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MINIREVIEW

State-of-the-Art Microbiologic Testing for Community-Acquired Meningitis and Encephalitis

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Meningitis and encephalitis are potentially life-threatening diseases with a wide array of infectious, postinfectious, and noninfectious causes. Diagnostic testing is central to determining the underlying etiology, treatment, and prognosis, but many patients remain undiagnosed due to suboptimal testing and lack of tests for all pathogens. In this article, we summarize the epidemiology, barriers to diagnosis, and current best tests for meningitis and encephalitis in developed countries. We end with a brief discussion of new test methods, such as multiplex panel-based tests and metagenomic sequencing, which are likely to alter diagnostic strategies for these conditions in the near future.

Community-acquired meningitis and encephalitis are potentially life-threatening syndromes due to inflammation of the meninges and brain parenchyma, with myriad infectious and noninfectious causes (1–6). Treatment, prognosis, and outcomes vary greatly between patients and depend primarily on the timely initiation of therapy based on identification of the underlying cause of inflammation, since clinical signs and symptoms are not pathogen specific (1–10). Nonetheless, many meningitis (15 to 60%) and encephalitis (40 to 70%) patients fail to receive a specific etiologic diagnosis leading to unnecessary or inappropriate treatment and potentially avoidable adverse events (8, 10–12). To address this issue, we discuss common reasons for failing to make an etiologic diagnosis in community-acquired meningitis and encephalitis and make general recommendations for the use of current and emerging microbiologic tests to maximize pathogen identification and appropriate treatment.

CLINICAL DEFINITIONS, MANIFESTATIONS, AND EPIDEMIOLOGY OVERVIEW

Meningitis is inflammation of the meninges, defined by an abnormal number of white blood cells (WBC) in cerebrospinal fluid (CSF) with few or no focal neurologic findings or brain abnormalities on imaging (3, 5, 13–15). Patients with meningitis typically present with some combination of fever, headache, meningeal irritation, and altered mental status, but CSF analysis is required to confirm the diagnosis and determine the underlying cause (1, 3, 5, 13). In contrast, encephalitis is defined as inflammation of the brain parenchyma with focal or global neurologic dysfunction, regardless of meningeal involvement (6, 16). In an effort to standardize the diagnosis and minimize overlap with other conditions, recent diagnostic criteria require altered mental status as a major criterion and two or more minor criteria (fever, seizures, focal neurologic findings, CSF WBC count of $\geq$5 cells/mm^3, abnormal brain imaging, or electroencephalogram) for encephalitis diagnosis (6).

As such, meningitis and encephalitis are uncommon, affecting 4 to 30 people/100,000 and 3 to 7 people/100,000, respectively, in developed countries each year, but the morbidity, mortality, and costs are substantial (4, 8, 10–12). For example, there are >70,000 meningitis-related hospitalizations in the United States each year, with an in-hospital mortality rate of 0.4 to 11.4% and cost of $1.2 billion (17). Encephalitis-related hospitalizations affect >20,000 people in the United States each year, with in-hospital mortality rates of 5.8 to 17.1% and cost of $2 billion (10). The rates for both illnesses are higher among infants and older adults (4, 10, 18).

Vaccines and public health interventions have had a dramatic impact on the epidemiology of meningitis and encephalitis over the past 50 years (19). For instance, mumps was the most common cause of viral meningitis prior to the measles, mumps, and rubella (MMR) vaccine but is now rare, and central nervous system (CNS) complications of varicella-zoster virus (VZV) and measles also declined after effective vaccines became available (19). Bacterial meningitis is a particularly striking example of the shifting epidemiology of these conditions. In the early 1980s, 10,000 to 20,000 cases of bacterial meningitis occurred in the United States each year, with the majority being due to *Haemophilus influenzae* type b (18–20). Twenty years later, the number of bacterial meningitis cases had declined to <4,200 annually in the United States, as a result of conjugate vaccines for *H. influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae*, and universal prenatal group B *Streptococcus* (GBS) screening (4).

