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Diagnostic Testing of Neurologic Infections

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Neuroinfectious diseases are a major cause of morbidity and mortality worldwide and have a sizable effect on local health care systems and economies.[1,2] A timely diagnosis and the institution of appropriate management can drastically improve mortality and morbidity when the precise organism is known.[3] In this review, the authors provide an overview of the current state of diagnostic techniques for neuroinvasive pathogens ranging from culture, polymerase chain reaction (PCR), to serology. The authors then review new diagnostic modalities, including unbiased metagenomic next-generation sequencing (mNGS). This overview is not meant to provide an exhaustive list of all possible diagnostics for all possible neuroinvasive pathogens. Instead, the authors hope that by reviewing the techniques in detail as they apply to particularly important and/or common neuroinvasive pathogens, including strengths and common pitfalls associated with each, the reader will be able to more judiciously select the most efficient and comprehensive diagnostic approach tailored to their particular patient.

CULTURE

Cerebrospinal fluid (CSF) culture is the gold standard for central nervous system (CNS) infections and can provide guidance for antimicrobial therapy. Bacterial cultures are critical in the management of meningitis with varying sensitivities depending on the causative organism. These range from 97% for *Haemophilus influenzae*, 87% for *Streptococcus pneumoniae*, and 80% for *Neisseria meningitidis*.[4] The timing of antibiotics in relation to the acquisition of CSF is crucial. A positive result decreases from 85% before antibiotics, to 73% when obtained less than 4 hours after therapy, 11% between 4 to 8 hours and 0% after 8 hours.[4,5] *Listeria monocytogenes* causes both meningitis and rhombencephalitis with associated abscess formation. Cultures are hampered by slow growth and are insensitive due to a low CSF bacterial load.[6,7] Sensitivities vary between studies and range from 55% to 90% and are as low as 41% in patients with rhombencephalitis.[6,8,9] Blood culture performs marginally better in cases of rhombencephalitis with rates reaching 61%.[9]

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In general, larger CSF volumes improve the sensitivity of culture. However, even with large volumes of CSF, visualization of acid fast bacilli (AFB) by microscopy is only 15% sensitive, and *Mycobacterium tuberculosis* (TB) can take 2 to 4 weeks to culture with a sensitivity of only 50% to 60%. Therefore, AFB culture cannot be relied on for time critical TB meningitis diagnoses.[10] In addition, an estimated 480,000 people developed multidrug-resistant TB in 2015.[11] Despite its low yield, TB cultures remain the gold standard for identifying drug sensitivities.

Viral cultures are performed with cell lines, including rhesus monkey kidney, African green monkey kidney, A549, and MRC-5. The patient sample is added to the culture medium, and cytopathic changes are observed in positive cases. These changes can take up to 30 days to appear depending on the virus.[12] Shell vial culturing, antigen detection, and immunofluorescent antibodies to specific viruses have improved this previously slow turnaround.[13] Enteroviruses are the easiest viruses to culture with 75% sensitivity and a 3- to 8-day test turnaround.[14] Other viruses fail to display equivalent results. Herpes simplex virus (HSV) is only cultured from CSF in less than 5% of HSV encephalitis cases.[15] Fortunately, with the advent of advanced molecular techniques, the need for viral cultures as a diagnostic tool for CNS infections has diminished.[16]

Fungal cultures can be performed on specific fungal mediums. However, the 3 most frequent neuroinvasive fungi: *Cryptococcus* spp, *Candida* spp, and *Aspergillus* spp, can be cultured on standard bacterial mediums with variable sensitivity. When more rare fungi are being considered, as may be the case with chronic meningitis, specific culture mediums are required.[17]

SEROLOGY

Syphilis

Syphilis is caused by the bacteria *Treponema pallidum* and can manifest with a variety of neurologic syndromes depending on the duration of infection and the host's immune status.[18] The diagnostic gold standard is rabbit infectivity testing, but this is expensive and limited to research laboratories. Dark field microscopy is operator dependent, time consuming, and not routinely used in the diagnosis of neurosyphilis. Treponemal nucleic acid detection by PCR in CSF is insensitive.[19] Antibody testing is therefore the standard tool for diagnosis and is divided into 2 groups, treponemal and nontreponemal testing. Treponemal tests include fluorescent treponemal antibody absorption (FTA-ABS), *T pallidum* particle agglutination (TP-PA), and enzyme immunoassay (EIA).[20] These tests detect antibodies to specific antigenic components of the bacterium. The latter two tests are more sensitive and specific than the older FTA-ABS test. These are opposed to nontreponemal tests, which detect antibodies to lipoidal material released from damaged host cells and cardiolipin-like material released by *T pallidum*.[21,22] The 2 most common nontreponemal tests are the Venereal Disease Research Laboratory (VDRL) slide test and the Rapid Plasma Reagin card test, in which reactive sera produce flocculation of the antigenic material.[21]

