Published in final edited form as:

JAMA Neurol. 2016 December 01; 73(12): 1389–1390. doi:10.1001/jamaneurol.2016.3617.

Emerging Diagnostic and Therapeutic Tools for Central Nervous System Infections

Michael Wilson, MD and

Department of Neurology, University of California, San Francisco.

Kenneth L. Tyler, MD

Departments of Neurology, Medicine, and Immunology-Microbiology, University of Colorado School of Medicine, Aurora.

The field of neurological infections is constantly changing, and the emergence of new pathogens including Zika virus (ZIKV),¹ already makes earlier commentaries look dated.² This commentary highlights 2 important “next-generation” approaches to diagnosis and therapy that are either already clinically relevant or likely to be tested in central nervous system (CNS) neurotropic viral infections in the near future.

Diagnostics

Even severe and seemingly intractable cases of encephalitis may be treatable if a specific etiologic agent can be identified. Unfortunately, using conventional diagnostic techniques this is possible in less than half of sporadic cases of patients with an “acute encephalitis syndrome.”³ Rare but treatable microbes are often missed with generally available tests, and autoimmune encephalitis is increasingly recognized as a potentially treatable condition that can mimic or even coexist with acute viral encephalitis. Treating empirically for a presumed autoimmune encephalitis poses the significant risk that an occult infection may be exacerbated when an immunomodulatory or immunosuppressive agent is used in the absence of concurrent antimicrobial therapy. Conversely, failure to recognize and appropriately treat patients with autoimmune encephalitis or inappropriate treatment of these patients with antimicrobial therapy may lead to significant morbidity and even mortality.

The recent ZIKV outbreak highlights not only that new threats are continuing to arise but also the substantial time delay that can occur before a newly emergent pathogen is identified. A recent phylogenetic analysis concluded that ZIKV arrived in Brazil 12 months prior to being detected and 18 months before suspicions about its link to microcephaly were raised.⁴ The prior known behavior of ZIKV, or of related flaviviruses, would not have offered clues

Corresponding Author: Kenneth L. Tyler, MD, Neurology B-182, University of Colorado School of Medicine, Research Complex II, 12700 E 19th Ave, Aurora, CO 80045 (ken.tyler@ucdenver.edu).

Conflict of Interest Disclosures: Dr Wilson is a coinvestigator on the Precision Diagnosis of Acute Infectious Diseases study. Dr Tyler has served as an expert consultant related to JC virus and progressive multifocal leukoencephalopathy for Genentech, Hoffman La Roche, Pfizer, and the PML Consortium.

Submissions: This Viewpoint is one in a series of Next Generation Neurology Viewpoints emphasizing innovative original concepts and approaches to understanding the causation of neurological disease and providing new and effective therapies. We welcome the submission of Viewpoints that incorporate these objectives.

to facilitate diagnosis. Experiences like this emphasize the importance of “unbiased” methods to search for novel or emergent pathogens.

Unbiased metagenomic next-generation sequencing (mNGS), with its agnostic or hypothesis-free stance toward what infectious agent(s) might be present in a tissue sample, offers a wholly different approach to infectious disease diagnosis compared with conventional “guided” serologies or nucleic acid amplification techniques.⁵⁻⁷ Using random hexamer primers to amplify all the nucleic acids in a biological sample like cerebrospinal fluid or brain biopsy tissue, massively parallel sequencing technologies can rapidly sequence all the contained genetic information. Coincident with the rapid advances in sequencing technologies, computer algorithms have developed apace. Bioinformatics pipelines can now analyze these enormous sequencing data sets to identify the microbial source of the nonhuman sequences present in the sample within minutes. Because the database used to search for the identity of the nonhuman sequences is typically the National Center for Biotechnology Information GenBank database, any type of organism, including viruses, fungi, bacteria, and parasites, can be identified with this single assay.

While mNGS has shown extraordinary promise in the diagnosis of unknown meningoencephalitis cases, it is not easy to transition a sophisticated research technique into widespread general laboratory use. A new diagnostic test for a particular pathogen must be benchmarked against the existing diagnostic test(s) for that microbe and shown to be equivalent or superior in sensitivity and specificity and the associated positive or negative predictive values before being deployed for general clinical use. However, it is challenging to devise a validation and benchmarking strategy for a test like mNGS that has the potential to identify potentially thousands of infectious agents. In addition, the unbiased approach of mNGS makes it particularly susceptible to microbial contamination from a number of sources including skin flora as well as laboratory and reagent contaminants.

A clinical study called Precision Diagnosis of Acute Infectious Diseases was recently launched by the University of California, San Francisco. Funded by the California Initiative to Advance Precision Medicine and multiple philanthropic foundations, this study is a first-of-its-kind demonstration project to deploy a laboratory-developed mNGS assay on cerebrospinal fluid to diagnose infectious causes of acute meningitis and/or encephalitis in patients presenting at hospitals in California and nationally. The mNGS assay is being performed as part of a clinical outcomes study in a Clinical Laboratory Improvement Amendments–certified laboratory, and as such, the test results are reportable in the medical record and can be used to guide medical decision making. As part of the Precision Diagnosis of Acute Infectious Diseases study, a multidisciplinary board including the primary clinician will be assembled in real time to evaluate mNGS test results.