Currently, most meningitis cases are infectious, but a sizable proportion have no infectious agent identified or may be due to noninfectious causes, such as medications, cancer, and systemic inflammatory conditions (1, 2, 13). Viral infections, including enterovirus, herpes simplex virus (HSV), and vector-borne virus (arbovirus) infections, are the most common causes, while bacterial, fungal, and parasitic causes are uncommon or rare but important to exclude due to their potentially life-threatening nature (12, 13, 17). The frequency of most microorganisms associated with meningitis also varies by host and geographic factors, season, and exposure history (21). For example, the type and incidence of

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arboviral infections vary markedly between geographic regions and both seasonally and year to year in regions of endemicity, depending on the climate, vector and reservoir population dynamics, and human behavior patterns (8, 21). Thus, arboviral infections are rare in the United Kingdom and northern Europe, occur seasonally in the summer and autumn in southern Europe and the United States, and occur year-round in the tropics (21). Bacterial meningitis is rare in healthy vaccinated populations but increased in infants and older adults and patients with persistent CSF leaks or basilar skull fractures, terminal complement deficiencies, and other immunocompromising conditions (4, 18). Agents that primarily affect immunocompromised patients and rarely cause meningitis in immunocompetent persons include *Cryptococcus* spp., cytomegalovirus (CMV), VZV, human herpesvirus-6 (HHV-6), and Epstein-Barr virus (EBV), among others (22, 23). *Mycobacterium tuberculosis* infections occur in patients with risk factors such as recent tuberculosis infection, prior residence in a region of endemicity, or immune-compromising condition (e.g., HIV infection). For a complete discussion of meningitis causes, including nosocomial and device-associated meningitis, which are distinct from community-associated meningitis, see references 13 and 24.

The epidemiology of encephalitis is also complex, with >100 infectious causes and a large proportion of patients with immune- or antibody-mediated disease or unknown etiology, despite extensive testing (6). About one-third of cases have a confirmed or probable infectious etiology as determined by comprehensive microbiologic testing. Viral infections are the predominant infectious cause in immunocompetent patients, with HSV (primarily HSV-1), VZV, enteroviruses, and arboviruses causing most cases, with some variation between regions (6, 8, 10, 25). Other viruses and bacteria are less common, but the number of potential causes is extensive, requiring multiple tests for diagnosis (6). Immunocompromised patients have additional agents that should be considered, including CMV, HHV-6/7, HIV, *Toxoplasma gondii*, *M. tuberculosis*, and fungi (6, 16, 25). Finally, encephalitis is similar to meningitis in that the likelihood of many infectious agents varies with host and geographic factors, season, and exposures, making it critical to consider local epidemiology and risk factors when selecting tests (6, 8). A recent consensus statement is an excellent reference for diagnostic testing in encephalitis (6).

COMMON BARRIERS TO ETIOLOGIC DIAGNOSIS AND TREATMENT

The clinical management of patients with meningitis or encephalitis is highly dependent on the underlying cause of infection or inflammation, making it necessary to obtain a specific etiologic diagnosis whenever possible. Delayed diagnosis and treatment are associated with increased mortality and adverse outcomes in patients with bacterial meningitis and HSV encephalitis (7, 9). Conversely, unnecessary hospitalization and treatment are common for patients with viral meningitis, resulting in potential harm and substantial avoidable costs in a population with a relatively benign and self-limited condition (11). Thus, it is important to identify the common reasons why physicians fail to make an etiologic diagnosis and take steps to optimize testing and diagnostic yield to improve management and outcomes.

The traditional lack of good rapid tests for most etiologies of meningitis and the reliance on nonspecific clinical signs and tests for initial treatment decisions have promoted a minimalist approach to managing patients that is a barrier to optimizing management as better tests become available. Lack of familiarity with the specific infectious and noninfectious causes of meningitis and encephalitis, risk factors, and best test(s) also likely play a role (26). Thus, failure to order the recommended CSF tests for common viruses (e.g., enterovirus nucleic acid test [NAT], West Nile virus [WNV] IgM) and the continued use of poorly performing tests, such as viral culture, decrease the likelihood of viral pathogen detection and contribute to continued empirical antibacterial use (26, 27). Unnecessary cranial imaging before lumbar puncture (LP) is an important cause of diagnostic failure and false-negative bacterial cultures when antibiotics are administered >1 to 2 h before LP and harmful treatment delays when antibiotics are withheld pending LP (9, 28). Finally, the large proportion of patients with an unknown cause of illness despite extensive testing, and an increasing recognition of immune-mediated causes of encephalitis, point to a need for additional studies to identify unrecognized causes of meningitis and encephalitis (6, 25, 29).

