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SUPPLEMENT ARTICLE

Metagenomic Next-Generation Sequencing in Central Nervous System Infections: Clinical Strategies, Evidence, and Best Practices

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Background. Central nervous system (CNS) infections are diagnostically challenging due to their nonspecific clinical presentations and wide array of potential pathogens. The rising population of immunocompromised patients further complicates this landscape, increasing the prevalence of atypical and opportunistic infections that are often missed by conventional testing.

Objective. This article provides guidance on the use and clinical interpretation of cerebrospinal fluid (CSF) metagenomic next-generation sequencing (mNGS) in suspected CNS infections.

Discussion. We highlight the paradigm shift from targeted molecular testing to agnostic mNGS, emphasizing key factors that impact diagnostic utility, including specimen handling, neuroanatomical factors, host inflammatory response, and pathogen kinetics. Using illustrative cases, we demonstrate how these biological and technical variables influence test sensitivity and result adjudication. We further discuss the impact of mNGS on clinical decision-making and current limitations regarding cost and turnaround time.

Conclusions. Cerebrospinal fluid mNGS is a transformative diagnostic tool, particularly for unusual presentations and in immunocompromised hosts. However, it does not replace clinical judgment and requires careful multidisciplinary interpretation. When integrated thoughtfully with clinical and laboratory data, mNGS can meaningfully reduce the diagnostic gap in CNS infections.

Keywords. metagenomic next-generation sequencing; mNGS; cerebrospinal fluid; CNS infections; neuroinfectious diseases.

THE IMPORTANCE OF MOLECULAR DIAGNOSTICS IN CENTRAL NERVOUS SYSTEM INFECTIONS

Nonspecific presentations of central nervous system (CNS) infections conceal a remarkable diversity of pathogens. Because these infections can be both treatable and fatal, clinicians must exclude infection across a wide range of clinical scenarios [1]. While viral pathogens remain the most common causes of meningitis, bacterial infections account for much of the mortality [2]. In the United States, an expanding immunocompromised population—including transplant recipients, patients on chemotherapy, and those treated with immunosuppressive agents for autoimmune disease—heightens the suspicion for opportunistic and atypical CNS infections, including neuroinvasive fungi, varicella zoster virus reactivation, and atypical bacterial pathogens [3, 4]. Furthermore, early empiric antimicrobial use often obscures conventional diagnostic results, particularly in critically ill or immunocompromised patients.

This landscape underscores the need for molecular diagnostics that are broad, sensitive, and rapid enough to meaningfully inform real-time clinical decision-making. Advancements in polymerase chain reaction (PCR), serologic testing, and metagenomic next-generation sequencing (mNGS) offer solutions to these challenges, but clinicians can face a paradox of choice as they navigate an expanding array of diagnostic modalities (Table 1) [3–5]. Recognizing that comprehensive resources already exist for the broader diagnostic approach to CNS infections [6–8], this article provides practical guidance on the use and interpretation of CSF mNGS, reinforced with 3 illustrative cases.

OVERVIEW OF METAGENOMIC SEQUENCING

Though the concept of sequencing mixed microbial DNA dates back to the 1990s, the field was revolutionized by the emergence of next-generation sequencing (NGS) technologies around 2005 [9]. The subsequent adoption of *clinical* metagenomic NGS (mNGS) gained significant traction over the past decade, following the maturation of high-throughput sequencing platforms and bioinformatic tools [10, 11]. The use of mNGS in CSF was an early and impactful example of this technology's

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Table 1. Examples of Nucleic Acid-Based Tests for CNS Infections

	Targeted PCR	PCR Panel	Universal PCR	CSF mNGS
Sample type	CSF, biopsy specimens	CSF	CSF, biopsy specimens	CSF
Target	RNA and DNA	RNA and DNA	16S rRNA (bacteria), 28S rRNA and ITS (fungi)	RNA and DNA
Types of pathogens detected	Varies by target, typically viruses, fungi, parasites	Viruses, bacteria, fungi	Bacteria, fungi	Viruses, bacteria, parasites, fungi
Number of pathogens detected	1	14–16	100s–1000s	1000 s within a reference database
Relative cost	$	$–$$	$$	$$$
Laboratory processing time	Usually hours for common pathogens, some may require send-out	Hours, usually performed in-house using commercially available panels	Hours to days, but usually sent out to specialized laboratory	48+ hours, usually sent-out to specialized laboratory
Also known as…	Conventional PCR, singleplex PCR	MEP, multiplex panel	uPCR, broad range PCR, 16S sequencing	NGS