Treatment

While acyclovir and other antiretroviral drugs have dramatically reduced the morbidity and mortality associated with CNS herpesviruses and human immunodeficiency virus infections, there is an urgent need to develop new therapies for other emergent CNS pathogens including flaviviruses and enteroviruses. An exciting new genome editing technology,

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)–associated endonuclease Cas9 offers a potentially exciting novel approach to antiviral therapy.

CRISPR-Cas9 natively functions as a bacterial adaptive immune system that removes incoming phage DNA from the host without harm to the bacteria's own genome. The CRISPR-Cas9 system has been widely adopted as a very precise, flexible, and programmable genome editing technique. When coupled with short guide RNAs (sgRNAs) designed against targets of interest, Cas9 binds to 3'NGG protospacer adjacent motif (PAM) sites and produces double-stranded breaks if the sgRNA successfully hybridizes with the adjacent target sequence. In theory this approach could be used to eliminate latent DNA viruses, including herpesviruses, from cells.

This approach has been used in vitro to inhibit the replication of JC polyomavirus,⁸ the agent responsible for progressive multi-focal leukoencephalopathy. In this study CRISPR-Cas9 technology was used to target a region in the N terminal of the viral T-antigen gene there by preventing expression of the encoded viral protein and reduced JC polyomavirus replication in cultured oligodendroglioma (TC620) and transformed human fetal glial (SVG-A) cells. The same approach has been used to inhibit herpes simplex virus 1 replication in TC620 cells by targeting the viral immediate early proteins (ICP0, ICP4, ICP27).⁹

Conclusions

The global health and biodefense communities are focused on the threat of emerging pathogens, many of which have severe neurologic sequelae.² Fueled by globalization and climate change, we will continue to experience a changing landscape of neurological infections. The technologies described earlier have already affected improved diagnosis of novel and unusual pathogens, and may eventually lead to dramatic new therapies. The clinical neurology community will continue to play a critical frontline role identifying, characterizing, and treating unusual neuroinfectious and neuroinflammatory syndromes. No technological advance will be successful without remaining closely linked to a careful clinical approach that can properly contextualize and thoughtfully apply these new diagnostic and treatment modalities. The California Encephalitis Project¹⁰ represented a novel approach to combining collection of standardized clinical data sets with advanced structured protocols for neurodiagnostic testing. However, this project used relatively nonspecific diagnostic criteria for encephalitis and suffered from the bias in ascertain of cases being evaluated. However, a similar approach with more robust definitions of encephalitis subtypes and an enhanced strategy for more comprehensive ascertainment and inclusion of cases could easily be coupled with mNGS. Accurate and rapid diagnosis remains the essential building block on which applications of novel therapeutics depend.

REFERENCES

1. Pastula DM, Smith DE, Beckham JD, Tyler KL. Four emerging arboviral diseases in North America: Jamestown Canyon, Powassan, chikungunya, and Zika virus diseases. *J Neurovirol.* 2016; 22(3):257–260. [PubMed: 26903031]
2. Nath A. Neuroinfectious diseases: a crisis in neurology and a call for action. *JAMA Neurol.* 2015; 72(2):143–144. [PubMed: 25485794]

3. Granerod J, Tam CC, Crowcroft NS, Davies NW, Borchert M, Thomas SL. Challenge of the unknown: a systematic review of acute encephalitis in non-outbreak situations. *Neurology*. 2010; 75(10):924–932. [PubMed: 20820004]
4. Faria NR, Azevedo RdoS, Kraemer MU, et al. Zika virus in the Americas: early epidemiological and genetic findings. *Science*. 2016; 352(6283):345–349. [PubMed: 27013429]
5. Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med*. 2014; 370(25):2408–2417. [PubMed: 24896819]
6. Wilson MR, Shanbhag NM, Reid MJ, et al. Diagnosing Balamuthia mandrillaris encephalitis with metagenomic deep sequencing. *Ann Neurol*. 2015; 78(5):722–730. [PubMed: 26290222]
7. Naccache SN, Peggs KS, Mattes FM, et al. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. *Clin Infect Dis*. 2015; 60(6):919–923. [PubMed: 25572898]
8. Wollebo HS, Bellizzi A, Kaminski R, Hu W, White MK, Khalili K. CRISPR/Cas9 system as an agent for eliminating polyomavirus JC infection. *PLoS One*. 2015; 10(9):e0136046. [PubMed: 26360417]
9. Roehm PC, Shekarabi M, Wollebo HS, et al. Inhibitor of HSV-1 replication by gene editing strategy. *Sci Rep*. 2016; 6:23146. [PubMed: 27064617]
10. Glaser CA, Gilliam S, Schnurr D, et al. California Encephalitis Project, 1998-2000. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. *Clin Infect Dis*. 2003; 36(6):731–742. [PubMed: 12627357]