Treponemal tests remain positive for life after primary infection and are not used as a marker for treatment response. Nontreponemal tests are quantitative and are used to assess treatment

response with the expectation that they will either revert to being negative or at least exhibit a 4-fold reduction in titer after successful treatment. However, the nontreponemal tests can be falsely negative either as a result of waning anti-body titers in late latent syphilis, or conversely, as a result of very high anti-body titers that interfere with the formation of the antigen-antibody lattice, called the prozone phenomenon. If the latter circumstance is suspected in a high-risk patient, the treating physician can ask the laboratory to dilute the biological sample and repeat the test. Because both treponemal and nontreponemal tests are susceptible to false positives and negatives, combined testing is recommended for an accurate diagnosis of syphilis (the syphilis testing algorithm for syphilis screening is outside the scope of this article).[23] A definitive diagnosis of neurosyphilis is based on a clinical syndrome suggestive of neurosyphilis, a positive serum TP-PA, and a positive CSF VDRL. However, CSF VDRL only has a sensitivity of roughly 70% and therefore cannot exclude neurosyphilis.[24] False positives can occur with traumatic taps resulting in contamination from peripheral blood. In the absence of a positive CSF VDRL, a probable diagnosis of neurosyphilis is made with a CSF white blood cell count >5 mm/μL or a protein greater than 45 mg/dL.[25] Treponemal tests are not routinely performed on the CSF despite some suggestions that the high sensitivity of the test should rule out syphilis.[26]

Varicella Zoster Virus

Varicella zoster virus (VZV) is a neurotropic virus that can cause a wide range of syndromes ranging from encephalomyelitis, multifocal polyradiculitis, and cranial neuritis to a vasculopathy affecting both small and large cerebral arteries leading to unifocal and multifocal strokes. Symptoms can present after a prolonged duration, and a rash can occur months before presentation and may not be present at all.[27] VZV serum immunoglobulin M (IgM) appears within 2 to 5 days of symptom onset. Levels begin to decrease by 3.5 weeks and cannot be detected by 1 year. IgG levels decrease with time but generally remain positive for life. Therefore, a positive serum IgM is usually indicative of active infection.[28] CSF evaluation displays a pleocytosis in two-thirds of patients, and the diagnosis is made with CSF VZV serology or PCR. VZV IgG levels have higher sensitivity in comparison to PCR, 93% versus 30%, respectively.[29] As most adults will have positive VZV IgG in serum, it is important to assess for a low-serum/CSF ratio to confirm intrathecal production.[27,29] CSF VZV IgM is also supportive of a diagnosis, despite its less robust sensitivity compared with IgG.[30,31] VZV PCR may also be dependent on the time of symptom onset and the time of CSF acquisition with decreasing sensitivity of PCR after 1 week. Time-dependent sensitivity is an important consideration given the protracted course and delayed presentation of most cases.[27,29]

Flaviviruses

Flaviviruses cause mosquito and tick-borne infections that are endemic to certain regions throughout the world. They can cause meningitis, meningoencephalitis, and anterior horn cell disease. West Nile virus (WNV) is endemic to Africa and Europe and arrived in North America in 1999. Viremia is detected as early as 1 to 2 days after the primary mosquito bite and persists for up to 1 week until the development of IgM neutralizing antibodies. Viremia is generally absent by the time neurologic symptoms appear in immunocompetent hosts, whereas immunocompromised patients demonstrate a prolonged viremia with a delay in