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; MEP, meningitis/encephalitis panel; mNGS, metagenomic next-generation sequencing; PCR, polymerase chain reaction; uPCR, universal PCR.

diagnostic potential, highlighted by a landmark 2014 case where it successfully diagnosed neuroleptospirosis in an adolescent boy after an extensive series of more than 30 conventional diagnostic tests proved non-diagnostic [12]. Since that time, access to mNGS has significantly expanded, with Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories now offering validated CSF mNGS assays.

Unlike traditional PCR, which targets specific nucleic acid sequences using primers, mNGS amplifies and sequences *any* nucleic acid fragments present. This includes host (human) DNA which is later bioinformatically filtered. Clinical mNGS platforms can sequence either DNA, RNA, or both, enabling the detection of viruses, bacteria, fungi, and parasites in a single test [10]. As a result, mNGS is an agnostic diagnostic modality that marks a paradigm shift from targeted assays (ie, PCR) to a broad, unbiased strategy. This hypothesis-free approach enables detection of common pathogens, fastidious and innately unculturable organisms, pathogens in sterilized specimens from antimicrobial exposure, and even novel neurotropic agents. Rather than triaging a narrow set of suspected pathogens based on pretest probability, clinicians now must interpret unbiased sequencing data within the clinical context. In this way, mNGS redefines the clinician's role—from choosing *what to test* to discerning *what the findings mean.* Far from replacing it, clinical judgment remains integral to the impact and utility of mNGS [11, 13–16].

KEY CONSIDERATIONS FOR CEREBROSPINAL FLUID METAGENOMIC NEXT-GENERATION SEQUENCING

The key to using CSF mNGS wisely is remembering its simplest principle: detection requires the pathogen's nucleic acid to be present in the CSF, and yet the presence of an organism's nucleic acid in the CSF does not necessarily indicate that it is the cause of the neurological illness. Many factors influence whether a causative pathogen's nucleic acid ever reaches the subarachnoid space or persists long enough or at high enough levels to be detected, directly impacting the sensitivity of mNGS as outlined below (Table 2) [14].

Specimen Handling

Nucleic acids—particularly RNA—degrade quickly at room temperature. Samples should be processed near the time of collection, stored at a temperatures below <4C (ideally < −20C), or mixed with nucleic acid preservation buffer [17–19]. This is a particularly important consideration for neuroinvasive RNA viruses such as enteroviruses, flaviviruses, orthomyxoviruses, and measles [20–22]. Sterile technique and aseptic conditions during collection are necessary to limit microbial contamination in the sample due to its potential impact to the sensitivity of mNGS.

Anatomical Factors

Importantly, mNGS performed on CSF tells us what is in the CSF—not what is in the entirety of the CNS. Pathogens confined to compartments without communication to the subarachnoid space, such as walled-off abscesses, dural-based lesions or epidural abscesses, may not shed detectable nucleic acids into the CSF [23]. At present, mNGS is not validated for use on human brain tissue or abscess drainage fluid; therefore, its clinical utility is limited in localized infections that require surgical sampling or debridement. In those cases, other methods validated for use in tissue or drainage fluid such as "universal PCR" (uPCR) testing may be more appropriate. Universal PCR utilizes primers to amplify and sequence conserved areas of the genome, most commonly the 16S rRNA gene (for bacteria) or the 28S rRNA or internal transcribed spacer (ITS) genes (for fungi), and thus offers a broad, untargeted approach to detect bacteria and fungi [24, 25]. Universal PCR shares with mNGS the limitation of requiring

