the number and functioning of B lymphocytes expressing the CD20 antigen, as well as a subset of CD3+ T cells.

The project aims to monitor the activity of NLRP3, which is a molecular indicator of inflammatory processes intensification in many diseases, including neuroinflammatory ones. The studies will include a multi-parametric analysis of the NLRP3 complex functionality in patients with RRMS receiving ocrelizumab - a humanized monoclonal antibody directed against the CD20 antigen or ofatumumab - a human anti-CD20 monoclonal antibody, differing in the method of administration, dosage and pharmacology, as well as tolerance, contraindications and safety profile. NLRP3 is a functional complex of 3 main component proteins, constituting a key element of the innate immune system and commonly found in immune cells. Its activation is responsible for the production of pro-inflammatory factors. The formation of demyelinating plaques and progression of neurodegeneration is a result of neuroinflammatory processes in MS regulated by the NLRP3 complex. The project is dedicated to determining the significance of the applied therapies on the NLRP3 complex by monitoring changes in the expression of its components (NLRP3, ASC, and caspase-1) at the mRNA and protein levels, through a strategy of longitudinal comparisons at 3 time points. NLRP3 activity will be determined by measuring the plasma concentration of selected pro-inflammatory effector cytokines (IL-1β and IL-18). Moreover, the study will cover the mechanism of epigenetic regulation of the NLRP3 complex by measuring the expression of exosomal miRNA molecules (7-5p, 22-3p, 30e-5p and 223-3p) which are involved in regulation of NLRP3 complex function. Exosomes are extracellular vesicles that actively participate in the mechanisms of intercellular communication. The content of active biological cargo makes exosomes effective carriers of signaling molecules, including miRNAs that regulate the expression of many genes important for the pathogenesis of MS, e.g. genes encoding NLRP3. However, this charge, including the expression profile of miRNA molecules, may reflect physio/pathological processes and, therefore, be modified due to treatment.

The obtained results will help answer the questions: (1) can the activity of the NLRP3 complex (important in the course of MS and many chronic diseases) be inhibited by selected anti-CD20 therapies and (2) can observed changes be used as parameters for assessing the effectiveness of treatment?

The advantage of the proposed project is full diagnostics of enrolled patients and monitoring of applied therapies impact (including at time points important for the project) based on clinical parameters, radiological features, disease relapse rate, and disability scale.