antibody production.[32] The pathophysiology of WNV mirrors the yield of laboratory diagnostics. CSF PCR may be helpful very early in the disease but generally has a low sensitivity (57%).[33] IgM capture enzyme-linked immunosorbent assay (ELISA) in either blood or CSF during the acute phase is the gold standard for the diagnosis of neuroinvasive WNV and is generally always present by the time neurologic symptoms manifest. The large IgM pentameter does not cross the blood-brain barrier, and therefore, its presence in CSF is suggestive of intrathecal production.[34] IgM may be falsely negative in the early phase in immunocompromised patients who have not yet mounted an antibody response, and PCR or repeat CSF IgM assay 7 to 10 days into the illness may be more appropriate in this setting.[35] Despite the high sensitivity of ELISA, the assay has poor specificity because of cross-reactivity with other neuroinvasive flaviviruses (eg, Zika virus, yellow fever virus, St. Louis encephalitis virus, and dengue virus). Confirmatory testing can be performed using plaque reduction neutralization testing (PRNT) for WNV and other flaviviruses. It is also notable that WNV IgM titers can remain positive for up to 1 year in serum and 7 months in CSF.[36] Therefore, demonstration of a 4-fold or greater increase in virus-specific antibody titer or elevated virus-specific IgG antibodies in the acute or convalescent serum sample confirms acute infection.

In 2016, Zika virus spread rapidly through South America and led to an increased incidence of microcephaly, Guillain-Barré syndrome, encephalitis, and myelitis.[37] Once Zika virus was recognized as the etiologic agent 2 years after it was first introduced to Brazil,[38] pathogen-specific reverse transcription-PCR and Zika virus IgM serology were used on CSF to detect neuroinvasive disease. Zika virus serology suffered from the same drawbacks as most flavivirus serologies with false positives due to cross-reactivity. However, the Euroimmun anti–Zika fever IgG and IgM ELISA tests demonstrated high specificity for the Zika virus.[39] The Centers for Disease Control and Prevention no longer recommends the PRNT in regions with high prevalence of multiple flaviviruses due to its low accuracy in this setting.[40] Instead, patients are tested for dengue virus and Zika virus on CSF to rule out cross-reactivity.[41]

Lyme Disease

Lyme disease is a tick-borne illness secondary to the *Borrelia burgdorferi sensu lato* group and is endemic to North America, Europe, and Asia. *Borrelia burgdorferi sensu stricto* is the main species found in North America, whereas 5 known species are endemic to Europe. Lyme disease can present with a wide range of neurologic syndromes, including polyradiculitis, multiple cranial neuropathies, myelitis, meningitis, brainstem encephalitis, and optic neuritis.[42] Diagnostic difficulties are encountered due to poorly performing assays, insensitivity of US assays against European *Borrelia* species, delayed serologic response early in the disease, and the inability for serology to delineate past and active infection. Hence, it is important to conduct tests in patients with an appropriate history and examination for neuroborreliosis, therefore increasing the pretest probability and yield from laboratory investigations. Direct identification of the spirochete is difficult with resultant low sensitivities for cultures and PCR. A diagnosis is achieved through a 2-tier system with EIA followed by Western blot. EIA is highly sensitive, and if positive or equivocal, the Western blot is performed.[43] If the symptoms have been present for less than 1 month, then IgM and

IgG are assayed. Two reactive bands constitute a positive IgM, and 5 or more out of 10 possible bands are a positive for IgG. If symptoms have occurred for longer than 1 month, then only IgG is performed, although like WNV, IgM antibodies to *B burgdorferi* can persist for months. IgM alone cannot be used to confirm a diagnosis, and evidence of seroconversion may be required.[43,44]

Both the EIA and the Western blot have significant flaws and may soon be superseded by newer assays. The current EIA was developed from whole cell sonicates of cultured *B burgdorferi* with no specific targeted antigen, which leads to a high degree of cross-reactivity. The Western blot has poor sensitivity; there are no bands that are more specific for the organism, and multiple antibodies with similar weights may colocate over the same band.[45] Both these tests perform very poorly with 50% sensitivity in early presentations, and serology may take up to 3 to 6 weeks to become positive.[46] Newer serologic tests target specific antigenic proteins, such as C6, on the "variable major protein–like sequence, expressed," a cell surface lipoprotein.[47,48] These new assays have demonstrated excellent sensitivity and specificity and may soon replace the Western blot in the 2-tier algorithm.[45,49] However, they suffer from similar issues of poor sensitivity in early disease, the inability to differentiate between active and past infection and false negatives in immunocompromised patients.[44,50,51]