Table 2. Key Considerations in the Use of CSF mNGS to Diagnose CNS Infections

	Consideration	Impact on Decision to Use mNGS
Anatomical	CSF mNGS may not identify infections located outside of the subarachnoid space.	Correlate with neuroimaging findings to assess for infection outside the subarachnoid space (eg, epidural or brain abscess). Direct tissue sampling may be more appropriate than CSF testing.
Host factors	High concentrations of white blood cells (WBC) in the CSF can lower the sensitivity of mNGS.	Review cell count; if pleocytosis is significant (several 100 s—1000 s cells/μL), prioritize conventional cultures/stains for common bacterial/fungal pathogens which may be more appropriate and rapid.
Temporal	Peak load of pathogen nucleic acids in the CSF differs by pathogen, and may not correlate with clinical symptoms.	Assess symptom duration; for presentations suggestive of arboviral infections, in which CSF clearance occurs rapidly (eg, arboviruses, like West Nile Virus), prioritize serology.
Therapeutic	Patients on prolonged antimicrobials may have lowered pathogen loads in their CSF.	Document therapy duration; recognize that while pretreatment lowers yield, mNGS/PCR remain more likely to be positive than routine culture in this setting.
Pathogen factors	Pathogen load must be high enough in the CSF to be detectable; low burden or pauci-cellular infections often do not reach detectable levels.	If tuberculosis, syphilis, or Lyme are suspected, order targeted PCR and/or serology in parallel, as mNGS often lacks the sensitivity to detect these low-burden infections.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing; PCR, polymerase chain reaction.

adequate microbial burden and may be affected by prior antimicrobial therapy [26]. Additionally, uPCR is limited to detecting organisms within the phylogenetic group targeted by the primer set, and thus does not detect viruses and may fail to identify unusual bacterial/fungal pathogens outside the primer's specificity.

Host Response

There are host-specific factors that influence the sensitivity of CSF mNGS. The performance of mNGS is intrinsically linked to the amount of pathogen nucleic acid that binds to the sequencing flow cell. The human nucleic acids extracted from the sample (eg, from leukocytes and red blood cells) compete with pathogenic nucleic acids for this limited sequencing space. Considering that the relative burden of microbial nucleic acids in a sample is often minute compared with host nucleic acids, a prominent pleocytosis can interfere with the ability to detect a pathogen, and thus lower the sensitivity of the test [27]. This may be of particular concern in infections characterized by a relatively low burden of pathogenic organisms such as tuberculous, fungal, and parasitic meningitis. "Subthreshold" or "low abundance" reads have been found in CSF samples initially reported as negative on mNGS, but later confirmed positive by conventional assays, illustrating this challenge [28]. On the other hand, the diagnostic yield of mNGS is often low in CSF samples without pleocytosis in immunocompetent hosts reflecting the lower (but non-zero) pretest probability for a neurologic infection [28–30]. In light of these considerations, the sensitivity of CSF mNGS may be highest in immunocompetent patients with a moderate degree of pleocytosis [31], as well as immunocompromised patients without pleocytosis (who frequently have muted inflammatory responses and little or no pleocytosis) [28], though further data is needed. This host-response limitation is not present in uPCR methodologies because of the use of targeted primers, resulting in the amplification and sequencing of *only* the nucleic acids of interest.

Pathogen Factors

The concentration of pathogen nucleic acid in bodily fluids fluctuates during the course of illness, impacting the diagnostic yield of mNGS. For example, paucicellular infections such as Lyme neuroborreliosis and neurosyphilis involve low pathogen loads that render serology more appropriate than mNGS and targeted PCR throughout the disease course [3]. Similarly, in arboviral infections, such as West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus, the CSF viral load often peaks in the CSF several days before symptom onset and clears rapidly thereafter. Consequently, serology is often more sensitive than PCR or mNGS by the time a lumbar puncture is performed [21, 32]. For other paucicellular infections like tuberculous meningitis that lack serologic options, mNGS is hindered by the same low pathogen loads that limit the sensitivity of front-line PCR testing. Thus, clinicians should consider repeat testing if clinical suspicion remains high despite initial negative testing [33]. Finally, pathogen nucleic acids can persist for several days following the sterilization of CSF with empiric antimicrobial therapy for many pathogens [34–36], making PCR and mNGS important tools in these circumstances.

