Methods of rapid diagnosis for the etiology of meningitis in adults

Infectious meningitis may be due to bacterial, mycobacterial, fungal or viral agents. Diagnosis of meningitis must take into account numerous items of patient history and symptomatology along with regional epidemiology and basic cerebrospinal fluid testing (protein, etc.) to allow the clinician to stratify the likelihood of etiology possibilities and rationally select additional diagnostic tests. Culture is the mainstay for diagnosis in many cases, but technology is evolving to provide more rapid, reliable diagnosis. The cryptococcal antigen lateral flow assay (Immuno-Mycologics) has revolutionized diagnosis of cryptococcosis and automated nucleic acid amplification assays hold promise for improving diagnosis of bacterial and mycobacterial meningitis. This review will focus on a holistic approach to diagnosis of meningitis as well as recent technological advances.

Keywords: aseptic meningitis • bacterial meningitis • *Cryptococcus* meningitis • diagnosis • diagnostic tests • tuberculosis meningitis

Nathan C Bahr^{*1,2} &
David R Boulware^{1,2}

¹Division of Infectious Disease & International Medicine, Department of Medicine, University of Minnesota, Minneapolis, MN 55455, USA

²Center for Infectious Diseases & Microbiology Translational Research, University of Minnesota, Minneapolis, MN 55455, USA

*Author for correspondence:

Tel.: +1 612 624 9996

Fax: +1 612 625 4410

bahrn026@umn.edu

Meningitis is a syndrome classically characterized by some combination of neck stiffness, headache, fever and altered mental status; other symptoms including nausea, vomiting and photophobia are frequently observed as well [1,2]. Meningitis may be due to bacteria, mycobacteria (e.g., *Mycobacterium tuberculosis*), fungi (predominantly *Cryptococcus neoformans*), viruses, parasites (e.g., cysticercosis due to *Taenia solium*), rickettsia or *Treponema* (e.g., syphilis) or noninfectious causes such as malignancy or rheumatologic conditions [2]. This review will focus on bacterial, mycobacterial, fungal and viral meningitis diagnostics. Prompt diagnosis is crucial in meningitis care as many causes of meningitis carry a high mortality, especially with any delay in diagnosis [3].

Adult mortality may vary widely according to cause and setting with rates of 3–30% for bacterial meningitis depending on the organism [4,5]. Aseptic meningitis (usually referring to viral meningitis but also encompassing other ‘culture-negative’ types of meningitis) is generally considered to a benign,

self-limited disease with low mortality [6], of note this does not include encephalitis due to herpes simplex virus (HSV) where mortality may be up to 70% without treatment, and still as high as 28% with acyclovir therapy [7]. Tuberculous meningitis (TBM) and cryptococcal meningitis carry high mortality rates of >50% in routine care [8,9].

Additional historical information such as duration of symptoms, sexual history, vaccination history, drug use history, personal history of TB, travel history and country of origin are extremely useful in considering the possible causes of meningitis [2,10]. Though helpful in narrowing the etiologic possibilities, symptoms and history alone are unreliable in terms of their ability to determine whether or not meningitis is present; much less its etiology [2]. One must combine this information with a good understanding of the basic epidemiology pertaining to the situation – knowing what types of meningitis might be common given a particular patient’s background informs diagnostic testing. This information, together, allows the provider to

efficiently order diagnostic testing to attempt to make a definitive diagnosis.

Epidemiology

The most common bacterial etiologies of meningitis are *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Listeria monocytogenes* and (in infants) Group B *Streptococcus* [5].

Streptococcus pneumoniae is the most common bacterial cause of meningitis worldwide among all ages, accounting for approximately 70% of isolates in US adults. *N. meningitidis* is second most common at approximately 11.5% of adult cases [4]. The incidence has decreased of both pneumococcal and meningococcal meningitis with vaccination in the US and England [11–13]. In South Africa, implementation of the 7-valent and then 13-valent pneumococcal conjugate vaccine has resulted in a 62% decline in invasive pneumococcal disease among children <5 years of age between 2009 and 2012 [14]. Meningitis accounts for 43% of invasive pneumococcal disease in South Africa [14]. Yet, the overall incidence of bacterial meningitis in low- and middle-income countries overall is greater than in high-income countries. For example, one systematic review found incidence of *S. pneumoniae* ranging from 5.8 to 12 cases per 100,000 adults (stratified by age group) in four countries in the African ‘meningitis belt’ as compared with a rate of 0.81 cases per 100,000 adults in the USA [4,15]. In South Africa during 2012, the incidence of confirmed pneumococcal meningitis was 2.6 per 100,000 total population [14]. Epidemics due to either bacteria may occur [16] but are noted more frequently with *N. meningitidis* where rates vary widely by region and may be historically as high as 1000 cases per 100,000 adults in the meningitis-belt outbreaks as compared with 0.27–1.43 cases per 100,000 adults by age group in the USA and 0.31 per 100,000 total population in South Africa [11,14,17–18]. However, the landscape of meningococcal disease has drastically changed since the implementation in 2010 of a new meningococcal A conjugate vaccine (MenAfriVac). This meningococcal serotype A vaccine in the ‘meningitis belt’ of Africa has drastically reduced rates of disease [16,19]. Between 2010 and 2012, the GAVI alliance vaccinated >100 million people in the meningitis belt, dropping the confirmed cases of meningitis A from a collective 1512 in 2009 in Burkina Faso, Mali and Niger to zero cases in 2012. For the MenAfriVac vaccine, the use of controlled temperature chain (at ≤40°C for ≤4 days), instead of traditional cold chain (2–8°C), should likely greatly accelerate deployment to even remote regions [20].

Haemophilus influenzae serotype b (Hib) was a major cause of meningitis in children until an effective

vaccine was developed [4,5]. Now, most *H. influenzae* meningitis cases occur in adults (~6% of US cases of bacterial meningitis) due to nontypeable strains [4,5]. The rarity of *H. influenzae* is likely related to herd immunity due to childhood vaccination [21]. As of 2013, national immunization programs include the Hib vaccine in 189 countries with approximately 50% global coverage of children. Notable exceptions include China, Thailand, South Korea and South Sudan. The GAVI alliance has been instrumental in implementation of the pentavalent vaccine, which protects against diphtheria–tetanus–pertussis, hepatitis B and Hib.

Group B streptococcus (*Streptococcus agalactiae*) and *L. monocytogenes* are responsible for 7 and 4%, respectively, of adult cases of bacterial meningitis in the US [4]. Vaccination is not available for either pathogen. Screening and treatment of colonized pregnant women in the US for group B streptococcus has led to a large decrease in neonatal cases [4,5] though this decrease has not been noted in adults where cases may have actually increased [22,23]. Little information is available about either cause of adult meningitis in resource-poor settings.