A diagnosis of neuroborreliosis is made with a suggestive history and examination consistent with Lyme disease, positive serum serology, CSF pleocytosis, and evidence of intrathecal antibody production. Most patients will display a CSF pleocytosis and elevated protein, except in cases of polyneuropathies.[52] In early neurologic disease, elevated intrathecal antibody production is evident in only 75% of patients but increases to nearly 100% within several months. The IgG index is elevated in 100% of all late neuroborreliosis cases.[53,54] The index can remain elevated for several years after treatment and cannot be used as a marker for follow-up nor clinical activity.[54] Measurement of C6 on CSF has had variable results and sensitivities.[55] CXCL13 is a B-cell–attracting chemokine that has a high sensitivity even before detectable intrathecal antibodies with decreased levels after treatment.[56,57] False positives have also been found with CNS lymphoma, TB meningitis, and neurosyphilis.[56,58]

Neurocysticercosis

Neurocysticercosis (NCC) is caused by infection with *Taenia solium*, a pork tapeworm. NCC is endemic to Central America, South America, Sub-Saharan Africa, and Asia.[59] Diagnosis is made on clinical, exposure history, and radiological characteristics with confirmatory laboratory diagnosis. The lentil lectin glycoprotein enzyme-linked immunoelectrotransfer blot (EITB) is a Western blot assay that is considered the test of choice.[60] This assay uses 6 glycoprotein antigens on a strip to detect antibodies to *T solium*. Appearance of any of the 6 bands is consistent with a systemic infection by the parasite.[61] In patients with 2 or more noncalcified or enhancing lesions on brain imaging, serum EITB carries a sensitivity of 98% and 100% specificity for NCC.[62] However, the EITB performs poorly on samples from patients with single lesions (28%) and calcified lesions. This may be due to a lack of an

antigenic response from dead calcified lesions compared with viable cysts. Serum carries a slightly higher sensitivity than CSF.[63]

Compared with the EITB, serum ELISA has poor sensitivity (89%) and specificity (93%) due to cross-reactivity with other helminthic infections.[64] This is less problematic in CSF due to fewer non-NCC antigenic components, allowing for a decreased test threshold and increased sensitivity.[63] CSF titers may also be higher in patients with subarachnoid, intraventricular, or malignant disease. ELISA also fares poorly with single or calcified brain lesions.[64–66]

The main drawback of serology is false positives in asymptomatic patients from endemic regions and an inability to differentiate between active and inactive infection. Some studies suggest that 40% of positive results in endemic regions are due to transient antibodies that become undetectable within 1 year.[67] For this reason, caution must be used when assessing patients from endemic regions, and weight should not be solely placed on serologic testing, but rather the entire clinical and neuroradiological information should be considered.

ANTIGEN TESTING

Antigen testing involves detection of antigenic proteins specific to a microbial source by immunologic methods, such as latex particle agglutination, coagglutination, and ELISA.

Neurocysticercosis

Monoclonal antibody-based antigen testing using ELISA is commonly used for NCC. Antigen levels are higher in patients with viable parasites, extraparenchymal disease, as well as the quantity and size of lesions. CSF samples have a higher sensitivity than serum. Sensitivity is again lower with calcified and single lesions.[68] Antigen testing is used to monitor treatment response because NCC antigen titers should normalize in successfully treated patients.[68–70]

Fungal Antigen Testing

Antigen testing is a rapid and accurate test for the diagnosis of *Cryptococcus neoformans.* This testing is done through latex particle agglutination or enzyme immunoassay. The test targets the cryptococcal polysaccharide capsule glucuronoxylomannan. The sensitivity of antigen testing is very high with 99% sensitivity and 97% specificity.[71] The introduction of the point of care lateral flow assay has allowed rapid and accurate diagnosis of Cryptococcus in resource limited settings. The lateral flow assay can be performed on serum, plasma, and CSF. It takes approximately 15 minutes for a result and has a higher sensitivity than standard latex particle agglutination.[72,73] Antigen titers decrease rapidly in response to treatment but may not normalize, with persisting low titers despite negative cultures, CSF normalization, and clinical improvement. Antigen testing should not be used to assess for cure.[74]

