Cystic fibrosis is an autosomal recessive inherited genetic disease associated with impairment of chloride channel function in cell membranes. It is one of the most common genetic diseases occurring in humans. Its incidence is 1 in 2,500 newborns, whereas patients who suffer from it live an average of 35 years. Thanks to the implementation of neonatal screening, it is possible to diagnose cystic fibrosis early. Over time, patients experience dysfunction of many organs including the pancreas, gastrointestinal tract, reproductive system and lungs (mainly due to the formation of thick mucus). Furthermore, patients suffer from exacerbations, which are recurrent deteriorations of the airways as a result of inflammation and co-occurring infections. Exacerbations are associated with a gradual loss of lung function, which ultimately leads to the premature death of patients.

Published research indicates a number of genetic molecules that affect cystic fibrosis. Some of them are transferred between respiratory epithelial cells via exosomes - extracellular vesicles containing bioactive molecules (e.g. microRNA or circRNA) in their interior. This type of transport of biologically active molecules is indicated as one of the possible ways of spreading inflammation in the respiratory tract. Currently published studies, among others, concern the transport of microRNA (miRNA). MiRNAs are short non-coding RNAs capable of inhibiting gene expression and influencing the course of diseases, including cystic fibrosis.

Our team, however, decided to analyse the expression profile of circRNA, long RNA with a closed, circular structure. These molecules are present in all eukaryotic cells, and due to their characteristic structure, they are distinguished by greater resistance to exonucleases, which potentially makes them good candidates for biomarkers. The main functional feature of circRNAs is the ability to complementary bind miRNAs and inhibit their activity (so-called "miRNA sponges"), alternatively, circRNA can act as miRNA reservoirs protecting them from the degrading effects of specific proteins.

So far, circRNAs associated with cystic fibrosis have not been identified, for that reason, we decided to study the expression of these molecules in serum samples from patients and compare them with the expression profile in healthy people. For our study, we recruited pediatric patients treated in the Pneumonology, Pediatric Allergology and Clinical Immunology Clinic in Poznań. All clinical data and material was collected, if it was possible, during the exacerbation and remission phases. Prior to the recruitment, criteria for inclusion and exclusion from the study were developed in detail (e.g. age below 6 years of age, autoimmune diseases excluded).

Our research process will start with an exosomal circRNA isolation procedure consisting of a series of reactions. Then, the resulting material will be subjected to the New Generation Sequencing, a modern technology enabling simultaneous testing of the sequence of many molecules in more than one sample. The accurate bioinformatic analysis will indicate the presence of circRNAs distinguishing patient’s material from control sample. Further research using the qPCR reaction will be expanded to include sputum material (coughing material not produced by healthy subjects) and will also verify the obtained sequencing results. Moreover, at this step, we want to investigate whether any of the discovered circRNAs differ in expression between remission and exacerbation in serum and sputum. The presented methodology of the study will allow an assessment of circRNA expression both peripherally in the blood and locally in the airways. The final stage aims to examine to verify if significantly different expression molecules in patients effectively capture specific miRNAs. To achieve this assumption, an experiment will be carried out using cultured and appropriately modified human bronchial epithelial cells.

We expect our project to identify circRNAs that are specifically expressed in cystic fibrosis. As a result, it will be possible to perform a thorough functional analysis of these molecules in the future, and thus to assess their actual impact on the course of the disease. In addition, we hope to detect circRNA that, through interactions with miRNAs, contribute to exacerbations in cystic fibrosis patients. This would be an introduction to the development of potential therapeutic methods that may slow down the progression of the disease and contribute to improving the quality of life and increasing the survival of patients in the future.