Tuberculosis is a rare condition in many high-income countries (35 cases per 100,000 persons in the WHO Americas region) but higher rates in regions, particularly those with high rates of HIV infection such as the WHO African (293 cases per 100,000) and south-east Asian regions (271 cases per 100,000) [24]. Among 8.7 million estimated new cases of tuberculosis, the WHO projected 13% of these were associated with HIV infection – of those approximately 80% occurred in Africa [24]. Among the reported approximately 6.2 million reported (as opposed to estimated) cases in 2011, 800,000 were classified as isolated extrapulmonary TB (which includes TBM among many other forms) [24]. Thwaites estimates approximately 1% of tuberculosis is TBM [8]. Individuals with TB and HIV co-infection have a higher rate of TBM than persons without HIV [8,25] as evidenced by one Spanish study where TBM was found in 10% (45/455) of TB patients with HIV and only 2% (38/1750) of TB cases without HIV [26]. In two South African studies (high HIV and TB prevalence) 28–44% of patients with meningitis had microbiologically confirmed TBM, 88–94% of those patients with TBM were HIV co-infected [10,27]. A meta-analysis including 15 studies in African countries with varying prevalence of HIV and TB found 5.2–28% of meningitis cases were due to TBM, with 55–88% of those cases being in patients with HIV (data not available for all studies) [28].

Cryptococcosis is primarily a disease seen in persons with advanced HIV although cases of *Cryptococcus gattii* and neoformans occur in HIV-negative patients (with or without immunosuppression) [29,30]. *Crypto-*

Table 1. Comparison of common etiologies of meningitis based on immune status.

Severe immune suppression	Not immune suppressed
Cryptococcal meningitis [†]	Aseptic meningitis
Tuberculosis meningitis [†]	Bacterial meningitis
Bacterial meningitis	Tuberculosis meningitis
Aseptic meningitis	
The table is listed in order of most common to least common causes of meningitis. The order of causes in the immunosuppressed column is based on persons living with AIDS but is relevant to other causes of T-cell immunosuppression.	
[†] Immune reconstitution inflammatory syndrome should be strongly considered in HIV-infected persons newly initiating antiretroviral therapy or in persons with iatrogenic immunosuppression discontinuing immunosuppression.	

coccus is the most common cause of meningitis in much of sub-Saharan Africa [31] where 75% of the estimated global burden of cryptococcosis occurs [32]. In settings with high rates of HIV, *Cryptococcus* is a significant cause of morbidity and mortality. The same two South African studies referenced earlier showed 45–63% of meningitis as being due to *C. neoformans* [10,27], while in one Malawian study the proportion was 43% [33]. 720,000 cases are estimated to occur per year in sub-Saharan Africa and a significant number of cases occur in south/southeast Asia and Latin America (120,000 and 54,400, respectively) [32]. In South Africa in 2012, the incidence rate of cryptococcal meningitis was 119 cases per 100,000 populations of HIV-infected persons with an in-hospital fatality rate of 32% [34]. With the substantial expansion of ART access in South Africa, the incidence rate has declined by only 10% from 2010 to 2012 [14,34].

Other causes of fungal meningitis that are more rare include *Coccidioides immitis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Candida* spp., *Aspergillus* spp., *Zygomycetes* [25], and the well-documented 2012 US outbreak of *Exserohilum rostratum* meningitis related to contaminated corticosteroid injections [35]. Of the endemic fungi, CNS involvement is generally rare (2%) and symptoms prolonged (i.e., months) [36–38]. This duration of progressive symptoms is an important detail to direct targeted testing.

The term aseptic meningitis encompasses vast etiologic possibilities, the majority of which are assumed to be viral in etiology. Enteroviruses cause 70–95% of those cases of aseptic meningitis in which an infectious etiology is found [39] with HSV and varicella zoster virus (VZV) being less common [40]. Yet, HSV and VZV are particularly important pathogens as acyclovir treatment exists. Nonviral causes include tick-borne illness such as Lyme disease, medications, rheumatologic disease and infections that more often are culture positive (e.g., tuberculosis, *Cryptococcus*) [41]. Local endemic diseases may shift proportions of etiologic agents of aseptic meningitis. For instance, in a dengue fever endemic region, 50% of cases were due to

enteroviruses, 15% HSV, 15% unknown etiology, 10% dengue virus and 10% cytomegalovirus (CMV) [42].

The host

The immune status of the patient presenting with meningitis is extremely important in narrowing or expanding the differential diagnosis. Table 1 shows a comparison of common etiologies of meningitis with and without immune compromise. The most obvious example of immune suppression is HIV infection. In patients living with AIDS, other CNS pathologies (e.g., CNS lymphoma, toxoplasmosis, etc.) should be on the differential; however, these conditions generally present differently than meningitis, most often presenting with focal neurologic deficits [25]. Clearly, meningitis due to *Cryptococcus* or *M. tuberculosis* should be considered very likely in a patient with advanced HIV disease [10,27]. In a patient with advanced HIV, but less severe immune suppression (e.g., CD4⁺ T-cell count >100 cells/μl), *Cryptococcus* becomes less likely, though still possible [25,43]. In HIV-infected patients, TBM frequently presents in patients with CD4⁺ counts <100 cells/μl [44], and although TBM may occur with any CD4⁺ count, CD4⁺ >200 cells/μl is less common [10,26–27]. TBM patients with HIV have a higher mortality rate than do those TBM patients without HIV [8] and in fact, risk of death from TBM is inverse to the CD4⁺ count [10], making prompt diagnosis all the more important.

Bacterial meningitis is also frequent in HIV-infected persons. In a study of 233 Malawian HIV-infected patients presenting with meningitis, 28 (12%) cases were caused by bacteria with a median CD4⁺ count of 119 cells/μl (interquartile range 56–457 cells/μl) [33], a study of South African patients (94% HIV+) found a similar proportion (8%) of meningitis cases were due to bacteria though the mean CD4⁺ count was 287 cells/μl [27]. In a study of 215 HIV-positive patients presenting with meningitis in the Central African Republic 31% had bacterial meningitis [45]. Of these 66 cases of bacterial meningitis, 22 (33%) were due to *S. pneumoniae*, nine (14%) due to *N. meningitidis*, six due to

Gram-negative rods and one each of *Staphylococcus aureus* and *Listeria* each – with 24 cases were presumed bacterial meningitis based on cell count and chemistry but culture negative [45]. Finally, in a Spanish study among 25 HIV-infected patients with microbiologically confirmed bacterial meningitis, etiologies were *S. pneumoniae* (60%), *N. meningitidis* (8%), *Staphylococcus aureus* (8%), *Listeria* (12%) and various Gram-negative bacteria (12%), respectively [46]. Although the proportion of cases attributed to each cause varies by region, *S. pneumoniae* is uniformly the most common bacterial cause of meningitis in HIV-infected persons. Further, patients with HIV have a six- to 324-times higher risk of invasive pneumococcal disease than patients without HIV, even on ART the risk is 35-times higher than the persons without HIV [5]. The increased risk of bacterial meningitis is thought to be due to disturbed humoral immunity [46]. However, the increased risk of bacterial meningitis compared with the general population needs to be put in the context of the incidence of Cryptococcal meningitis in an HIV-infected population, whereby *Cryptococcus* is more common than all causes of bacterial meningitis combined [27,47]. Figure 1 shows an algorithm for diagnosis of meningitis in patients with severe immune suppression with this context in mind.