OVERVIEW OF CLINICAL USE OF MICROBIOLOGIC TESTING IN MENINGITIS AND ENCEPHALITIS PATIENTS

There are several goals of diagnostic testing in patients with suspected community-acquired meningitis or encephalitis, although occasional patients are treated empirically before testing is performed when clinical suspicion of infection is particularly high. The first goal is to confirm or exclude the presence of a CNS inflammatory process by CSF analysis (e.g., WBC count and differential, glucose, and protein), in combination with blood tests and other biomarkers, such as procalcitonin or CSF lactate. A related goal is to determine the initial likelihood of life-threatening infection, such as bacterial meningitis or HSV encephalitis, and the need for empirical treatment, based on the clinical presentation, CSF and blood parameters, CSF Gram stain, and other biomarkers or rapid NAT, if available. The next goal is to definitively confirm or exclude bacterial meningitis and other treatable or potentially life-threatening infections while patients are treated empirically or observed based on the level of clinical suspicion. This is typically done with blood and CSF cultures along with additional tests in meningitis patients, depending on the clinical presentation and disease severity, risk factors, and physician practice. Encephalitis patients get a large battery of tests for infectious and noninfectious causes, as directed by consultants, guidelines, risk factors, and imaging, electroencephalogram, and test results (6). Once definitive microbiologic results are available, patients with a specific etiologic diagnosis get standard antimicrobial treatment or supportive care, as appropriate. However, most patients have no infectious agent identified and receive a nonspecific diagnosis and empirical therapy or have treatment stopped, depending on the severity of illness and confidence in negative test results. Thus, the type and extent of microbiologic testing are key factors in determining the likelihood that an infectious agent will be identified and appropriate therapy will be administered.

CURRENT DIAGNOSTIC TEST METHODS

CSF cell count, glucose, and protein measurements play a fundamental role in confirming the presence and type of CNS inflammation and the likelihood and type of infection that may be present. However, the diagnostic accuracy of these parameters is limited by overlap between clinical conditions and is subject to important exceptions. For example, a CSF WBC count of >5 cells/

mm^3 is a common diagnostic threshold for CNS infection, but rare patients with meningitis and occasional patients with encephalitis have a lower CSF WBC count due to early, fulminant, or subcortical infection or immunocompromising condition (5, 6). Similarly, while a raised CSF neutrophil count typically suggests bacterial meningitis, many viral infections have an initial neutrophilic predominance, which transitions to lymphocytic predominance after one or more days (AIDS patients may never transition) (3, 15, 30–32). CSF glucose level must be evaluated with a simultaneous blood glucose for correct interpretation. A low CSF-to-blood glucose ratio (<0.6) suggests a nonviral cause, but results are nonspecific (3, 14, 15, 32). CSF protein elevation is common and also nonspecific (3, 15). Antibiotic pretreatment reduces CSF glucose and protein abnormalities fairly quickly (hours) in bacterial meningitis, with less effect on WBC and neutrophil counts (15, 33).

Other biomarkers have also been explored in an effort to identify a rapid single test to rule out bacterial meningitis and differentiate bacterial from viral meningitis. Of these, serum procalcitonin (PCT) and CSF lactate have the most potential to be useful clinically, with performances that are similar to or better than conventional CSF parameters in research studies (34, 35). However, both biomarkers are nonspecific and can be affected by prior antibiotic treatment and noninfectious conditions, making it unclear how generalizable these results are to routine clinical practice. Thus, most experts recommend that these biomarkers be used in combination with conventional CSF parameters and microbiologic tests until more data are available.

