Advance laboratory diagnosis of fungal meningitis: A mini review

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Abstract

Meningitis is an infection of the membranes (meninges) surrounding the brain and spinal cord. Meningitis is usually of multiple aetiology-bacterial, fungal or viral yet bacteria remain the common etiological agent. Fungal meningitis is rare, but can be life threatening. Although anyone can get fungal meningitis, people at higher risk are those who have AIDS, leukemia, or other forms of immunodeficiency. The most common cause of fungal meningitis in HIV is Cryptococcus spp. Recent efforts to improve the sensitivity and specificity of diagnostic tests have focused on culture-independent methods, in particular nucleic acid-based methods, such as PCR assays. Numerous studies have highlighted the advantages of using PCR technology to detect viable and nonviable fungal pathogens in a variety of clinical specimens.

Keywords: fungal meningitis; fungal meningitis diagnostic test; nucleic acid assay

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Introduction

Meningitis is an infection of the membranes (meninges) surrounding the brain and spinal cord. Meningitis is usually of multiple aetiology-bacterial, fungal or viral yet bacteria remain the common etiological agent (Reid & Fallon, 1992). Meningitis can be acute, with a quick onset of symptoms, or chronic, lasting a month or more, or can be mild or aseptic, but the emphasis should be on identification of cause so that appropriate interventions can be applied. One form of fungal meningitis is cryptococcal meningitis. Patients treated for cancer or chronic illnesses where treatments and/or the disease itself compromise the patient's immune system are particularly vulnerable. Most common in AIDS patients, cryptococcal fungal meningitis cases have increased globally during the past decade. Fungal meningitis is rare, but can be life threatening. Although anyone can get fungal meningitis, people at higher risk are those who have AIDS, leukaemia, or other forms of immunodeficiency. The most common cause of fungal meningitis in HIV is Cryptococcus spp. In the last two decades, more elaborative use of intensive care units for serious medical disorders, advancements in transplant procedures and concomitant use of immunosuppressive therapies as well as the pandemic spread of HIV, etc. have increased the incidence of Central Nervous System (CNS) fungal infections which present with various clinical syndromes: meningitis commonly. The clinical picture may mimic TBM and therefore, needs careful evaluation. The CNS mycoses carry higher risks of morbidity and mortality as compared to other infective
processes and therefore promptly require precise diagnosis and appropriate medical and/or surgical management strategies to optimize the outcome (Raman Sharma, 2010).

Laboratory diagnosis of fungal meningitis

Fungal meningitis is rare, but can be life threatening. The most common cause of fungal meningitis among people with immune system deficiencies, like HIV, is Cryptococcus, Candida, which can lead to meningitis in rare cases, especially in pre-mature babies with very low birth weight. People with immunodeficiency are at a higher risk for histoplasma meningitis. Histoplasma is found primarily in soil or bird/bat droppings in the Midwestern United States, although it can be seen in other places. Soil in South-western United States and northern Mexico contain the fungus Coccidioides, which can cause fungal meningitis. People at higher risk include African Americans, Filipinos, and pregnant women in the third trimester, and immunocompromised persons.

Lumbar puncture is also part of the routine evaluation. CSF is tested for opening pressure, WBC and differential, glucose, protein, culture, antibodies/antigens, India ink stain (Cryptococcus). However, repeated sampling is often required because diagnosis of non-HIV-associated cryptococcal meningitis, coccidioidal meningitis, histoplasmosis, and candidal meningitis can be difficult. Up to three sets of blood cultures should be taken in all patients; they may be positive when candidal, histoplasmal, or cryptococcal meningitis is associated with disseminated disease. CSF analysis usually reveals lymphocytic pleocytosis with raised protein and low sugar levels. The diagnosis of cryptococcal meningitis can be established with India ink stain in > 50% of the cases of cryptococcal meningitis in HIV-negative cases and in > 90% of patients with AIDS (Satishchandra et al., 2007).

The India ink or Nigrosin should be shaken well before every wet mount preparation. Too much stain makes the background too dark and the stain should be regularly checked for quality control, contamination by examining just the stain under a microscope. It will be positive when about $10^3$-$10^4$ colony-forming units (CFU)/ml are present in a CSF sample. AIDS patients have larger concentrations of yeast ranging between $10^5$-$10^7$ CFU/ml (Satishchandra et al., 2007). The CSF sample should also be evaluated for cryptococcal antigen assay that is positive in almost all cases except very early in the disease or in those with very high titers due to prozone effect and in certain patients with cryptococcomas (Satishchandra et al., 2007). The methods used for antigen detection are latex agglutination test and enzyme immunoassay and are > 90% sensitive and specific. Cryptococcal antigen titers usually decrease with treatment, but it can remain at low titers for long periods even after effective therapy (Satishchandra et al., 2007).

