

Central nervous system (CNS) infections including encephalitis and meningitis are caused by a variety of viruses, bacteria, fungi and parasites. There are currently over 100 known viruses capable of CNS infection and the list keeps growing each year. Consequences of neuroinfections can be severe and include permanent brain injury and even death. Therefore, early determination of the etiology is critical for the implementation of appropriate and often life-saving therapy. Unfortunately, there are many pathogens that can cause encephalitis and meningitis and testing for such a large number of infectious agents is not feasible from a practical point of view. Current serological and molecular diagnostic tests are aimed at the detection of particular pathogens and thus only the most common microorganisms are targeted.

Consequently, in up to 85% of cases of CNS infections the etiology remains unknown. Next-generation sequencing (NGS) combined with metagenomics could provide a solution to these problems. Metagenomics provides culture-independent sequencing and analysis of all nucleic acids recovered from a sample. The emerging field of metagenomics has the potential to revolutionize pathogen detection in encephalitis/meningitis by allowing for a simultaneous detection of all microorganisms in a cerebrospinal fluid (CSF) sample, without any prior knowledge of their identities. This approach allows for a fast detection of almost any microorganisms including rare and emerging pathogens.

In the present project we plan to verify the usefulness of metagenomic approach in the diagnostics of CNS infections. For this purpose we will perform metagenomic analysis of CSFs collected from patients with encephalitis (100 patients) and meningitis (100 patients) and compare these results with serological and molecular test results performed as part of routine diagnostics. Altogether 150 various control samples will be similarly processed.

In our project all CSF samples will be digested with the mixture of nucleases. Then, DNA and RNA will be extracted and preamplified to generate an efficient amount of DNA to prepare sequencing libraries. All reads obtained in next-generation sequencing will be analyzed using bioinformatic pipeline involving quality and quantity analysis, removing artifact sequences and mapping all reads to human genome, as well as to NCBI-nt database. All metagenomic results will be confirmed using PCR/RT-PCR and then compared to the results of routine serological and molecular testing.