MULTIDISCIPLINARY INTERPRETATION

For the reasons discussed, clinical interpretation of mNGS results in suspected CNS infection cannot be reduced to a simple yes/no readout and requires careful integration with the patient's presentation and other diagnostic data (Figure 1). Although mNGS is highly sensitive and capable of detecting common, rare, and unexpected pathogens, this breadth can introduce challenges—particularly when detection of organisms appears at low abundance or represent latent viruses, commensals, or environmental contaminants. Because standardized interpretive frameworks remain limited, collaboration among neurology, infectious disease, and microbiology is essential [28, 37]. Clinicians should not

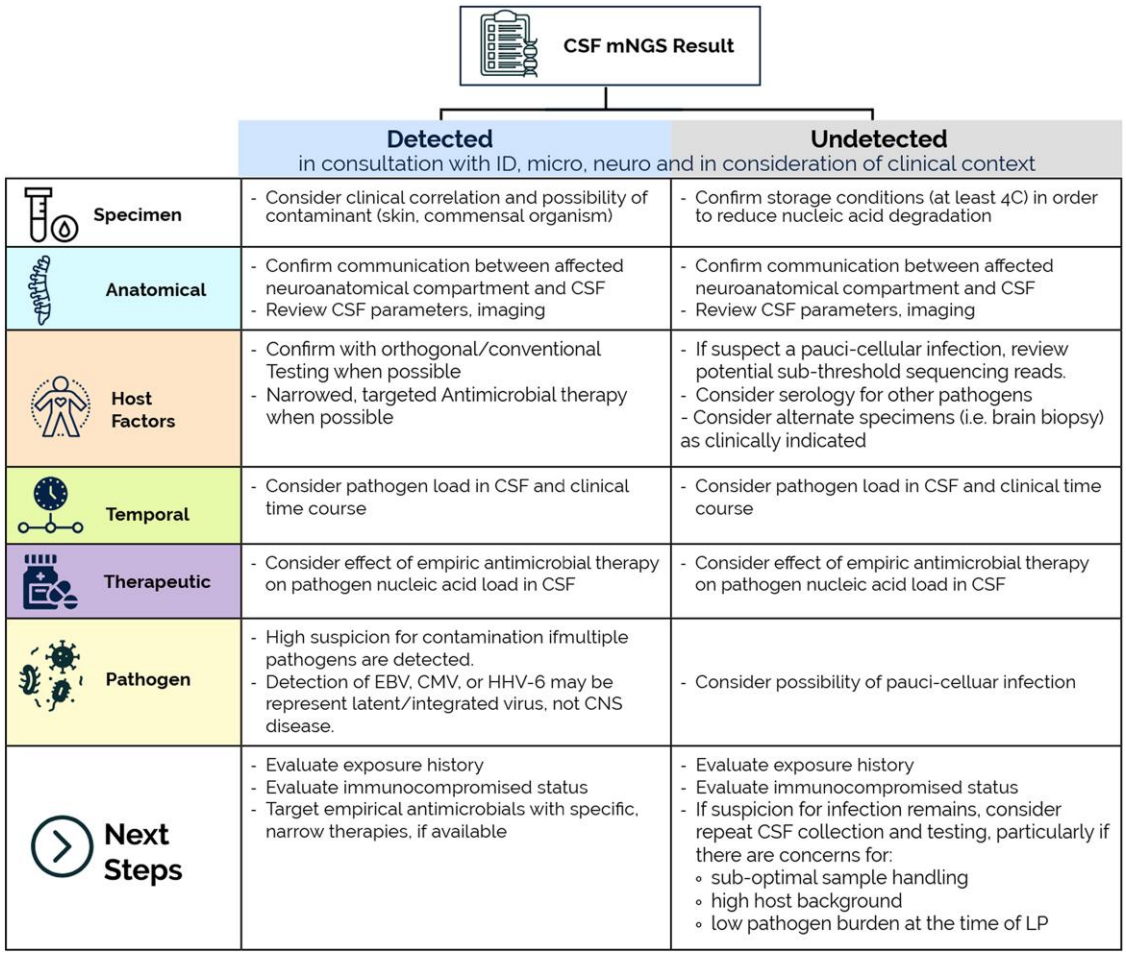

Figure 1. Clinical interpretation of CSF mNGSresults. Abbreviations: CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing.

hesitate to contact the performing laboratory to discuss specific findings; in many cases, this dialogue is needed for accurate interpretation. Ultimately, the clinician's judgment is paramount: in a hypothesis-free testing framework, it is the interpretation that determines whether an mNGS result advances clinical decision-making.