A positive fungal culture is the gold standard for diagnosis of Cryptococcal infection and CSF samples show fungal growth in almost all the cases. In our series, fungal cultures grew C. neoformans in 100% of the cases. Fungal cultures also help to determine the species of the infecting organism and sensitivities to various antifungal agents. Globally, all cases of Cryptococcosis in AIDS patients are due to var grubii, followed by var neoformans. Our observations highlight the fact that the rate of C. gattii in this part of the country is comparatively low (2.8%) (Nagarathna et al., 2010). Drug susceptibility testing of the fungal isolates is not routinely done except in cases of recurrent disease (Satishchandra et al., 2007). It has become important due to emerging antifungal resistant fungi causing infections in patients with AIDS. However, in a pilot study conducted by us did not reveal resistance to
any of the routine antifungal drugs. The routine processing of CSF for chronic meningitis and sub acute meningitis cases for fungal culture is a good surveillance procedure so as not to miss any Cryptococcal, Candidial and occasionally the Cladosporial CNS infections. This is more essential because of the incidence of HIV. In disseminated Cryptococcal infections, additional sampling of sputum, urine and blood is useful. The fungus often isolated is Cryptococcus. The other CNS fungal agents are almost never isolated except rarely the Cladosprium. Cytospin studies can identify the yeasts when India ink preparation is negative. Pan-fungal PCR has been a promising aid in rapid, early diagnosis of invasive fungal infection (IFI). On the other hand; it has the potential to detect all fungal species. Epidemiological studies now indicate that the spectrum of fungal pathogens has expanded well beyond Aspergillus fumigatus and Candida species (Lau et al., 2007). However, current culture-based phenotypic methods are insensitive and slow, may initially be nonspecific, and require considerable expertise for correct morphological identification of less common or unusual fungi (Lau et al., 2007). Additional drawbacks of conventional culture include the failure of zygomycetes to grow:
- When hyphal cells have been damaged during processing (Lau et al., 2007)
- Or the collection of tissue biopsy specimens directly into formalin fixative for paraffin embedding when IFIs are not suspected clinically (Lau et al., 2007).
- Or when limited material is available.

Recent efforts to improve the sensitivity and specificity of diagnostic tests have focused on culture-independent methods, in particular nucleic acid-based methods, such as PCR assays. Numerous studies have highlighted the advantages of using PCR technology to detect viable and nonviable fungal pathogens in a variety of clinical specimens. The majority of assays target multi copy genes, in particular the ribosomal DNA (rDNA) genes (18S, 28S, and 5.8 S) and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2), in order to maximize sensitivity and specificity.

To date, most assays have been designed to detect Candida or Aspergillus species only. Given that more than 200 fungal species have been reported to cause disease in humans and companion animals, the clinical utility of a species-specific or even a genus-specific assay is limited. Sequence-based identification of PCR products is a sensitive alternative, provided that accurate sequences have been submitted to public databases, e.g., Gen Bank. A panfungal PCR assay targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene cluster successfully detected and identified the fungal pathogen in 93.6% and 64.3% of culture-proven and solely histological proven cases of IFI, respectively. A diverse range of fungal genera were identified, including species of Candida, Cryptococcus, Trichosporon, Aspergillus, Fusarium, Scedosporium, Exophiala, Exserohilum, Apophysomyces, Actinomucor, and Rhizopus. The results support the use of the panfungal PCR assay in combination with conventional laboratory tests for accurate identification of fungi in tissue specimens (Lau et al., 2007).

Galactomannan (GM) is a component of the cell wall of the mould Aspergillus and is released during growth. Detection of GM in blood is used to diagnose invasive aspergillosis infections in humans. Eg Platelia galactomannan enzyme immunoassay (Bio-Rad). Although the test is approved by the FDA for use with patients with neutropenia and that undergoing stem cell transplantation, controversy about the test's utilization exists. Although
initial results were promising, various sensitivities and specificities (29 to 99%) have been reported recently in prospective studies (Zedek and Miller 2006). The Aspergillus GM test was performed on CSF and serum. Detection of \textit{Aspergillus} GM in CSF may be diagnostic of cerebral aspergillosis. It is suggested that the Aspergillus CSF GM index might be diagnostic for cerebral aspergillosis in patients at high risk for aspergillosis and with a compatible neurological disease. (Claudio Viscoli et al., 2002). A positive result supports a diagnosis of invasive aspergillosis (IA) and should be considered in correlation with clinical condition, microbiologic culture, histological examination of biopsy specimens, and radiographic evidence, and other laboratory parameters.

A negative result does not rule out the diagnosis of IA. When there is a strong suspicion of IA, repeat testing is recommended. Patients at risk of IA should have a baseline serum tested and should be monitored twice a week for increasing GM antigen levels. GM antigen levels may be useful in the assessment of therapeutic response. Antigen levels decline in response to antimicrobial therapy. False-positive results are reported to occur at rates of 8% to 14%.

The Glucatell (1\textsubscript{r3})-\textbeta-D-glucan (BG) detection assay was studied as a diagnostic adjunct for IFIs and a serum BG level of 60 pg/mL was chosen as the cut off. IFIs included candidiasis, fusariosis, trichosporonosis, and aspergillosis. Absence of a positive BG finding had a 100% negative predictive value, and the specificity of the test was 90% for a single positive test result and 96% for 2 sequential positive results. The Glucatell serum BG detection assay is highly sensitive and specific as a diagnostic adjunct for IFI. Glucatell assay may be a useful diagnostic adjunct for the diagnosis of invasive fungal infection, particularly in high-risk populations. The positivity of this test, particularly when used in a serial fashion, often precedes the microbiological or clinical diagnosis of invasive fungal infection. This cell wall component has the advantage of being present and detectable in a variety of fungal infections (Zekaver et al., 2004).

\textbf{Conclusion}

Fungal meningitis is rare, but can be life threatening, particularly in persons co infected with HIV. Early diagnosis and treatment can dramatically reduce the high mortality associated with this disease. There is a need to urgently address deficiencies in the diagnostic service for fungal meningitis. There have been many advances in methodology for fungal meningitides diagnosis and earlier diagnosis is of value clinically, and through the early institution of appropriate drug therapy is of public-health benefit. Nevertheless, many diagnostic tests have given promising results initially only to prove less effective in routine use. This is frequently due to bias resulting from non-independent interpretation of test results. While, at present many of these techniques are only economically viable in the developed nations, it is to be hoped that recent advances will lead to the development of novel diagnostic strategies applicable to use in developing nations, where the burden of fungal meningitis is greatest and effective intervention most urgently required.

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