Galactomannan is a cell wall polysaccharide that is released by *Aspergillus* species during growth. Galactomannan antigen testing uses antibodies directed against b(1r5)-linked galactofuranosyl residues found on the side chains of galactomannan.[75] Its use for the detection of invasive aspergillosis in immunocompromised patients has been extensively

studied in serum and recently in CSF with a sensitivity of 88% in the latter. Specificity is 96% due to cross-reactivity with Trichocomaceae family, *Fusarium* spp, and *Histoplasma capsulatum.*[76] Serum false positives can occur from antibiotic therapy (piperacillin-tazobactam), bacterial infections, blood transfusions, and dialysis. Sensitivity of the assay increases in patients with hematologic malignancy and severe neutropenia in comparison to solid organ transplant patients and those with mild immunosuppression.[75]

1,3-beta-D-Glucan (BDG) is the major cell wall component of most fungal species, and BDG antigen testing is used as a broad test for detection of fungal pathogens. *Cryptococcus* spp do not contain high levels of BDG in their cell walls and therefore are not detected. BDG antigen testing is helpful for detecting invasive aspergillosis and candidiasis. Most studies were conducted on serum that displayed 60% to 100% sensitivity with a recommended test cutoff of 60 to 80 pg/mL.[77] After a recent outbreak of fungal meningitis secondary to contaminated intrathecal methylprednisolone,[78] studies have suggested that CSF BDG at a cutoff of 138 pg/mL has a 100% sensitivity and 98% specificity for *Aspergillus fumigatus, Exserohilum rostratum, Cladosporium cladosporioides, Epicoccum nigrum*, and with decreasing titers suggestive of an effective treatment response.[79,80]

POLYMERASE CHAIN REACTION

Herpes Simplex Viruses

Over the last 2 decades, the advent of PCR has revolutionized the diagnosis of infectious diseases. Its ability to detect common viral and bacterial pathogens has made it the gold standard in clinical diagnostics.[81–83] DNA is extracted from a biological sample and heated to separate the nucleic acid. Oligomeric primers for the organism-specific sequences are added with DNA polymerase, leading to transcription of new DNA, which is complementary to the target sequence. This process is repeated multiple times with each new strand undergoing the same process, leading to exponential amplification and increasing sensitivity. Labeled nucleotides are added during the final run to confirm the suspected genomic sequence.[84] The diagnosis of herpes simplex encephalitis (HSE) was revolutionized by the development of a CSF PCR assay.[81,85] Before this, diagnosing HSE required a brain biopsy because viral culture had only 5% sensitivity. HSV-1, 2 PCR has a sensitivity of 98% and 94% specificity.[86] False negatives may occur within the first 72 hours or after 7 to 10 days of antiviral treatment. If high clinical suspicion exists for HSE, then repeat lumbar puncture (LP) and PCR are required despite an early negative CSF HSV-1, 2 PCR.[87] PCR is available for numerous pathogens, including standard bacterial meningitis pathogens, VZV, enterovirus, human herpesvirus-6, Epstein-Barr virus, cytomegalovirus, JC virus, and WNV. Each PCR has different test performance characteristics, so both negative and positive results have to be interpreted in clinical context.[15]

Mycobacterium tuberculosis

The Xpert MTB/RIF is a rapid PCR used as the standard molecular test for the diagnosis of pulmonary TB. Xpert sensitivity for TB meningitis is approximately 50% depending on CSF volume and processing technique.[88] The Xpert MTB/RIF also allows detection of rifampicin resistance, a key drug in TB antimicrobial regimens. The new Xpert MTB/RIF Ultra is the

next generation of the Xpert MTB/RIF and has recently been adopted by the World Health Organization as the test of choice for the diagnosis of TB meningitis.[89] Preliminary studies found a sensitivity of ~95% for TB meningitis when compared with Xpert MTB/RIF or TB cultures combined. However, when tested against the current uniform case definition for TB meningitis, Xpert MTB/RIF Ultra demonstrated a sensitivity of only 70%.[90]

Multiplex Polymerase Chain Reaction

Multiplex PCR is a technique in which multiple primers are used allowing detection of several organisms by a single assay. The FilmArray meningitis and encephalitis panel is a rapid, multiplex PCR panel that tests for 14 common viral, bacterial, and yeast pathogens. A recent prospective multicenter trial evaluating the FilmArray displayed a range in sensitivity of 85% to 100% depending on the organism. However, there was also a high rate of false positives and several false negatives.[91] The US Food and Drug Administration has approved this multiplex panel, and some hospitals are using it as a stand-alone test. Conventional agent-specific confirmation testing by PCR may be more appropriate in some circumstances.