Equally important in patients with HIV infection is considering whether or not the patient is receiving antiretroviral therapy (ART). If the patient has initiated ART within the prior 3 months, immune reconstitution inflammatory syndrome (IRIS) should be considered. IRIS is the worsening of symptoms due to inflammation caused by immune activation in patients previously treated for an opportunistic infection (paradoxical IRIS) or an exaggerated presentation of a previously unrecognized opportunistic infection upon starting ART (unmasking IRIS) [48]. Paradoxical CNS IRIS occurs in 20–30% of cryptococcosis patients who survive to receive ART and up to 47% TBM patients who are subsequently started on ART [48]. Thus, in a patient who has recently started ART with worsening CNS symptoms and a recent history of CNS infection, paradoxical IRIS should be strongly considered along with treatment failure/relapse due to drug resistance. In patients with CM, IRIS is seen more commonly in patients with CD4⁺ counts <100 cells/μl, less evidence of cerebrospinal fluid (CSF) inflammation (e.g., low WBC count and normal protein), and greater organism burden (evidenced by high CrAg titer and/or quantitative culture) at baseline [48]. In patients with TBM predictors of IRIS include higher CSF neutrophil counts and increased bacillary load at baseline [48].

The most common unmasking IRIS etiology due to ART that occurs with CNS manifestations is

Cryptococcus. The prevalence of subclinical Cryptococcal antigenemia (i.e., serum or plasma cryptococcal antigen [CrAg] positivity) ranges from 4 to 12% in persons with CD4 counts <100 cells/μl, with lower prevalence above this threshold [43,48–49]. Unmasking cryptococcal manifestations can be diverse extending beyond meningitis to include parenchymal disease with cryptococcomas and unusual neurologic or cerebellar deficits. Cryptococcoma(s) can appear identical as toxoplasmosis with ring-enhancing lesions on head computed tomography (CT). In contrast, unmasking of toxoplasmosis among persons receiving trimethoprim–sulfamethoxazole prophylaxis is exceedingly rare. Unmasking of TBM is also possible, and these persons typically often have disseminated TB outside of the CNS as well [48]. For any unmasking IRIS event occurring within 3–4 months of starting ART in an HIV-infected person with AIDS, CrAg testing of blood is an essential first diagnostic step.

Asplenic patients are at high risk for bacterial meningitis, in particular *S. pneumoniae*, *N. meningitidis* and *H. influenzae*, because they lose the splenic macrophages that phagocytize pathogens [50]. Vaccination against these pathogens is recommended for asplenic patients [50]. A cohort study showed that 24 out of 965 patients with microbiologically proven bacterial meningitis were asplenic; all 24 of these patients grew *S. pneumoniae* [51].

Diabetes mellitus, alcoholism, chemotherapy, medications and cancers also effect on the types of meningitis one might encounter as they cause immune dysfunction [5]. Bacterial meningitis remains a significant concern with some causes occurring more frequently in immunosuppressed patients than normal hosts. B-cell dysfunction with humoral immunoglobulin deficiency is a risk factor for encapsulated organisms, such as *S. pneumoniae* and *H. influenzae* [52]. In a case series from France, 38% of persons with invasive pneumococcus or *Hemophilus* had an immunoglobulin abnormality [52]. Additionally with group B streptococcal meningitis in adults, a literature review reported immunosuppression in 42% of 64 adults [53]; whereas in a case series of *Listeria* meningitis all 30 patients had immunosuppression or were >50 years of age [54]. Although these two organisms are more common in immunosuppressed populations than in the general population, typical organisms such as *S. pneumoniae* and *N. meningitidis* should still be considered more likely culprits [55]. Often, the immunodeficiency will not be discovered until after the infection occurs. TBM is also an important consideration with non-HIV-related immune compromise; particularly in association with TNF-α inhibitor use [56]. TBM should also be considered in patients with hematologic malignan-

Figure 1. Algorithm for diagnosis of meningitis in patients with known immune compromise.

†Most likely bacterial meningitis, institute empiric antibiotics after lumbar puncture (or before if the patient is unstable). If Gram stain and culture are negative may consider 16s rRNA PCR (if available).

‡Most likely tuberculous meningitis. If acid fast bacilli smear unremarkable and duration of symptoms correlate strongly consider empiric treatment and/or nucleic acid amplification tests, ideally testing a large volume (>5 ml) of centrifuged cerebrospinal fluid.

§Most likely aseptic meningitis. Consider nucleic acid amplification tests for viral pathogens if serum inflammatory biomarkers (e.g., C-reactive protein, procalcitonin) are minimally elevated or normal.

AFB: Acid-fast bacilli; CM: Cryptococcal meningitis; CrAg: Cryptococcal antigen; CSF: Cerebrospinal fluid; LFA: Lateral flow immunochromatographic assay.

cies, those who undergo solid organ or bone marrow transplant or other patients with immune compromise who have untreated, latent TB infection [57,58]. *Cryptococcus* is a significant concern in immune compromised populations as cases have been described in patients receiving chemotherapy, high-dose corticosteroids, biologic agents such as infliximab, immunosuppressive drugs such as azathioprine, as well as patients with both hematologic and nonhematologic malignancies, diabetes mellitus, alcoholism and/or liver cirrhosis and solid organ transplant [48,59–61]. Viral etiologies of meningitis in non-HIV-infected persons with immune compromise include human herpes virus 6 (particularly in bone marrow transplant patients), HSV, VZV, CMV, Epstein–Barr virus, JC virus (associated with progressive multifocal leukoencephalopathy) and adenovirus [62]. Additional considerations that act as defects in host defense are recent neurosurgical history or shunt placement – in these cases nosocomial organ-

isms such as *S. aureus*, coagulase-negative *Staphylococcus* species, *Acinetobacter* and *Pseudomonas aeruginosa* are much more common [25].

Incidence of meningitis in the ‘normal host’ is described in some detail above and Figure 2 provides an algorithm for diagnosis in these patients. In short, 70% of bacterial meningitis is due to *S. pneumoniae* [51], although bacterial meningitis overall is still a minority of etiologies [1,24]. Aseptic viral etiologies predominate in nonimmune compromised populations. *Cryptococcus* is much less common in these patients than in persons with severe immune compromise [30]. Other causes of fungal meningitis are also rare in normal hosts though the outbreak of meningitis due to *Exserohilum rostratum* is a notable exception [35]. In non-HIV populations, TBM typically occurs at the extremes of age (i.e., infants, elderly); however, TBM can occur in non-HIV-infected adults, but likely at approximately 100-fold less frequently than among HIV-infected per-

sons, extrapolated from HIV prevalence rates in recent diagnostic studies [63,64]. Aseptic meningitis is the most common cause of meningitis in the patient with a normal immune system and thankfully, mortality is quite low [6]. Thus, it is important to rule out conditions with higher mortality such as bacterial, cryptococcal and TB meningitis when the history and epidemiology make these conditions possible diagnoses. In any adult with meningitis, HIV status should be checked.

Duration of symptoms

One additional way to narrow the possible etiologies in a patient with meningitis is by the duration of symptoms. Generally, bacterial meningitis presents much more acutely than do either TBM or fungal meningitis, which are subacute in onset. Viral aseptic meningitis duration of symptoms may vary. In a 2002 study in Vietnam, Thwaites *et al.* observed a mean duration of illness at TBM presentation of 12 days

(range: 4–34 days) compared with bacterial meningitis patients' mean duration of illness of 3 days (range: 1–11 days) [65]. A study in Turkey found a median duration of illness of 7 days in TBM and ≤ 2 days in bacterial meningitis [3]. Although broadly these differences in duration are clear, there can be overlap and so this is by no means a single entity that can accurately predict the TBM versus bacterial meningitis. *Listeria monocytogenes* is an important exception to the broad characterization of rapid onset of bacterial meningitis. Approximately a third of *Listeria* meningitis patients have symptoms for ≥ 4 days [54,66].