IMPACT ON CLINICAL DECISION-MAKING AND PATIENT OUTCOMES

When used in the appropriate clinical context, CSF mNGS can meaningfully influence diagnostic clarity and treatment decisions. A large US single-laboratory analysis reported positive results in 16.2% of samples, and among patients who ultimately had an infectious diagnosis, 21.8% were diagnosed by mNGS alone [28]. More than 70% of pathogens detected were viruses, underscoring the utility of mNGS for identifying viral etiologies of CNS infection.

Across prospective and retrospective studies, CSF mNGS results contributed to changes in clinical management in roughly 20%–30% of cases, most often through targeted adjustments to antimicrobial therapy [4, 38, 39], including both narrowing or broadening of antimicrobial coverage, initiation of specific antivirals or antifungals, and earlier recognition of atypical or unexpected pathogens. Also, in line with antimicrobial stewardship efforts, mNGS findings results supported discontinuation of unnecessary broad-spectrum antibiotics and facilitated earlier escalation to organism-directed therapy, contributing to improved clinical trajectories and fewer adverse drug effects [40]. Importantly, the added diagnostic contribution of mNGS appears most pronounced in complex settings—such as immunocompromised or pediatric populations—where conventional diagnostics often fall short. In these groups, mNGS identified pathogens missed by standard testing in over 20% of infections and frequently helped guide or de-escalate therapy [41, 42].

PRACTICAL CONSIDERATIONS AND COST

Because mNGS is relatively new and expensive, the field continues to refine where its use provides the greatest clinical value relative to cost and turnaround time. Laboratory processing times, while faster than many microbiologic methods (such as

fungal cultures), still range from 48 hours to several days, which may limit immediate decision-making in critically ill patients [14, 43]. Costs typically range from $2000 to $3500 per test, with inconsistent reimbursement across insurers and regions.

In current practice, CSF mNGS is often reserved for cases with nondiagnostic/negative initial testing, for immunocompromised hosts, or in presentations that fall outside typical pathogen presentation. Many institutions also limit mNGS to specimens with pleocytosis (particularly in immunocompetent hosts) to improve pretest probability and manage testing volume [28, 44]. This reflects both practical constraints (limited availability, higher cost, and the need for specialized interpretation) and the lack of consensus on whether mNGS should be used early, reflexively, or only after conventional tests are exhausted.

Emerging evidence suggests that earlier use of CSF mNGS may meaningfully reduce diagnostic burden. In a recent study that modeled the use of mNGS within 48 hours of the first lumbar puncture, the addition of mNGS could have reduced the number of microbiological tests by 64% and shortened time to diagnosis by an average of 6.9 days per patient with infectious meningitis [45]. In suspected autoimmune encephalitis there was an even greater reduction in unnecessary infectious testing (92%) and a 10.2-day reduction in time to diagnosis, driven largely by a negative mNGS result that supported earlier initiation of immunosuppressive therapy [45]. There is experience with this "rule out" approach in high-throughput tertiary care centers, where results can be thoughtfully integrated with clinical, radiographic, and laboratory data. However, this application remains controversial, particularly in isolated cases managed in settings without substantial familiarity in test performance and interpretation.

ILLUSTRATIVECEREBROSPINAL FLUID METAGENOMIC NEXT-GENERATION SEQUENCING CASE EXAMPLES

Case 1: Atypical Presentation of Central Nervous System Infection

Presentation

A 61-year-old woman with a history of lung transplant presented to the hospital with 1 month of headache, 2 weeks of progressive confusion, and new-onset seizures. Medications included prednisone, mycophenolate, tacrolimus, and prophylactic dose trimethoprim-sulfamethoxazole and valacyclovir. One week earlier, she was started on levetiracetam due to 2 episodes of transient unresponsiveness and hemibody weakness, most suggestive of seizure. However, her mental status continued to decline, prompting a return to the hospital. On admission, her neurologic exam was notable for severe encephalopathy; her eyes opened to voice, but she was unable to regard or track, did not have any verbal output, and was unable to follow any commands. Initial evaluation including vital signs, complete blood count, comprehensive metabolic panel, and magnetic resonance imaging (MRI) of the brain with and without contrast was unrevealing. Cerebrospinal fluid profile was notable for mildly elevated protein (83 mg/dL). Electroencephalogram demonstrated intermittent asymmetric rhythmic slowing (Figure 2).

