Metagenomic sequencing of cerebrospinal fluid from children with meningitis

Charles Grose *

Division of Infectious Diseases/Virology Laboratory, Department of Pediatrics, University of Iowa, Iowa City, IA, USA

eBioMedicine 2022;84: 104287
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.104287

The metagenome of a sample of cerebrospinal fluid (CSF) includes all the genomes found within that sample. Prior investigations have examined CSF samples of meningoencephalitis patients by metagenomic second generation sequencing, after preparation of both RNA and DNA libraries.[1,2] An important proof-of-concept clinical study has now been published where the authors applied metagenomic third generation sequencing toward the diagnosis of meningitis in children and adults.[3] The results were both informative and promising. A total of 52 subjects were enrolled, with 49 patients having a positive result on their CSF sample when tested by either the Biofire Filmarray meningitis/encephalitis PCR kit or the Oxford Nanopore MinION sequencer. Because meningitis remains an important infection in children and because pediatric infectious disease is my area of specialization, this comment will concentrate on the results from the children enrolled in the study.

Thirteen of the 52 patients were children.[3] Of the 13 children, 8 were neonates and the other 5 ranged in age from 2 to 14 years. The 8 neonates had meningitis caused by 3 different bacteria:*Streptococcus pneumoniae*, *Hemophilus influenzae* and *Streptococcus agalactiae* (formerly called group B Streptococcus). Infections in the older children were caused by *Streptococcus pneumoniae* (3 cases), Neisseria meningitidis (1 case) and herpes simplex virus (1 case). The Biofire Filmarray correctly identified the pathogen in all 13 cases, whereas the MinION sequencer failed to identify one of the 5 cases of pneumococcal meningitis.[3] In short, this study has revalidated the importance of the Biofire Filmarray for diagnosis of meningitis and encephalitis in children.[4] The Biofire meningitis/encephalitis kit contains primers for 6 bacteria (*Escherichia coli*, *Hemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*), one yeast (cryptococcus), and 7 viruses (cytomegalovirus, enterovirus, herpes simplex virus-1, herpes simplex virus-2, human herpes virus-6, human parechovirus, and varicella-zoster virus). Note that 5 of the 7 viruses are herpesviruses with DNA genomes: herpes simplex virus types 1 and 2, varicella-zoster virus (VZV; human herpesvirus-3), cytomegalovirus (human herpesvirus-5), and human herpesvirus-6 (roseola infantum virus), while enteroviruses and parechoviruses (formerly called Echoviruses 22 and 23) are closely related RNA viruses.

What the authors could have emphasized more was the power of both second and third generation sequencing to subtype viruses in a single assay. The authors detected VZV in the CSF of one young adult.[3] The VZV strain was shown to be wild-type VZV by their MinION sequencer. In the United States, where universal varicella vaccination has been carried out since 1995, a small number of cases of varicella vaccine meningitis have been identified.[5] In one CSF collected from a 7-year old boy, metagenomic next generation sequencing clearly defined enough DNA reads with single nucleotide polymorphisms to identify the virus as a varicella vaccine strain.[6] The Biofire Filmarray had detected VZV in the CSF of the above 2 cases, but that assay cannot discriminate wild-type from vaccine-type VZV.

Another important aspect of metagenomic sequencing is the detection of CSF pathogens not part of the Biofire primer set and not previously suspected in patients living in a particular geographic area. One excellent example is the newly emerging tick-borne flavivirus called Powassan virus.[2] A second excellent example is the mosquito-borne flavivirus called St. Louis encephalitis virus.[1] Two more examples are mentioned because of their medical importance, although blood rather than CSF is tested by the third generation MinION sequencing apparatus; the diseases are Ebola infection in children and adults in West Africa and dengue infection in Indonesia.[7,8]

A further discovery not made in the current report but widely recognized is the detection of two pathogens in the same CSF sample by metagenomic sequencing. This pattern appears especially common among herpesviruses. For example, the CSF that contained numerous DNA reads specific for varicella vaccine virus also contained a small number of reads specific for human herpesvirus-7 (HHV-7).[6] An extremely similar scenario was described in a second report, in which a small number of HHV-7 reads were detected in a patient with

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2022.104247

Abbreviations: CSF, Cerebrospinal Fluid; VZV, Varicella-zoster Virus

*Correspondence to: University of Iowa Hospital, Room BT2001, 200 Hawkins Drive, Iowa City, IA 52242, USA.
E-mail address: Charles-grose@uiowa.edu

HSV-2 neurologic disease.[2] Since HHV-7 establishes latency within a lymphocyte, these lymphocytes within the central nervous system presumably are stimulated to reactivate by the same process that stimulated varicella vaccine virus or HSV-2 to reactivate. Epstein-Barr virus (human herpesvirus-4) sequences have also been detected in CSF samples from people with meningoencephalitis caused by other viruses.[2] These patients deserve long-term follow-up into late adulthood.[9]

In summary, the advantages of diagnostic metagenomic sequencing are readily apparent.[1–3] At the present time, however, the above studies have also shown that the Biofire Filmarray correctly detects common pathogens in most cases of meningitis in children in the United States and France.[4] Although the Biofire Filmarray cannot define antimicrobial sensitivities in a single assay, most pathogens found in the CSF of these children are easily treatable. Therefore, in the coming years, a tandem approach is suggested where metagenomic sequencing is conducted shortly after the Biofire test whenever there is a diagnostic ambiguity. As a final comment, I have been privileged to witness and participate in these advances in diagnosis of viral infections over the past decades.[6,10] I commend the authors of the current article for taking the next step forward.

Contributors

Conceptualization, literature search, writing and editing: C.G.

Declaration of interests

The author has no potential conflicts of interest to disclose.

References

1 Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N Engl J Med*. 2019;380(24):2327–2340.
2 Piantadosi A, Mukerji SS, Ye S, et al. Enhanced virus detection and metagenomic sequencing in patients with meningitis and encephalitis. *mBio*. 2021;12(4):e0114321.
3 Morsli M, Boudet A, Kerharo Q, et al. Real-time metagenomics-based diagnosis of community-acquired meningitis: a prospective series, southern France. *eBioMedicine*. 2022;84:104247.
4 Leber AL, Everhart K, Balada-Llasat JM, et al. Multicenter evaluation of biofire filmarray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol*. 2016;54(9):2251–2261.
5 Heusel EH, Grose C. Twelve children with varicella vaccine meningitis: neuropathogenesis of reactivated live attenuated varicella vaccine virus. *Viruses*. 2020;12(10):1078.
6 Ramachandran PS, Wilson MR, Catho G, et al. Meningitis caused by the live varicella vaccine virus: metagenomic next generation sequencing, immunology exome sequencing and cytokine multiplex profiling. *Viruses*. 2021;13(11):2286.
7 Quick J, Loman NJ, Duraffour S, et al. Real-time, portable genome sequencing for Ebola surveillance. *Nature*. 2016;530(7589):228–232.
8 Stubbs SCB, Blacklaws BA, Yohan B, et al. Assessment of a multiplex PCR and Nanopore-based method for dengue virus sequencing in Indonesia. *Virol J*. 2020;17(1):24.
9 Rizzo R. Controversial role of herpesviruses in Alzheimer's disease. *PLoS Pathog*. 2020;16(6):e1008575.
10 Grose C, Henle W, Henle G, Feorino PM. Primary Epstein-Barr-virus infections in acute neurologic diseases. *N Engl J Med*. 1975;292(8):392–395.