Duration of viral meningitis symptoms are variable but often short, overlapping with bacterial meningitis. One French study of 18 patients with bacterial meningitis and 133 patients with aseptic meningitis found equivalent durations of symptoms median 1 day (95% CI: 1–2 days), and 3 days (95% CI: 0–5 days) [67]; however, a Brazilian study of 20 patients with asep-

Figure 2. Algorithm for diagnosis of meningitis in patients without known immune compromise.

*If appropriate consider rapid HIV test, If HIV-infected, refer to Figure 1 algorithm.

**If mild immune compromise include cryptococcal antigen lateral flow assay, treat bacterial etiology accordingly if positive.

†Likely bacterial meningitis, continue empiric antibiotics, await definitive etiology.

‡Most likely aseptic meningitis, consider stopping empiric antibiotics, consider sending appropriate nucleic acid amplification tests (NAATs).

§Most likely tuberculosis meningitis. If duration of symptoms are compatible, strongly consider empiric treatment and/or NAATs, ideally testing a large volume (>5 ml) of centrifuged cerebrospinal fluid.

¶Most likely aseptic meningitis, although may be tuberculosis meningitis as well. If strong clinical suspicion, consider TB NAATs.

AFB: Acid-fast bacilli; CrAg: Cryptococcal antigen; CSF: Cerebrospinal fluid.

Table 2. Comparison of common laboratory tests in bacterial, viral, cryptococcal and tuberculosis meningitis.

Laboratory test	[†] Bacterial meningitis	Aseptic meningitis [†]	Tuberculosis meningitis	Cryptococcal meningitis [†]
Total protein	245–270 mg/dl	75 mg/dl	191 [†] –314 [†] mg/dl	90 mg/dl
CSF/blood glucose ratio	0.20–0.36	0.54	0.27 [†] –0.28 [†]	0.38
Total WBC	Approximately 500–2500 cells/ μ l	98 cells/ μ l	300 [†] –375 [†] cells/ μ l	53 cells/ μ l
Neutrophils (%)	80–90%	37%	37% [†]	<20%

[†]Median values.
[‡]Mean values.
CSF: Cerebrospinal fluid; WBC: White blood cell.

tic meningitis showed a mean duration of symptoms of 7.5 days [42]. Thus, clearly the duration of symptoms in aseptic meningitis may vary. Duration of symptoms in cryptococcal meningitis is generally at least 1 week, in the absence of HIV therapy. In two separate cohorts from Uganda and Vietnam of 434 patients with cryptococcal meningitis, the median duration of antecedent headache was approximately 14 days (interquartile range [IQR]: ~7–21 days) prior to hospital presentation [68,69]. Unmasking cryptococcal-IRIS with HIV therapy can have a much more rapid onset, mimicking the acute onset of bacterial meningitis [49].

Common diagnostic laboratory & imaging tests

CT and MRI may be considered as adjunctive diagnostics tests but are generally nonspecific and show meningeal enhancement [70]. Imaging may be helpful in cases of focal neurologic deficits, particularly when a tuberculoma or cryptococcoma is suspected [70]. In the absence of trauma, altered mental status or focal neurologic deficit, imaging increases healthcare costs and has minimal yield in providing a definitive diagnosis. PET has even greater expense with no ability to provide a definitive etiologic diagnosis in a person with meningitis.

Standard diagnostic testing of CSF includes: white blood cell (WBC) count with differential, total protein, and CSF/blood glucose (or CSF glucose itself), used in conjunction with patient history and epidemiology to support potential diagnoses. Total protein and WBC counts reflect inflammation in the CSF while decreased glucose CSF/blood ratio is a sign of glucose consumption by an active infection. These common laboratory tests cannot be the lone laboratory method of diagnosis and while overlap in their values among different diagnoses does occur, general trends emerge and are useful as they help the clinician to focus on particular possible diagnoses. **Table 2** shows median and/or mean values for these laboratory tests stratified

by condition [65,67,71–72]. Importantly, up to 40% of persons with *Cryptococcus* may have an unremarkable CSF WBC <5 cells/ μ l, which can mistakenly delay the diagnosis [73]. For other endemic fungi (e.g., histoplasmosis, blastomycosis, coccidioidomycosis), CSF typically shows lymphocytic pleocytosis with protein elevation (~80%), and a variably depressed glucose (approximately 80%). Additionally, TBM-related and cryptococcal-related IRIS generally have high CSF protein levels and high WBC counts as IRIS is an inflammatory process [48,73].

More specific technologies have been, or are under development to enable more confident diagnosis of the major etiologic agents of meningitis (**Table 3**). One example of a technology that could potentially be applied broadly to meningitis is that of a PCR multiplex panel. These panels allow for detection of multiple different pathogens that might cause a particular syndrome with one test – ideally in rapid, easy to use and accurate manner. Panels have successfully been trialed for use in respiratory illnesses and blood cultures [74,75], a panel developed for sepsis has been trialed in patients with meningitis [76], and another multiplex PCR panel has been developed for use in meningitis (although not yet thoroughly studied) [77]. Technologies such as multiplex PCR are exciting, but in the case of meningitis, not yet ready to aide clinicians. Below we discuss the use of specific diagnostic tests and their roles for diagnosing various causes of meningitis.

Diagnostic tests for fungal meningitis

Diagnosis of cryptococcal meningitis has been revolutionized by the development of an extremely accurate lateral flow immunochromatographic assay (LFA (Immuno-Mycologics [Immy], Norman, Oklahoma, USA) to detect CrAg. While CSF culture is thought of as the gold standard for detection of *Cryptococcus*, CrAg had been used (latex agglutination or enzyme immunoassay) for some time as a rapid surrogate [43]. CrAg LFA is a ‘dipstick’ test, costing \$2 per test in

Table 3. Comparison of major diagnostic tests commercially available or under investigation for bacterial, viral, cryptococcal and tuberculosis meningitis.

Etiology	Test	Description	Time to Results	Positive attributes	Negative attributes	Commercial availability
Bacterial	Gram stain	Stain for bacteria by microscopy	1 h	Cheap, easy to perform	Sensitivity 70–90% prior to antibiotics for pneumococcal meningitis	Yes
	Culture	Standard bacterial aerobic culture	1–3 days	May grow quickly, easy to perform, adaptable to rapid identification methods	Yield decreased by antibiotics prior to culture, may be days to results, variable sensitivity by organisms	Yes
	Procalcitonin, CRP	Serum inflammatory biomarkers	1 h	Good differentiation between bacterial and aseptic meningitis	Cost, lab requirements, no studies on <i>Cryptococcus</i> or TB (with probable overlap in CRP)	Yes
	Lactate	Biomarker measure in CSF	<5–60 min	Rapid, sensitive and specific if obtained prior to antibiotics	Not very sensitive if measured after antibiotics given	Yes
	16s rRNA PCR	PCR detection of 16s ribosomal RNA to elicit specific pathogens	Hours to days	Rapid, more sensitive than culture, very specific, rapid automated assays being developed	Extremely costly, requires lab infrastructure and expertise.	Yes
	NAATs	Specific RT-PCR and LAMP assays have been tested for particular pathogens	1–2 h	Rapid, specific, potentially quite sensitive	Cost, lab infrastructure, lack of large studies	In some cases
	LFA	Rapid, usually card or dipstick based tests for specific etiologies	<15 min	Rapid, cheap, easy to use, no significant lab infrastructure necessary	Variable specificity, sensitivity – dependent on quality of monoclonal antibody and target analyte	Yes
<i>Mycobacterium tuberculosis</i>	Ziehl-Neelsen AFB staining	Staining for acid fast bacilli by microscopy	1 h	Cheap	Very insensitive, extremely technician dependent	Yes
	Culture, Löwenstein-Jensen (LJ)	Traditional culture on solid LJ media	3–5 weeks	Reliable, somewhat sensitive	Very slow growth, still many false negatives, costly, labor intensive	Yes
	Culture, MGIT	Liquid based culture	1–2 weeks	As sensitive and quicker than LJ culture	approximately 2 weeks to growth, costly	Yes
	Culture, MODS	Kit based liquid culture	1 week	More rapid than MGIT, detects resistance concurrently	Sensitivity may be slightly less than MGIT and LJ cultures	yes