Considerations for the Use of Metagenomic Next-Generation Sequencing

Given the broad differential diagnosis in this immunocompromised host a broad workup was pursued including serum and CSF infectious and autoimmune testing, systemic imaging, and an empiric decrease in the tacrolimus dose to assess for the possibility of tacrolimus-induced neurotoxicity. Cerebrospinal fluid mNGS returned positive for human polyomavirus 2, more commonly known as John Cunningham (JC) virus. The diagnosis of JC virus meningoencephalitis was confirmed with JC virus-specific CSF PCR, and her immunosuppression regimen was cautiously decreased in conjunction with her transplant physicians. Her symptoms gradually resolved, and follow-up CSF JC virus PCR 6 months later demonstrated a dramatic decrease in viral load.

Considerations for the Use of Polymerase Chain Reaction-Based Diagnostics

In this case, the diagnosis was ultimately confirmed with singleplex JC virus PCR, demonstrating the high clinical utility of targeted PCR when the suspected pathogen is known. However, such testing depends on a priori clinical suspicion. The hypothesis-free approach was therefore critical for identifying the virus in an atypical presentation where JC virus would not have been initially considered. Broad-range uPCR of the CSF would not have detected this pathogen, as uPCR assays are not designed to identify viral targets.

Key Takeaways

Cerebrospinal fluid mNGS can be an important tool to clinch the diagnosis in situations where there are uncommon presentations of known neuroinfectious diseases. Although progressive multifocal leukoencephalopathy is the most common neurologic manifestation of JC virus reactivation, other manifestations, including JC virus meningitis/encephalitis, are increasingly recognized and can occur in the absence of classical white matter involvement [46]. This case also serves as a reminder that the absence of pleocytosis should not reassure against CNS infection, particularly in immunosuppressed hosts.

Case 2: Neuroanatomic Mismatch

Presentation

A 66-year-old woman with a history of migraine and hypertension presented with 7 days of headache, fever and chills. The headaches were more severe than typical migraines, did not respond to over-the-counter analgesia and were associated with nausea and vomiting. MRI demonstrated a complete ring enhancing lesion in the left occipital lobe with internal diffusion restriction and surrounding vasogenic edema without mass