Due to the potential lethal nature of bacterial meningitis, microbiologic testing for aerobic bacteria is often a routine part of CSF examination, regardless of the level of suspicion for infection. CSF Gram stain and culture and blood cultures are the primary methods of testing in the United States, while NATs are increasingly used in the United Kingdom and elsewhere (18). Without rapid NAT, a concentrated CSF Gram stain is the best rapid test for bacterial meningitis, with an approximate limit of detection of 10^4 CFU/ml and sensitivity that ranges from 10 to 93%, depending on the microorganism, severity of infection, and bacterial load (18). For instance, CSF Gram stain sensitivity is relatively high for *S. pneumoniae* and GBS meningitis (60 to 90%) but much lower in *Listeria monocytogenes* meningitis (10 to 35%) (18). CSF culture is the traditional reference test for bacterial meningitis, with a limit of detection of 10^2 to 10^3 CFU/ml, but only 60 to 90% of cultures are positive when clinical diagnostic criteria are used (18). CSF culture sensitivity decreases further within 1 to 4 h of antibiotic administration (18, 28). Blood cultures are useful in bacterial meningitis patients and should be collected prior to antibiotic treatment in all patients when the diagnosis is suspected, especially when LP is delayed (18). Blood cultures are often more sensitive than CSF cultures in listeriosis. The performance of blood and CSF cultures in encephalitis is unknown. NATs have the potential to improve the speed and frequency of bacterial meningitis diagnosis, but there is limited clinical experience outside the United Kingdom. However, published data suggest that NATs perform similarly to culture or better for most bacteria, and they perform much better in *N. meningitidis* cases and patients with prior antibiotic exposure (18). It is likely that NAT use will expand dramatically as rapid commercial multiplex NAT assays are adopted (see below). The detection of fastidious, slow-growing, and uncultivable bacterial infections, such as *Borrelia burgdorferi*, *Treponema pallidum*, *Bartonella* spp., *Rickettsia* spp. and other tick-borne bacteria, and *Leptospira* spp. requires a combination of tests, including serology from serum and CSF, NAT, and specialized culture techniques (6, 36–39). Testing for these agents should be guided by clinical signs and symptoms, exposures, risk factors, and the duration and severity of illness (6, 13, 16).

Specific viral testing is essential in patients with encephalitis and has been shown to improve clinical management and reduce costs in children with meningitis (6, 40). Viral infections are generally detected by a combination of NATs and/or serology, depending on the syndrome, virus, host, and duration of illness (3, 6, 21, 41). Viral culture is no longer recommended for clinical diagnosis but may be indicated when viral isolation is desired for antiviral resistance testing or typing (27). Qualitative NAT testing is the standard of care for the detection of enterovirus, HSV-1/2, and VZV from CSF samples in patients with meningitis or encephalitis (6, 41). However, HSV is one of the only viruses for which the clinical sensitivity and specificity of CSF testing have been confirmed relative to brain biopsy in encephalitis patients (42). Even so, occasional patients with early-stage HSV encephalitis have a negative initial NAT result. Serologic tests (CSF IgG and IgM) detect additional HSV and VZV encephalitis cases when NAT results are negative (6). Acute CNS infections with other herpesviruses, such as CMV, EBV, and HHV-6/7, are rare in immunocompetent patients, and testing is typically limited to immunocompromised patients. Quantitative NATs are preferable to qualitative NATs for these viruses to allow distinction of low-level positive results due to influx of latently infected leukocytes versus active CNS viral replication in clinical disease (6, 41). In contrast, the diagnosis of arboviral infections is primarily based on geographically appropriate serology (CSF IgM and IgG), not NAT, for individual viruses (6). NATs are less sensitive because immunocompetent patients typically do not have virus in their CSF at the time of presentation. Immunocompromised patients may have virus or antibodies, however, and should be tested by CSF serology and NAT.

Testing for *M. tuberculosis* and fungi, such as *Cryptococcus neoformans*, is usually limited to patients with recognized risk factors or immunocompromising conditions (6, 22). *Cryptococcus gattii* occurs occasionally without obvious risk factors. The diagnosis of tuberculous meningitis or encephalitis is challenging and typically requires multiple tests. Large-volume CSF culture is the traditional standard but is of limited value clinically, due to the length of time required for detection and limited access to testing in many high-prevalence areas. Concentrated CSF stain for mycobacteria is rapid but insensitive. Numerous laboratory-developed and commercial NATs have been developed in an effort to achieve a rapid diagnosis, but the sensitivities of most of these have been less than desirable (56%) (43). More recently, a commercial nested NAT, the Xpert MTB/RIF (Cepheid), has become available, with a sensitivity approaching that of culture for sputum samples that may also be useful in patients with suspected tuberculous meningitis, but additional studies are needed to investigate this (44). Cryptococcal polysaccharide antigen (CrAg) detection by latex agglutination or enzyme-linked immunosorbent assay (ELISA) is currently the most common method used for the diagnosis of cryptococcal meningitis, but a newer lateral-flow immunochromatographic assay (LFA) is more sensitive and specific (45). CSF culture and serology (e.g., *Coccidioides* spp.) remain the primary diagnostic methods for other fungal infections, although

direct detection of (1,3)-β-D-glucan from CSF may occasionally be useful, in conjunction with traditional methods.