Test is meant to describe test category, not each specific commercial test. The description notes how the test works in principle. 'Pro' and 'Con' refer to positive and negative aspects of each tests performance and utility. Assays dealing with *M. tuberculosis* organisms require increased biosafety apparatus. AFB: Acid-fast bacilli; CrAg: Cryptococcal antigen; CRP: C-reactive protein; CSF: Cerebrospinal fluid; LAMP: Loop-mediated isothermal amplification; LFA: Lateral flow immunochromatographic assay; MGIT: Mycobacteria growth indicator tube; MODS: Microscopic observation drug susceptibility; NAAT: Nucleic acid amplification test.

Table 3. Comparison of major diagnostic tests commercially available or under investigation for bacterial, viral, cryptococcal and tuberculosis meningitis (cont.).

Etiology	Test	Description	Time to Results	Positive attributes	Negative attributes	Commercial availability
<i>Mycobacterium tuberculosis</i> (cont.)	Adenosine deaminase activity (ADA)	Detectable enzyme released by during T-cell activation	<1 h	Rapid, low cost	Variable sensitivity and specificity, lab infrastructure	Yes
	Interferon-gamma release assay (IGRA)	Interferon-gamma secretion by host memory T cells on exposure to TB antigen	24–36 h	Limited data on CSF	Labor intensive, costly, high numbers of indeterminate results, variable studied cut-points, rely on T-cell function	Yes
	NAATs	Traditional nucleic acid amplification tests such as PCR	Hours	Fast, nearly as sensitive as culture, very specific	Commercially available tests less sensitive than 'in-house' tests, cost, lab expertise, lab apparatus	Yes
	LAMP	DNA amplification detected by color change	1 h	Less lab expertise and infrastructure required than typical PCR, isothermal	No data on performance	No
	GeneXpert	Cartridge based, automated PCR	2.5 h	Quick, similar sensitivity to culture, highly specific	Costly, requires significant infrastructure, easy to use on sputum	Yes (for use on sputum)
<i>Cryptococcus neoformans</i>	TB-LAM LFA	Dipstick test detects lipoarabinomannan antigen	25 min	Quick, inexpensive	Sensitivity related to CSF volume and organism burden	Yes, urine
	CrAg LFA	Dipstick test detects CrAg	10 min	Very sensitive and specific, inexpensive, no lab infrastructure needed	Insensitive, minimal data on CSF	Yes
	Culture	Fungal culture on Sabouraud agar	3–14 days	Very accurate, can decide active from past infection	Cannot differentiate active from past infection	Yes
	CrAg latex agglutination or ELISA	Latex agglutination or enzyme immunoassay detection of CrAg	1–48 h	Sensitive and specific. Requires lab infrastructure	Slow, labor intensive, requires lab infrastructure	Yes
	India ink	Staining for <i>C. neoformans</i> capsule by microscopy	15 min	Inexpensive, easy to perform	Costly, lab capacity requirement, cold chain of reagents, cannot differentiate active from past infection	Yes
<p>Test is meant to describe test category, not each specific commercial test. The description notes how the test works in principle. 'Pro' and 'Con' refer to positive and negative aspects of each tests performance and utility. Assays dealing with <i>M. tuberculosis</i> organisms require increased biosafety apparatus.</p> <p>AFB: Acid-fast bacilli; CrAg: Cryptococcal antigen; CRP: C-reactive protein; CSF: Cerebrospinal fluid; LAMP: Loop-mediated isothermal amplification; LFA: Lateral flow immunochromatographic assay; MGIT: Mycobacteria growth indicator tube; MODS: Microscopic observation drug susceptibility; NAAT: Nucleic acid amplification test.</p>						

Table 3. Comparison of major diagnostic tests commercially available or under investigation for bacterial, viral, cryptococcal and tuberculosis meningitis (cont).						
Etiology	Test	Description	Time to Results	Positive attributes	Negative attributes	Commercial availability
Aseptic (viral)	16s rRNA amplification	PCR detection of 16s rRNA to elicit specific pathogens	Days	Rapid, very specific	Extremely costly, requires lab expertise and infrastructure. Delay in sequencing	Research and reference labs
	NAATs	Specific PCR and RT-PCR assays have been developed for certain pathogens	1–6 h	Rapid, specific, automated assays in development	Cost, lab infrastructure and expertise	In some cases
Test is meant to describe test category, not each specific commercial test. The description notes how the test works in principle. 'Pro' and 'Con' refer to positive and negative aspects of each tests performance and utility. Assays dealing with <i>M tuberculosis</i> organisms require increased biosafety apparatus. AFB: Acid-fast bacilli; CrAg: Cryptococcal antigen; CRP: C-reactive protein; CSF: Cerebrospinal fluid; LAMP: Loop-mediated isothermal amplification; LFA: Lateral flow immunochromatographic assay; MGIT: Mycobacteria growth indicator tube; MODS: Microscopic observation drug susceptibility; NAAT: Nucleic acid amplification test.						

low-income countries (US\$5 in high income), and is ideal for the environments (warm climate, low–middle income countries) where much of the world’s cryptococcosis occurs [43]. The LFA requires only a drop of blood or CSF, is quick (10 min), and is easy to interpret. More importantly, the CrAg LFA can be shipped and stored at room temperature, and does not require lab infrastructure or cold chain transport that the traditional CrAg latex agglutination did [43]. In a multisite study in Africa of 666 persons with suspected meningitis, the sensitivity of the LFA was 99.3%, specificity 99.1%, positive predictive value 99.5% and negative predictive value 98.7% [78]. Importantly, CSF LFA CrAg was actually more sensitive than culture in this study, whereby a composite reference standard defined cryptococcal infection [78]. Titers can be performed with the LFA, which are a median 2.5-fold higher titers than CrAg latex agglutination, and titer level corresponds with mortality [78,79]

The LFA has now been validated in CSF, urine, serum, plasma, whole blood and by finger stick, and has European CE marking for these specimen types [78,80–82]. One important limitation of any CrAg assay (LFA included) is that the antigen persists long after culture sterility in the range of months to years [83,84]. Thus, in a patient with a history of cryptococcosis, CSF CrAg may indicate culture-positive relapse, cryptococcal-IRIS or a prior resolved infection, distracting from another, true cause of meningitis.

