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Review Article

Contemplating the False Negative CSF Cryptococcal Antigen Testing in Early Meningoencephalitis: Clinical relevance of Alternative Lab Testing

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Abstract:

In poorly controlled HIV patients, clinicians should maintain a high index of suspicion for cryptococcal meningitis and have a low threshold for initiating antifungal therapy. There are high chances of CSF cryptococcal antigen to be negative negative in the early disease phase. This is can partially attributed to limitations or technical issues with antigen-capture assays and discrepancies between cryptococcal antigens and antibodies. Despite a negative or inconclusive initial test there should not be delay in starting treatment, particularly because this early cryptococcal meningitis can progress rapidly and is commonly associated with substantial inpatient mortality. To circumvent this initial ambiguity, clinicians can rather rely on additional diagnostic including CSF polymerase chain reaction (PCR), β-D-glucan (BDG) testing, and next-generation sequencing (NGS), which may provide complementary evidence of infection. Further research is needed to develop diagnostic assays with greater sensitivity and specificity we can accurately identify and confirm the diagnosis of cryptococcal meningitis during its earliest stages. Such advances would help clinicians resolve diagnostic uncertainty promptly and enable timely initiation of antifungal therapy, potentially reducing disease progression, complications, and mortality.

Key words: hiv; cryptococcal; lateral flow assay

Introduction

Cryptococcal meningitis commonly occurs in patients with HIV/AIDS patients and severely immunocompromised patients. Diagnosis mainly depends on detection of cryptococcal antigen in both serum and CSF. We present this clinical case to discuss the diagnostic skepticism and indecisiveness for starting treatment in cryptococcal meningitis in HIV patients.

Clinical Case

40-year female with PMH of AIDS with who is non-compliant with anti-retroviral therapy (Biktarvy & Prezcobix) and seizures on Keppra presented to the emergency medicine department with headache and persistent hypotension. On admission, RPR was negative, TB GOLD TEST was negative and HIV viral load 317000. Serum crypt antigen being positive at 1:16. Lumbar puncture was performed with CSF WBC: 0, glucose: 9, protein: 83, and CSF cryptococcus antigen negative. With the negative CSF cryptococcus antigen, it is highly unlikely that this patient has clinical cryptococcal meningitis. CT scan head non-contrast showed no acute intracranial hemorrhage, cerebral edema, new white matter lesions, midline shift and accelerated cerebral atrophy. However, given markedly abnormal CSF glucose and protein values, there is a possibility that patient has sub-clinical cryptococcal meningitis. Therefore, patient was started on liposomal amphotericin and flucytosine.

Discussion

The diagnosis of cryptococcal meningitis in HIV patients is usually accomplished with positive serum cryptococcal antigen reactivity [Sensitivity 94.2%], CSF cryptococcal antigen positivity by latex agglutination test or latex flow assay (LFA) [Sensitivity 99.3%], positive CSF culture as well as positive fingerstick point-of care test [Sensitivity 100%] for cryptococcus Ag. Furthermore, PCR of the CSF detecting > 100 cryptococcal CFU [Colony forming Units] is confirmatory in 96% of cases [1]. In 4.3% of HIV population, cryptococcal antigen will be unable

