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O1114 Mapping the cerebrospinal fluid virome in patients with suspected infectious neurological syndrome

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Background: The aetiology of most neuroinvasive infections remains undetermined. Next Generation Sequencing Metagenomics (mNGS) represents a valuable approach to characterise the microbial population, to estimate the viral diversity (virome), and to complement classical diagnostics. In this study we analyzed the viruses present in cerebrospinal fuid (CSF) from patients with clinical suspect of infectious meningo-encephalitis, using mNGS. CSF samples from 22 patients that resulted negative to routine diagnostic procedures were analyzed.

Materials/methods: After RNA extraction, cDNA was prepared by Sequence Independent Single Primer Amplification technique (SISPA). NGS libraries were prepared by fragmentation, adapters ligation and amplification, and quantified with Bio-analyzer. Sequencing was performed using Ion-Torrent S5 platform. A median of 40,2(range 23,6-52,8) million reads, 180 nt length (range 30-300 nt) per sample were obtained.

High-quality reads were filtered using Bowtie2 with high stringency parameters (90% of homology over 80% of read sequence), to subtract human-derived reads (99% of reeds). Filtered reads were then mapped to NCBI Nucleotide Database with an E-value cut-off of 10^-5. All results were then merged and grouped by taxonomy using Megan5, collecting the overall numbers of reads mapped to every taxa. Finally, the presence of the identified viruses was confirmed by targeted PCR. Experimental protocol and pipeline were optimized using either samples spiked with known RNA and DNA viruses or clinical specimens with known viral content.

Results: In the majority of samples sequences from human-endogenous-retroviruses and phages were detected. In addition, sequences of other human viruses were detected in individual samples, including: HIV (3), EBV (1), TTV (2), circoviruses (3) and a virus belonging to the Genomoviridae family (1). In 1 sample mNGS detected CMV sequences, but the presence of this virus was not confirmed by specific PCR.

Conclusions: mNGS represents a new promising opportunity to investigate the potential role of viruses in the pathogenesis of nervous system disorders through an unbiased method able to identify known and novel pathogens and to characterize the virome. Extensive effort is needed for method standardization and data interpretation, including the role and abundancy of viral sequences detected, mostly with respect of possible exploitation for diagnostic purpose.