In addition to LFA, the India ink microscopy, CrAg latex agglutination and ELISA CrAg assays also exist. In high-quality lab settings, India ink microscopy has sensitivity of approximately 85% [78]. India ink is particularly problematic for early and/or low burden infections, whereby sensitivity is only 40% with CSF cultures having <1000 *Cryptococcus* CFU/ml [78]. Culture is a mainstay, yet not rapid. Cryptococcal-IRIS is a particularly difficult diagnosis. History (timing of ART) is crucial but as noted above, CrAg can be difficult to interpret in this setting and so one relies on culture. A paucity of initial CSF inflammation (i.e., normal CSF WBC count and protein) is predictive of higher probability of future cryptococcal-IRIS [73,85].

Diagnosis of meningitis due to endemic fungal infection (e.g., histoplasmosis, blastomycosis, coccidiomycosis) has traditionally relied on culture or identification from another body site in the presence of CNS inflammation – neither of these options has adequate sensitivity. *Histoplasma* and *Blastomyces* antigen (Mira Vista Diagnostics, IN, USA) can be detected in CSF (relatively unknown sensitivity: 35% (5/14) for *Histoplasma* and 3 of 3 in a case series of CNS *Blastomyces*) as can *Histoplasma* antibodies (approximately 75% sensitivity) [37,38,86]. The 1,3-beta-D-glucan (Fungitell®,

Cape Code Associates, MA, USA) testing of CSF performed well in the 2012 US. *Exserohilum* outbreak [87], and likely is possible for other mycoses including *Aspergillus* [88]. Diagnostic performance of beta-D-glucan on CSF is limited; however, specificity in CSF appears very high [BOULWARE DR, UNPUBLISHED DATA], thus a positive 1,3-beta-D-glucan result most probably reflects CNS fungal disease.

Diagnostic tests for TBM

Diagnosis of TBM is extremely difficult and so case definitions and clinical prediction rules based are often employed in clinical studies to help understand the probability of TBM [89,90]. Ziehl-Neelsen staining for acid-fast bacilli (AFB) is of limited utility, though fast, sensitivity is poor (often 10–20%) and even with meticulous examination of large CSF volumes (which are not plausible in most settings) may be only as high as 60% [8,26,91]. A recent study describing 37 culture-proven TBM patients showed a sensitivity of just 3.3% (95% CI: 1.6–6.7%) with traditional AFB staining but with an altered protocol involving cytospin slides and triton processing a sensitivity of 83% (95% CI: 77–87%) while an early secretory antigen target (ESAT)-6 immunostain allowed for 75% (95% CI: 69–81%) sensitivity [92]. Traditional Lowenstein-Jensen (LJ) solid media culture is generally 60–70% sensitive, but its clinical utility is limited by the 4–5-week average time to a positive result [91,93], whereas mycobacterial growth indicator tube (MGIT) culture often takes approximately 2 weeks [94]. Culture is too slow to direct treatment of TBM. Another culture technique, microscopic observation drug susceptibility (MODS) was found to be 65% sensitive while MGIT and LJ cultures both were 70% sensitive in a study where 42% of CSF specimens in persons suspected to have TBM grew *M. tuberculosis* [94]. In this study, the major advantage of MODS was median time to diagnosis of 6 days for MODS (IQR: 5–7 days) versus 15.5 days (IQR: 12–24 days) for MGIT and 24 days (IQR: 8–25 days) for LJ solid media cultures [94]. Although 6 days is an improvement, this is still a significant delay – often deterioration of the patient's condition necessitates empiric treatment long before 6 days. Exclusion of all other treatable conditions and empiric TB therapy remain the norm; however, some novel TB assays appear promising which may change the paradigm.

Adenosine deaminase (ADA) was evaluated in a small Spanish retrospective study of 16 TBM patients with 62.5% sensitivity among culture-proven TBM, using ADA threshold of >10 IU/l [26]. A 2010 systematic review of 10 TBM studies reported a mean ADA sensitivity of 79% (95% CI: 75–83%) with a mean specificity of 91% (95% CI: 89–93%), with two substantial

caveats [95]. Across studies, there was substantial variation in the ADA threshold considered abnormal (range of 5–15.5 IU/l) and substantial variation in the range of sensitivity reported (50–100%) [95]. The concept of ADA has been present for some time, but has not gained widespread use in TBM.

Interferon gamma release assays (IGRAs) have been evaluated on CSF for the diagnosis of TBM in multiple studies. One Korean study of HIV-negative patients (25 with definite or probable TBM and 57 classified as not having TBM by clinical characteristics) showed a sensitivity of 72% (95% CI: 51–88%) and specificity of 79% (95% CI: 66–89%) [96], the same group published a study 2 years prior with 59% sensitivity (95% CI: 36–79%) and specificity of 89% (95% CI: 72–98%) [97]. A South African study of 86 patients (87% HIV positive), 38 with TBM by culture or PCR (median CD4: 84; IQR: 53–173) and 48 classified as non-TBM (median CD4: 161; IQR: 54–261) showed sensitivity of 84% (95% CI: 69–94%) and specificity of 73% (58–85%) [98]. This is an important study as IGRAs rely to some degree on T-cell function, yet despite advanced HIV infection, the IGRA was relatively sensitive. All studies had high numbers of inconclusive results and different cut-points with the same assay (T-spot: 6 vs 20 positive spots) [96–97]. IGRA are currently relatively expensive, requires overnight processing and specialized equipment [98] limiting the utility of IGRA for TBM at present.

A lipoarabinomannan (LAM) antigen detection test (Clearview® TB ELISA, ME, USA) was evaluated on CSF in 150 patients (84% were infected with HIV) 39 were classified as definite TBM and 54 as not having TBM [90]. Sensitivity was 31% (95% CI: 17–48%) and specificity 94% (95% CI: 85–94%), performance improved with decreased CD4 count [90]. A Brazilian study of 83 patients (19 with TBM, 62 without TBM) evaluated the Lionex TB ELISA kit along with numerous *M. tuberculosis* antigens (MPT-64 [Rv1980c], MT10.3 [Rv3019c], 16 kDa [2031c] and 38 kDa [Rv0934]) (Lionex Diagnostics and Therapeutics, Germany) in various combinations on CSF [99]. Sensitivity ranged from 10.5 to 63.2% with specificities from 78.1 to 96.7% [99].

Nucleic acid amplification tests (NAATs), both RNA and DNA, are very specific. A 2003 meta-analysis of 14 commercial and 35 'in-house' nucleic acid amplification tests found mean sensitivity was 71% (95% CI: 63–77%) with mean specificity of 95% (95% CI: 92–97%) [100]. Interestingly commercial tests (primarily RNA based) showed mean sensitivity of 56% (95% CI: 46–66%) while 'in-house' tests (primarily DNA based) had a mean sensitivity of 76% (95% CI: 67–83%), mean specificities were 98%

(95% CI: 97–99%) and 92% (95% CI: 88–95%), respectively [100]. A 2014 meta-analysis of NAATs included studies from 2003–2013; pooled estimates of 9 commercial tests showed sensitivity and specificity were 64% (95% CI: 56–72%) and 98% (95% CI: 96–99%), respectively [101]. Though it appears that sensitivity has improved in commercially available tests since the 2003 meta-analysis above, among 40 commercially available tests sensitivity and specificity were very more or less unchanged [101]. Unfortunately cost and the significant lab infrastructure and expertise required to run these tests (even the less onerous commercial tests) limits their utility to some degree.