Bacterial and Fungal Polymerase Chain Reaction

The 16s recombinant ribosomal RNA (rRNA) gene is a highly conserved genetic region that is found in all bacteria. The sequence is approximately 1550 base-pairs long and contains both hypervariable and conserved regions. Universal primers are used to complement either end of the conserved region. The hypervariable regions contain specific signature sequences useful for bacterial identification at a species level.[92] Fungal pathogens can be identified using a similar process with universal fungal primers targeting the ITS1 and ITS4 conserved regions on the 18s and 28s rRNA sequences, respectively. The amplified sequences include the variable ITS2 region for species identification.[93] The use of 16s rRNA PCR for bacterial meningitis has been encouraging. In culture-proven cases of meningitis, 16s rRNA PCR demonstrated a sensitivity of 94%, a specificity of 94% confirming a bacterial cause, and was positive in 30% of culture-negative cases.[94] In cases of suspected CNS infection with a CSF pleocytosis greater than 500 cells/μL (to increase likelihood of bacterial pathogens), universal primers to 16s and 18s had a 65% sensitivity compared with 35% by microscopy and culture. The main reason for discordance was pretreatment with antibiotics before LP leading to diminished culture results.[95]

BRAIN BIOPSY

Before the advent of advanced molecular and immunologic testing, brain biopsy was considered the gold standard for diagnosis for certain encephalitides. However, the yield from brain biopsies is moderate, and its ability to identify a clear cause in encephalitis is poor.[96] Indeed, a recent study demonstrated that the most common initial pathologic diagnosis after biopsy was “encephalitis of unclear origin.” Despite this, diagnostic yield may be increased with re-review by a neuropathologist, careful clinical evaluation with appropriate follow-up, and more advance molecular and immunologic testing.[97] Neuropathology review adds to the hypotheses of what type of encephalitis might be present even if a specific cause is not identified.

HYPOTHESIS-FREE TESTING

The performance characteristics of a pathogen-specific test are irrelevant if that organism is not on the treating physician's differential diagnosis, and thus, the test is not ordered. The candidate-based diagnostic approach relies on the unrealistic expectation that a clinician will have complete knowledge of all pathogenic, local microorganisms and their clinical manifestations. Although certain pathogens are native and endemic to specific regions, a constant flux of novel and mutated microorganisms commonly occurs.[98] This ever changing microbial landscape is becoming increasingly evident in the era of globalization and climate change. Migration and travel have led to rapid spread of pathogens into new regions, increasing the potential for epidemics and pandemics. Zika virus is a recent example of a virus that spread rapidly, with 2 years elapsing before its neurologic manifestations became apparent.[38]

Over the last decade, the cost of whole genome sequencing has fallen drastically and can now be achieved for less than $1000 with the data being generated in a day rather than the 10 years and $3 billion it took to sequence the first draft of the human genome. Unbiased mNGS provides a hypothesis-free and agnostic approach to the diagnosis of infectious meningoencephalitis. Total DNA and RNA are extracted from a patient's biological sample (ie, CSF and/or brain biopsy material) and both host and nonhost nucleic acid are amplified and then sequenced in a massively parallel manner with NGS technologies. After human and environmental contaminant sequences are computationally filtered out, the remaining sequences are rapidly matched against publicly available databases to identify the infectious cause.[99] Instead of multiple, targeted PCR tests being performed, mNGS allows testing of thousands of pathogens including novel organisms within a short timeframe.[100] mNGS can identify early outbreaks and the arrival of novel organisms to a region before large epidemiologic studies can detect definitive trends.[101,102] The final promise of mNGS is to identify previously overlooked neurotropic pathogens that cause meningoencephalitis, thereby gradually discovering the organisms responsible for some of the large percentage of cases that are deemed as unknown origin.

Potential Drawbacks

As all the nucleic acid within a sample is amplified in the mNGS assay, invariably there will be amplification of host and environmental contaminant sequences. The latter can originate from the patient's skin flora, microbial nucleic acid present in the collection tube, and laboratory reagents. This significant "background noise," which frequently includes many bacterial and fungal species that have pathogenic potential, can make interpretation difficult. Thus, stringent measures should always be taken during sequencing library preparation to minimize cross-contamination. A mock sequence library is created from water samples and is used as a control, thereby characterizing the environmental and background microbiome.[103,104]

In addition to computational solutions to mitigate difficulties discriminating between signal and noise, molecular depletion and enrichment techniques have been developed. Commercially available human ribosomal and mitochondrial RNA depletion kits are not useful for CSF samples because of the very low RNAyields (typically pico-gram quantities).