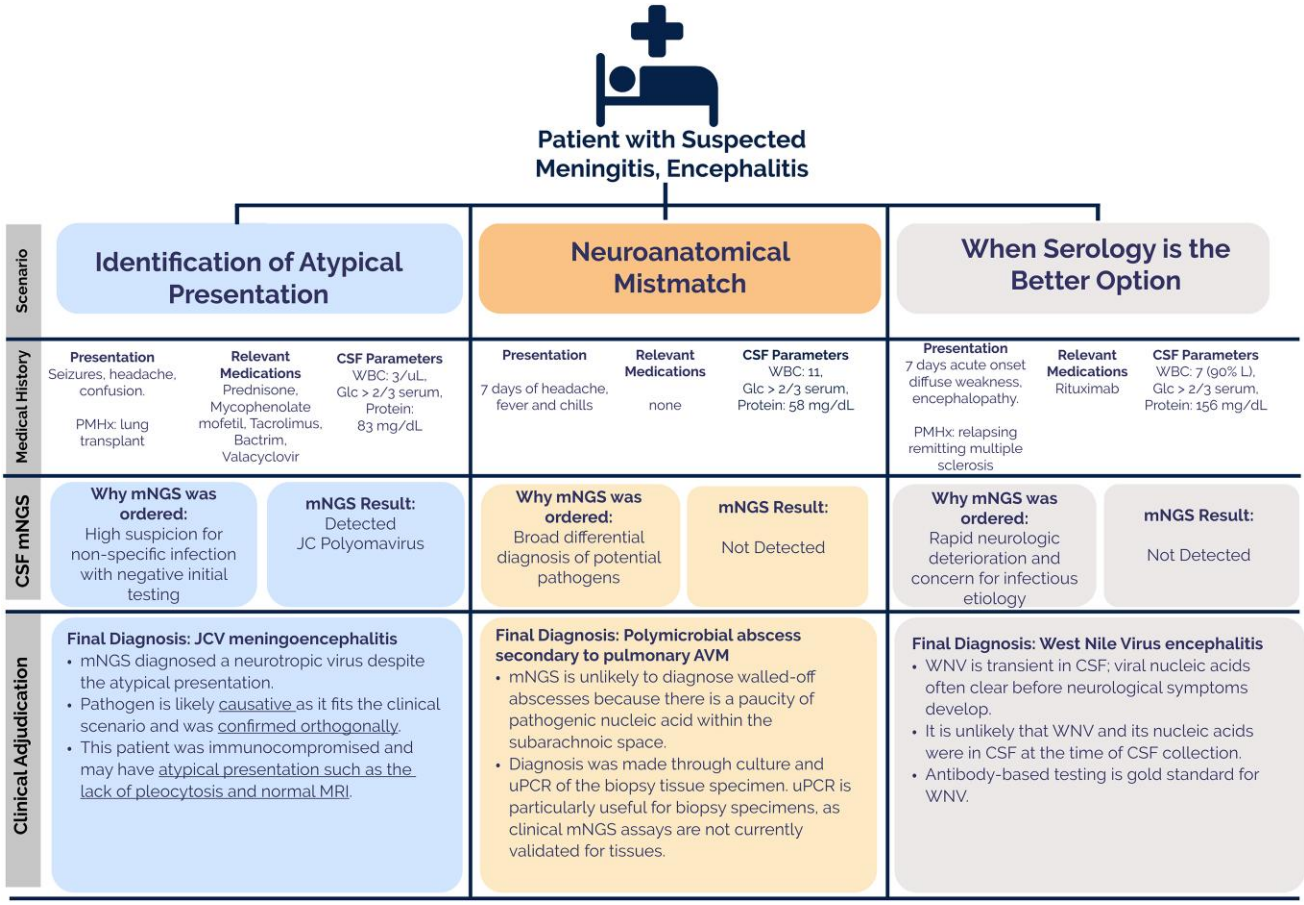

Figure 2. Clinical case summaries.

effect. Lumbar puncture was notable for mild pleocytosis (11 WBC), but was otherwise unrevealing. Broad serum and CSF infectious testing was negative, including CSF mNGS. Neurosurgical biopsy and drainage were performed given concern for abscess, and abscess culture demonstrated a polymicrobial abscess (*Streptococcus constellatus, Streptococcus anginosus, Bacillus* sp.). This finding was confirmed with uPCR. Subsequent systemic evaluation suggested that the polymicrobial abscess was likely due to a pulmonary arteriovenous malformation (Figure 2).

Considerations for the Use of Metagenomic Next-Generation Sequencing

Cerebrospinal fluid mNGS was of limited utility, given that the imaging was suggestive of a walled-off abscess. mNGS is not validated for brain tissue or abscess fluid.

Considerations for the Use of Polymerase Chain Reaction-Based Diagnostics

Targeted PCR testing of abscess material can be highly informative when specific pathogens are suspected based on clinical or epidemiologic context (eg, *Toxoplasma gondii*, *Mycobacterium tuberculosis*, or fungal organisms). However, as with all singleplex or limited-panel PCR assays, diagnostic yield depends on a priori clinical suspicion and selection of appropriate targets. Broad-range bacterial or fungal PCR performed on abscess tissue remains particularly when the differential diagnosis remains broad, and helped to confirm the abscess culture results in this case. This can be particularly helpful after multiple days of empiric antimicrobial therapy, where culture is more likely to be sterile.

Key Takeaways

Anatomic compartment matters—CSF testing, including mNGS, has limited diagnostic value in walled-off intracranial abscesses where pathogens are confined to parenchymal tissue. Alternative molecular nucleic acid testing and standard culture of biopsy tissues have higher diagnostic yield in these cases.