Loop-mediated isothermal amplification (LAMP) is a DNA amplification technology that requires much less infrastructure than traditional PCR and so is very attractive for use in low-middle income countries where much of the world's TBM occurs. One small Indian study of 27 patients (17 with TBM, 10 without TBM) found 88% sensitivity (95% CI: 64–98.5%) and 80% specificity (95% CI: 44–98%) based on clinical diagnosis [102]. LAMP requires further evaluation.

The MTB/Rif cartridge used with the GeneXpert system (Cepheid, CA, USA) has been a significant breakthrough in TB diagnostics [8]. This system is expensive (\$16,000 in low/middle income countries), but its cartridge-based platform (\$10/cartridge discounted cost, \$60 regular cost) decreases the need for lab expertise. The only requirement is reliable electricity. GeneXpert has been extensively studied on sputum but less so on CSF. Three studies of extrapulmonary TB suspects in Italy and Spain reported variable sensitivity but high specificity in GeneXpert use in a small number of cases [103–105]. All three studies looked at multiple extrapulmonary sites in aggregate, and CSF contributed a relatively small amount of samples, thus it is difficult to draw firm conclusions from these studies. However, GeneXpert appeared promising. More recently, in a South African study of 54 patients with TBM (by microbiology) and 65 non-TBM patients found sensitivity of the Xpert MTB/Rif cartridge was 67% (95% CI: 53–79%) while specificity was 94% (95% CI: 85–98%), sensitivity was significantly improved with centrifuged samples (Xpert positive in 22/27) versus uncentrifuged samples (Xpert positive in 20/39) [63]. A Vietnamese study of 379 patients (182 with definite or probable TBM and 197 who did not have TBM) showed 59% (95% CI: 52–67%) sensitivity for patients with definite or probable TBM by clinical criteria with 99% specificity (1 false positive of 197 negative samples) [64]. As a reference, sensitivity for MGIT culture was 66% (95% CI: 59–73%) and 79% (95% CI: 72–84%) with AFB staining described as meticulous with large volumes [64]. A 2014 meta-

analysis showed pooled sensitivity of 70% and specificity of 97% [101]. More data are required; however, GeneXpert appears to be helpful due to its ease of use, rapidity of the test and high specificity. Negatives of the GeneXpert include cost of the assay and the imperfect sensitivity. The key aspect to GeneXpert sensitivity is that GeneXpert detection threshold is approximately 80–100 *M. tuberculosis* organisms per test cartridge [106]. Regardless of the specimen type, one needs sufficient DNA present. Unfortunately, TBM has a paucity of organisms, thus there is imperfect sensitivity with nonconcentrated CSF.

Diagnostic tests for bacterial meningitis

Gram stain and CSF culture are the most common tests (specific to bacterial meningitis) used to diagnose bacterial meningitis. Culture is the gold standard, aerobic culture is generally sufficient although in special cases (such as those patients with recent neurosurgery) anaerobic culture is warranted [5]. Culture is generally a good diagnostic test with sensitivities of 60–90% commonly quoted, if collected prior to antibiotics [5,107]. Gram stain is less reliable but is quick and inexpensive. Sensitivities are frequently between 40 and 70% and may yield a helpful result in 30–50% of culture negative patients [5,107], particularly in the setting of antibiotic treatment prior to culture (although Gram stain yield decreases to some degree in this setting as well) [5]. Blood culture may also be used and yields results in 50–90% of patients with bacterial meningitis (varying by organism) [5]. Latex agglutination tests for bacterial antigens have been used for many years for a variety of common organisms, including: *S. pneumoniae*, *N. meningitidis*, Group B *Streptococcus*, *H. influenza* type B and *Escherichia coli*. Their increase in sensitivity over Gram's stain and culture is relatively modest, and these require similar cold chain and lab infrastructure as the CrAg latex agglutination, thus are not used widely in low- and middle-income countries. Lateral flow immunochromatographic assays have also been developed for meningococcal and pneumococcal meningitis with excellent sensitivity and specificity [108,109], although LFA performance is more variable for *N. meningitidis* [110].

Procalcitonin and C-reactive protein (CRP) are serum biomarkers that may have some roll in differentiating bacterial meningitis from other meningitides. A 1999 study of 23 patients with bacterial meningitis and 57 patients with viral meningitis showed substantial differences in procalcitonin between bacterial meningitis (mean: 13.8 ng/ml; range: 0.22–101 ng/ml) and viral meningitis (mean: 0.03 ng/ml; maximum: 0.1 ng/ml) [107]. Another 2007 study of patients with acute meningitis showed significant differences in CRP and procalcitonin values between bacterial

meningitis ($n = 18$) and aseptic meningitis ($n = 133$). Patients with bacterial meningitis had a median CRP value of 162 mg/l (IQR: 39–275 mg/l) and a median procalcitonin level of 3.75 ng/ml (IQR: 0.1–6.16 ng/ml) compared with patients with aseptic meningitis with median CRP levels of 13 mg/l (IQR: 9–17 mg/l) and procalcitonin of 0.07 ng/ml (IQR: 0–0.8 ng/ml), respectively [67]. A 2011 meta-analysis (33 studies) of CSF lactate (cut-off 35 mg/dl) showed sensitivity of 93% (95% CI: 89–96%) and specificity of 96% (95% CI: 93–98%) [111]. Sensitivity dropped to 49% (95% CI: 23–75%) if antibiotics were given prior to lumbar puncture [111]. These three tests (procalcitonin, CRP, CSF lactate) are generally used as adjunct tests when they are used. However, the relatively low cost of a serum CRP may be useful to guide whether more extensive testing is sought and the response to therapy.

In culture-negative, pyogenic meningitis with elevated inflammatory serum biomarkers (e.g., procalcitonin, CRP), 16s ribosomal RNA (rRNA) PCR is an exciting new technology that allows for definitive identification of bacteria. A 2012 meta-analysis of 14 studies of 16s rRNA PCR (448/2780 CSF samples were culture positive) for use in bacterial meningitis showed 92% (95% CI: 75–98%) sensitivity and 94% (95% CI: 90–97%) specificity compared with culture [112]. Further, 16s rRNA PCR identified pathogens in 33% of 360 cases of presumed culture negative bacterial meningitis though definitions of presumed bacterial meningitis varied broadly and sensitivities of individual studies varied widely (3–100%) [112]. The major role of 16s PCR is for culture negative meningitis, particularly when antecedent antibiotics have been received and/or with elevated biomarkers. CSF collection and processing must be strictly sterile, as any contamination of CSF at any point can introduce environmental flora.

Lastly, a number of assays have been tested for specific bacterial etiologies though their use is not widespread. LAMP use in separate studies detecting *H. influenza* and *S. pneumoniae* actually outperformed conventional PCR and specificity was 100% [113,114]. RT-PCR developed to combat the changing serotype epidemiology of *H. influenza* showed proof of concept in detecting all known samples of *H. influenza* nonserotype B [115] and has been used to detect *L. monocytogenes* [116].