Therefore, depletion of abundant sequences by hybridization (DASH) is a tool now being utilized to remove unwanted sequences. DASH uses CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology to target human complementary DNA (cDNA) within an already amplified sequencing library to reduce background noise in a highly specific and programmable manner that is completely agnostic to the input sample type and quantity.[105]

Conversely, VirCapSeq-VERT is a method for enriching viral sequences in metagenomic sequencing libraries by up to 10,000-fold. Approximately 2 million oligonucleotide probes that are designed to bind to the coding site of all viral taxa known to infect vertebrae are hybridized to a cDNA library. Streptavidin magnetic beads are added to the probes and their associated cDNA components. The beads are magnetically captured, cDNA removed, followed by posthybridization PCR. However, this method only enriches for known viral pathogens.[106,107]

The delay in processing massive amounts of data used to be the bottleneck in the timely delivery of clinically pertinent information. However, with rapid development in bioinformatics pipelines, the time required to process these data has been reduced drastically. Several pipelines currently exist, including Sequence-based Ultrarapid Pathogen Identification (SURPI), which is a cloud-compatible, open-access, computational pipeline used for pathogen identification from complex mNGS data.[108] SURPI was tailored for clinical use, and its speed is suited for clinical application where results are required within hours. The algorithm initially matches the sequence library against viral and bacterial databases and can process 7 to 50 million reads within 10 to 30 minutes. If this is negative, a comprehensive review of all pathogens in GenBank is performed within in 1 to 5 hours. The simultaneous development of both mNGS and bioinformatics has allowed exponential progress in the field of infectious diagnostics with promise for ongoing advancements.

Clinical Application

The use of research-based mNGS in the sphere of meningoencephalitis gained momentum after several notable cases and case series.[35,104,109–113] Until recently, astrovirus was considered only as a gastrointestinal infection and was not a standard test in the investigation of meningoencephalitis. Several recent cases have demonstrated a divergent genotype of astrovirus (HAstV-VA1/HMO-C-UK1) that has highly neurotropic characteristics in immunocompromised patients.[100,114–116] Given this discovery, it is now recommended that astrovirus should be considered during the workup of patients with meningoencephalitis.[100] These cases demonstrate the ability of mNGS to discover new neuroinvasive organisms that have not been previously considered pathogenic.

The first evaluation of a clinically validated CSF mNGS assay whose results are reportable in the patient medical record has now been conducted. The Precision Diagnosis of Acute Infectious Diseases study enrolled 204 patients with idiopathic meningitis, encephalitis, or myelitis at 8 hospitals, and the study results are currently under review. This and other studies promise to guide clinicians and health policy experts as they seek to understand the proper context in which mNGS testing is most appropriate.

SUMMARY

Neuroinfectious diseases continue to play a major role in morbidity and mortality worldwide, with many emerging or reemerging infections resulting in neurologic sequelae.[117,118] There is a growing need for rapid and accurate diagnostics that can lead to meaningful results and curb the significant burden of these diseases. Careful clinical evaluation of the patient coupled with the appropriate laboratory investigations leads to the correct diagnosis and implementation of appropriate management. mNGS is a promising new tool as its ability to identify multiple pathogens in a single test leads to an unbiased and agnostic approach in the diagnosis of infectious diseases. Prospective studies are forthcoming and will help to answer urgent questions about the overall performance characteristics of mNGS relative to conventional diagnostic modalities. As with other direct detection assays, it is likely that CSF mNGS will be relatively insensitive for detecting pathogens that are traditionally diagnosed with serology (eg, WNV and syphilis), that are anatomically localized (ie, brain abscess), or that have very low titers in the CSF.

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KEY POINTS

- A thorough clinical evaluation of a patient with meningoencephalitis should guide the physician toward a thoughtful differential diagnosis that will guide the selection of appropriate diagnostic tests to rule in or rule out suspected infections.
- Knowledge of the role and accuracy of each of the many diagnostic tests for identifying neurologic infections is crucial for accurate diagnosis.
- Multiplex assays, including unbiased metagenomic next-generation sequencing, promise to increase diagnostic yield in patients with meningitis and encephalitis.