

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M.tb*) bacteria remains a global health emergency with 9 million new cases and 1.5 million deaths annually. The increase in TB morbidity and mortality is caused by the HIV pandemic and spread of multi-drug resistant *M.tb*. As estimated 30% of the world population is infected with *M.tb*. These are latent tuberculosis infections (LTBI), which means that the lifelong *M.tb*-infected individuals do not have TB symptoms because mycobacteria are in a dormant stage. However, in approximately 5%-10% of LTBI individuals *M.tb* are reactivated and TB disease arises. The risk of active TB developing is increased in *M.tb*-infected children. Clinical TB can be developed in 40% of *M.tb*-infected infants, 24% of children between 1-10 years and 16% of adolescents of 11-15 years of age. Diagnosing TB in children is more difficult than in adults. BCG vaccine has limited efficacy in preventing pulmonary TB, albeit it protects against disseminated disease in children. The project will use a very up to date technology in order to select correlate biomarkers of anti-TB protection versus tuberculosis disease in children.

The responses to *M.tb* are complex and multifaceted involving cascade reactions occurring in mycobacteria and in host cells and tissues. The course of these interactions determines the formation of a balance between bacteria and host, when the weakened metabolism of mycobacteria prevents their growth in LTBI, or on the contrary host defense mechanisms are inefficient and allow mycobacteria grow leading to tissue destruction in active TB. In our biomarker studies, using current knowledge and our own experience, we plan to select pre-defined biological molecules, cytokines, chemokines and several inflammatory mediators, whose role in *M.tb* infections is not clear, but it has been already suggested. Taking into account the vast complexity of the interactions of mycobacteria with the host organism we intend to apply a randomized omics approach to select large sets of small biological molecules of bacterial origin and host metabolomics in respect of anti-TB protection, pathogen-driven inflammation and clinical disease. The prospective study will be performed on the cohort of 300 children including children with active TB and latent *M.tb* infection. Use of the latest achievements in metabolomics allows analysis of vast sets of small molecules (metabolites) in biological samples. We expect that profiling about 400 small molecules such as amino acids and their derivatives, metabolites of glycolysis and tricarboxylic acid cycle, lipids and steroids and many of others will reflect differences between healthy children and suffering from TB. In the project metabolomic profiles (signatures) of sera and soluble products of activated immune cells will be defined by liquid chromatography tandem- mass spectrometry, in children groups in the study. This techniques allow the specific and sensitive structural analysis of many biological components present in low concentration in body fluids. To know the effector activity of immune cells in uninfected healthy children and patients with active TB or LTBI, cultures of whole blood stimulated with specific antigens of *M.tb* will be performed and analyzed by Multiplex Arrays quantification technology. This system offers a possibility of simultaneous evaluating the set of 39 of cytokines, chemokines and inflammatory mediators with high sensitivity and specificity in children sera and blood cultures. These studies will provide new knowledge about cytokine/chemokine network, which differentiate measured variables in healthy versus active TB and LTBI children. We also expect to identify important links defensive anti-mycobacterial immune responses, which are weakened by virulent *M.tb* leading to active TB.

The potential biomarkers for active tuberculosis and latent M. tuberculosis infection that we would like to identify by cytokine and metabolomic profiling of sera and soluble effectors released by immune cells responding to *M.tb* specific antigens will provide new basic knowledge about the mechanisms underlying the outcome of the infection with *M.tb* bacteria in children. The new insight into TB mechanisms might facilitate the discovery of new diagnostic tests and speed up an appropriate treatment of children with active TB. Moreover, data analysis can subsequently inspire appropriate experimental models aimed at developing new anti-tuberculosis vaccines superior to current vaccination BCG and new anti TB drugs.