NEW AND EMERGING TEST METHODS

In addition to the tests discussed above, there are new tests and methods on the horizon that are likely to dramatically alter the approach to meningitis and encephalitis diagnosis and to expand the number of patients with a microorganism identified. The first rapid commercial multiplex NAT for the detection of pathogens causing meningitis and encephalitis received *de novo* clearance for use as an aid in the diagnosis of these conditions by the U.S. Food and Drug Administration (FDA) in October 2015. This assay, the FilmArray meningitis/encephalitis panel (FilmArray) from BioFire Diagnostics, simultaneously detects 14 pathogens, including six bacteria (*S. pneumoniae*, *N. meningitidis*, *H. influenzae*, GBS, *Escherichia coli* [K1 strains only], and *L. monocytogenes*), seven viruses (enterovirus, HSV-1/2, VZV, CMV, HHV-6, and human parechovirus), and *C. neoformans/C. gattii* from ≤200 μl of CSF in about 1 h. At the time of this review, there were no peer-reviewed publications with performance data for the FilmArray, but unpublished results from the pre-FDA multicenter clinical evaluation showed good correlation with standard laboratory methods. In prospective clinical specimens, the sensitivity/positive percent agreement (PPA) was ≥95.7% for all FDA-cleared targets except HHV-6 and GBS, and the specificity/negative percent agreement (NPA) was ≥99.2% for all targets (46). The HHV-6 PPA was somewhat lower than that obtained with current clinical comparator NATs, at 85.7% in the prospective clinical cohort. The FilmArray performance for GBS was unclear, as 0/1 (0.0%) GBS positives were detected in the prospective cohort, and 2/2 (100.0%) GBS positives were detected in archived specimens. Another challenge in the interpretation of data from the FilmArray trial is the fact that several targets were underrepresented despite collection at multiple centers and the use of prospective and archived specimens, pointing to the difficulty involved in evaluating performance of new tests for rare organisms. Still, it is expected that the FilmArray will substantially improve the number of pathogens detected and the speed of identification of common infectious causes of meningitis and encephalitis, in particular in the setting of prior antibiotic therapy. The impact on patient care and outcomes with use of this assay will need to be investigated. The main limitations of the FilmArray are the potential cost and utilization issues and the inability to detect arboviral infection by this assay and all NAT tests in general. Additional limitations are described in the package insert (47), including the possibility of false-negative results when the concentration of organism(s) in the specimen is below the limit of detection and false-positive results due to contamination at the time of collection or laboratory testing.

Unbiased metagenomic deep sequencing is another new diagnostic approach that is further away from routine clinical use but has the theoretical potential to detect and identify any microorganism(s) with nucleic acid present in a clinical sample, with important caveats (48, 49). The approach is conceptually similar to methods used to sequence the first human genome and for microbiome and environmental studies in which all nucleic acid fragments in a sample (DNA and RNA) are sequenced, assembled, and matched with existing sequences in electronic databases. Matching sequences are screened to identify potential pathogens, which are then evaluated for clinical significance. At the moment, the method is relatively expensive and time and labor-intensive, but costs continue to drop, and protocols, bioinformatics pipelines, and user-friendly analytic interfaces are being developed to standardize, accelerate, and simplify the process. Enthusiasm for the approach has been fueled by high-profile case reports in which previously unrecognized and difficult-to-detect organisms were identified and treated with remarkable clinical recovery in some patients (49). However, overall performance data, including information regarding the limit of detection for important pathogens, sensitivity relative to existing test methods, and yield of clinically significant organisms in different patient populations, are lacking. Finally, the cost, time, and difficulty involved in distinguishing clinically significant organisms from contaminants and nonsignificant organisms and the clinical impact of this approach need to be understood.

SUMMARY AND FUTURE DIRECTIONS

Community-acquired meningitis and encephalitis are potentially life-threatening diseases caused by a diverse array of infectious and noninfectious causes. Accurate identification of the underlying etiologic cause of these conditions is essential to provide optimal treatment and minimize negative outcomes. Multiple tests and methods are currently required to detect the majority of infections, but new and emerging test methods, such as multiplex NAT panels and metagenomic sequencing, have the potential to simplify testing and increase the number of pathogens identified. This should lead to more effective treatment and better patient outcomes, but well-designed studies will be necessary to evaluate the performance, impact, cost, and value of new tests before and after clinical implementation. Finally, more work is needed to understand why etiologic diagnoses are often not achieved in routine clinical practice and to determine if better utilization of existing tests or new tests can reduce the frequency of nonspecific diagnoses and empirical treatment in meningitis and encephalitis patients.

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