Case 3: Disease Stage/Pathogen Kinetics Mismatch

Presentation

A 59-year-old woman with a history of relapsing remitting multiple sclerosis, treated with rituximab, was admitted for

diffuse weakness and encephalopathy. One week prior, she presented to the emergency department with these same symptoms, but was discharged after MRI of the brain and cervical spine showed no new findings. However, she returned due to rapid progression of her symptoms and required intubation almost immediately. Repeat neuroimaging demonstrated new symmetric T2/FLAIR hyperintensities in the thalami, midbrain, pons, and cerebellar hemispheres, long segment central cord signal abnormality with associated enhancement in the cervical and thoracic cord, and enhancement of the ventral cauda equina nerve roots. Cerebrospinal fluid analysis was notable for lymphocytic pleocytosis (47 WBC, 90% lymphocytes), and elevated protein (156 mg/dL, Figure 2).

Considerations for the Use of Metagenomic Next-Generation Sequencing

Given the fulminant clinical decline requiring mechanical ventilation and the patient's B-cell depleted state, the neuroinfectious differential diagnosis remained broad. Cerebrospinal fluid mNGS was utilized as a "safety net" to potentially capture unusual or unexpected pathogens in this context, but this testing was ultimately negative. Ultimately the diagnosis of West Nile Virus (WNV) encephalomyelitis was made using the gold standard technique, positive WNV IgM serology from the same CSF specimen. The diagnosis was corroborated by the neuroimaging characteristics, the seasonal timing, and the known geographic distribution of the virus at the time of presentation.

Considerations for the Use of Polymerase Chain Reaction-Based Diagnostics

Given the clinical presentation—particularly lymphocytic pleocytosis and brainstem/spinal cord neurotropism—viral etiologies rank highest on the differential. Multiplex or targeted singleplex PCR assays may still be considered, but doing so would require a broad panel of individual tests to cover the possibility of an atypical presentation of a common viral infection. However, for some of these neurotropic viruses, serology remains the diagnostic gold standard because viral kinetics often limit nucleic acid detectability in CSF. Universal PCR is also unlikely to be helpful in this context, as it does not detect the viral pathogens.

Key Takeaways

Understanding the temporal relationship of the pathogen in the CSF and the clinical course of the disease is essential for interpreting mNGS results. West Nile Virus is an RNA flavivirus with a transient viremic/CSF phase ("hit and run") [32]. By the time neurologic symptoms are severe, the viral load is often below the limit of detection for nucleic acid amplification tests (both PCR and mNGS). In such scenarios, detection of the host immune response (via WNV IgM antibodies) remains the gold standard [32].

CONCLUSION AND FUTURE DIRECTIONS

Cerebrospinal fluid mNGS has emerged as a promising diagnostic modality that overcomes several limitations of conventional testing and offers a path toward reducing the large fraction of encephalitis and meningitis cases that remain unexplained. Its clinical value, however, depends on thoughtful ordering and multidisciplinary interpretation, grounded in the principles outlined in this paper: detection requires that the pathogen's nucleic acid be present in the CSF, and the presence of nucleic acid does not inherently establish causality. These biological and technical realities are shaped by specimen handling, host inflammatory background, pathogen lifecycle, and neuroanatomical compartmentalization, and directly influence test sensitivity and decisions about when and how to deploy mNGS. Within this framework, CSF mNGS offers advantages in cases with (1) negative conventional testing despite high clinical suspicion for infection, (2) immunocompromised patients at risk for uncommon infections, (3) broad differential diagnoses in which multiple targeted assays would otherwise be required, and (4) investigation of emerging pathogens or outbreak scenarios.

Despite its potential, several knowledge and implementation gaps must be addressed to fully integrate mNGS into routine practice. Prospective cost-effective analyses and structured diagnostic algorithms are needed to clarify its role within established diagnostic pathways. Evidence remains sparse regarding how mNGS influences downstream testing, antimicrobial use, and overall healthcare utilization. Standardized clinical workflows should guide specimen selection, incorporating factors such as disease stage and pretest probability to maximize diagnostic yield while avoiding low-value testing. Equally important is clinician education, as accurate interpretation requires understanding assay limitations, background signal, and the distinction between incidental detection and true clinical causation.

Technological hurdles also persist. While turnaround time and cost remain prohibitive in many acute settings, both are expected to improve as sequencing platforms scale and accessibility increases. Finally, validating mNGS for non-CSF specimens, including brain parenchyma and meningeal tissue, is an important frontier that could further advance the clinical utility of this technique in CNS infections.

Notes

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