Diagnostic tests for aseptic meningitis

A number of very specific (conventional PCR, 16s rRNA, RT-PCR) tests have been developed for detection of the etiologies responsible for aseptic meningitis [117,118]. We will not, however, devote significant space to these tests as with some exceptions, the specific etiology is not treatable and so the clinical utility is minimal for many of these tests [119]. Further, as noted

above, the important distinction is bacterial versus aseptic meningitis. Gram stain, culture, glucose and cell count, and to a lesser degree procalcitonin, CRP, or lactate provide compelling evidence to assist the clinician. Newer rapid multiplex assays are in development, but there is as yet published data.

Barriers to adoption

Cost remains a significant barrier for many new molecular diagnostics both in high-income and low/middle-income countries. Excluding reference laboratories, most local hospital microbiology labs are costs to a healthcare system, not a revenue generator. An integrated health system approach needs to be considered for decision making. A new relatively expensive assay may cost more for a microbiology laboratory budget, yet accrue significant cost savings in avoidance of other unnecessary procedures/testing, decreased pharmaceutical costs for empiric treatments and shorter duration of hospitalization. A second, ironic barrier to adoption is the standard Good Clinical Lab Practice of every laboratory internally validating a new assay. For fully automated US FDA-approved molecular assays, this slows adoption for relatively rare diseases where validation takes significant time and effort. Third, as new molecular tests become available, how best to utilize such testing in a cost-effective manner in high- and low-income settings needs to be explored [47]. Lastly, the patchwork of individual country regulatory requirements, which are often unclear or unknown in low- and middle-income countries, creates a substantial disincentive for manufacturers to bring new FDA-approved and/or CE-marked diagnostic assays to markets where there is minimal financial return. These barriers unfortunately impact vulnerable patients with deadly diseases, foremost.

Conclusion

Diagnosing the specific etiology of meningitis is crucial in bacterial, TB and cryptococcal meningitis – delays in diagnosis increase mortality. In order to rationally select which additional diagnostic tests might prove useful in making the proper diagnosis, physicians should pay close attention to time course of symptoms, past medical history, travel history, country of origin, sexual history, history of drug use, regional epidemiology and the patient's immune status. Thankfully, for bacterial meningitis and cryptococcal meningitis, excellent diagnostic tests exist which can help make a rapid diagnoses. Progress is being made with TBM diagnostics but delay in diagnosis still costs many lives. Further dissemination of technologies such as GeneXpert and 16s ribosomal PCR will certainly lead to improved care. Worldwide, point-of-care lateral flow assays, such as the

CrAg LFA, an ideal test for meningitis diagnostics that stakeholders should strive to emulate.

Future perspective

The field of diagnostics for infectious diseases is currently undergoing a renaissance in the development of new molecular assays with increased automation. GeneXpert is the first such broadly rolled out platform technology, and others such as the Biofire Diagnostics FilmArray platform are in development. In the next 5 years, these automated nucleic acid amplification tests will likely become more commonplace in high-income countries. Lateral flow assays will likely become more commonplace in all of infectious disease diagnostics, including meningitis. An unresolved question is the degree to which new diagnostics will

become available in low-income countries. Significant regulatory and market barriers exist for actual implementation in resource-limited settings.

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Executive summary

Background

- Meningitis may be due to bacteria, mycobacteria (e.g., *Mycobacterium tuberculosis*), fungi (predominantly *Cryptococcus neoformans*), viruses, parasites or noninfectious causes such as malignancy or rheumatologic conditions.
- Mortality is high except in aseptic meningitis.
- Prompt diagnosis is key to successful treatment.

Epidemiology

- *Streptococcus pneumoniae* is the most common cause of bacterial meningitis worldwide though rates of this meningitis due to this and other bacterial pathogens shift in relation to vaccinations.
- Rates of bacterial meningitis, fungal meningitis, tuberculosis meningitis and aseptic meningitis vary widely by geographic location.
- Rates of tuberculosis and cryptococcal meningitis tend to be much higher in areas with high burdens of HIV and low-income levels.

The host

- In patients with severe immune compromise; cryptococcal meningitis is by far the most common cause of meningitis.
- Tuberculosis and bacterial meningitis are also more common in patients with abnormal immune systems than in those with normal immune function.
- Cryptococcal meningitis becomes less likely as the CD4⁺ T-cell count rises above >100 cells/ μ l.
- In addition to HIV a number of additional causes of immune compromise such as solid organ or stem cell transplant, diabetes mellitus, alcoholism, cirrhosis of the liver, use of biologic immune-suppressive medications, asplenia, cancer and chemotherapy should be considered as influences of the relative likelihood of meningitis etiologies.

Duration of symptoms

- Tuberculosis and cryptococcal meningitis tend to present as subacute meningitis.
- Bacterial meningitis is generally rapidly progressive. If the patient has had symptoms for >1 week they are very unlikely to have bacterial meningitis.
- Symptoms related to aseptic meningitis vary widely.

Common diagnostic laboratory & imaging tests

- Most often patients with tuberculosis or bacterial meningitis have high cerebrospinal fluid (CSF) white blood cell and protein values with low glucose values.
- Patients with tuberculosis meningitis are less likely to have neutrophilic CSF than are those patients with bacterial meningitis.
- Patients with aseptic meningitis or cryptococcal meningitis are less likely to have inflammatory CSF.
- Imaging is generally not helpful unless persistent or focal neurologic symptoms are present.

Diagnostic tests for fungal meningitis

- Cryptococcal antigen detection by lateral flow assay is the mainstay of diagnosis in patients without previous history of cryptococcal meningitis as a fast, cheap and accurate test.
- Culture is extremely important in patients with previous history of cryptococcal meningitis.

Executive summary (cont.)**Diagnostic tests for tuberculous meningitis**

- Traditional staining for acid fast bacilli with microscopy is rapid but insensitive.
- Culture has improved sensitivity (though still poor) but growth takes weeks.
- Nucleic acid amplification tests offer some promise with sensitivity similar to culture with much improved speed.

Diagnostic tests for bacterial meningitis

- Gram staining and culture are the mainstays of diagnosis. Gram stain is more rapid while culture is more sensitive.
- Both Gram staining and culture have better sensitivity if CSF is collected prior to administration of antibiotics.
- Nucleic acid amplification tests may offer some promise in rapid, sensitive detection of bacterial meningitis.
- Rapid tests have been developed for detection of specific pathogens with variable efficacy reported.

Diagnostic tests for aseptic meningitis

- Numerous nucleic acid amplification tests are available but cost and lack of actionable treatments in many cases (combined with generally good outcomes) makes the values of these tests less clear than that of tests for other forms of infectious meningitis.

Barriers to adoption

- Major barriers to adoption of specific diagnostic tests most often include cost, required laboratory infrastructure and/or disparate regulations between countries that prevent rapid adoption.

Conclusion

- Early diagnosis of the etiologic agent of meningitis leads to improved outcomes.
- Patient history, epidemiology, patient symptoms and diagnostic tests need to be used in combination with each other to provide rapid and accurate diagnosis.
- Progress has been made in diagnostic tests for cryptococcal and bacterial meningitis while improved detection of tuberculosis meningitis remains a major priority.

Future perspective

- Increased automation in assays and use of lateral flow assays will improve the applicability of diagnostic tests to disparate settings.
- Barriers such as cost and variable regulations across countries slow widespread use of diagnostic advances.

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