to be detected in CSF serology and culture but will be detected in the serum [2]. These subsets of patients were considered to have early CNS cryptococcal infection and were found to have in-hospital mortality of 39% [2]. Since cryptococcal antigen is highly specific in diagnosing cryptococcal CNS infection, it is best to be mindful of having false negative cases particularly when there is high suspicion of harboring CNS infection [3]. Diagnosis of cryptococcal meningitis mainly relies on the identification of cryptococcal antigen in the CSF, which is mainly accomplished by Lateral Flow Assay (LFA). LFA has a very high sensitivity and specificity (99%) in detecting cryptococcal antigen in the CSF [4, 5]. Serum Ag is very sensitive and be very helpful in detecting the cryptococcal antigen that can be performed in instances there is delay in performing the lumbar puncture [6, 7]. Some of the possible reasons for false negative cryptococcal antigen testing in the CSF include low fungal burden, prozone effect, post-zone phenomenon, infection with atypical acapsular strains or cryptococcus gattii and symptomatic antigenemia [8, 9]. In prozone and post-zone phenomenon, antigens and antibodies are out of proportion of each other, thus engendering attenuated agglutination titer [8, 9]. In prozone phenomenon, there is excess of antibodies in comparison to antigens, thus attenuating the formation of antigen-antibody complexes and hindering their detection by immunoassay [10]. Sometimes prozone effect can be rectified by diluting the sample, as diluted sample relays a better signal as compared to undiluted sample [10]. Prozone is predominantly reported in syphilis, however there is possibility for occasional happenstance during cryptococcal meningitis [11, 12]. In the contrary, post-zone phenomenon materializes due to outnumbering of antigens as compared to antibodies, thus thwarting the aggregation of antigen-antibody complexes and anti-cryptococcal antibodies, thus leading to a false negative result [3, 10]. For immunoprecipitation tests, there should be a precise balance in the ratio of antigens and antibodies for eventuation of precipitation, antigen-antibody complexes, and positive result. Any imbalance in the above-mentioned ratio might hinder the detection of antigens in the CSF, thus accounting for false negative tests [13]. In our clinical case, serum cryptococcal antigen was 1:16 and CSF cryptococcal antigen was negative. In line with our findings, there are few clinical case reports published that encountered similar lab findings, thence sparking a clinical dilemma where the decision to initiate antifungal therapy is ambiguous [8, 14]. Any delay in starting antifungal therapy for cryptococcal meningitis in HIV patients is deleterious as it can increase the risk of complications in these patients. If clinicians encounter such clinical scenarios of cryptococcal meningitis in HIV patients, then it would be prudent to dilute the sample to counteract post-zone phenomenon [4]. Recently in disseminated gastrointestinal cryptococcosis detection of cryptococcal antigen by lateral flow assay (LFA) was false negative secondary to occurrence of post-zone phenomenon [3]. Since its initial discovery, post-zone phenomenon has been documented in few clinical scenarios, all of which were rectified by sample dilution [15-21]. Other aberrations that interfere with antigen-antibody binding include antibody interference, cross-reactivity and signal interference [22]. It is not uncommon for post-zone phenomenon to be confused with pro-zone phenomenon [23]. If the clinical suspicion is high, and post-zone phenomenon is suspected, it would be prudent to dilute the sample to increase the chance of detection of Cr Ag in the CSF [18]. In symptomatic antigenemia, Ramachandran et al had utilized ultrasensitive metagenomics next generation sequencing (mNGS) which detected low levels of cryptococcus DNA, thus indicating an early phase of meningoencephalitis [24]. As a result of these phenomena, confirmation of diagnosis and treatment of cryptococcal meningitis might be delayed, thus increasing the morbidity and mortality in the HIV population. As a result of these findings, clinicians should rather rely on clinical evaluation and a combination of tests including CSF culture, Indian Ink, and polymerase chain reaction for increasing the chances of confirming the diagnosis. Fungal capsules can be visualized with Indian microscopy [5]. When fungal capsules are mixed with Indian Ink and examined in the light microscopy, capsules becomes more evident with darker background. Indian Ink microscopy might not be applicable in all clinical scenarios, due to lower sensitivity in early-stage disease and in non-HIV population [25, 26]. Furthermore, preservatives like thiomersal added to Indian Ink might be toxic to the fungal capsules, thus making it a less viable option in real time clinical applications [27]. Fungal capsule diameter can be indirectly measured with Indian Ink preparation [28]. (1→3)-β-d-Glucan (BDG) has also used in certain clinical scenarios for diagnosing invasive fungal infections [29]. The sensitivity and specificity of BDG assay in CSF for diagnosing cryptococcal meningitis is 89% and 85% respectively [29]. CSF BDG levels of 343 picograms/ml are high suggestive of and levels greater than 500 picograms/ml were associated with higher 10-week mortality in cryptococcal meningitis. Importantly, the levels of BDG were shown to be highly connected with higher cryptococcal fungal burden, cryptococcal antigen levels and monocyte chemoattractant levels [29]. Since sensitivity of CSF BDG increases at higher fungal load (>10,000 CFU), its measurement might be useful in monitoring treatment response and detecting relapses [29]. Next CSF fungal cultures can be performed to confirm the diagnosis, reinforce the presence of fungal spores and also provides an opportunity to perform drug susceptibility testing in treatment resistance and relapses [5]. CSF fungal cultures are more reliable than serum for endorsing the diagnosis of cryptococcal meningitis in HIV patients [25, 26]. Positive fungal cultures and higher fungal burdens do not always translate into higher mortality rate. In a prospective clinical trial, patients with CSF cryptococcal antigen (+) but sterile CSF cultures were linked with increased levels of CSF interferon-gamma (IFN-γ), IFN-α, interleukin (IL)-6, IL-17, G-CSF, GM-CSF, and chemokine CXCL2 and higher 30-day mortality rate as compared to those with higher fungal burden [30]. It is speculated that patients with sterile CSF culture with higher cytokine profile have more proclivity to develop immune reconstitution inflammatory syndrome (IRIS) due to distorted immune attack against the cryptococcus antigens, thus increased fatality rate in this patient group [30-33]. Brain CT/MRI can also be performed in cryptococcal meningitis which can unravel findings such as brain atrophy (34%), cryptococcomas (11%), hydrocephalus (9%), diffuse cerebral edema (3%) and gelatinous pseudocysts [34]. Brain imaging should be considered in all patients with cryptococcal meningitis in HIV patients particularly. Non-HIV patients presenting with visual disturbances, seizures, generalized weakness, gait disturbances, focal neurological deficits, IRIS and post-inflammatory immune response syndrome should be screened [5, 34, 35]. Brain imaging might also be useful in surveillance after therapy to monitor new symptoms and to detect complications that might develop in long run. It is particularly notable to comprehend that brain MRI is much more sensitive in discovering cystic lesions and parenchymal disease as compared to CT scan [34]. In false negative CSF cryptococcal antigen testing, newer clinical applications like metagenomic next generation sequencing (mNGS) has yielded diagnosis of mixed fungal infections in HIV patients, thus increasing the chances of delivering focused therapies and improved clinical outcomes [36]. This testing specifically holds true promise in the clinical setting where there is lower fungal burden, negative fungal

cultures, prior antifungal treatment, mixed species, and improper species identification are increasingly common [36]. Utilization of mNGS in CSF might increase the diagnostic yield by recognizing the fungal strains when routinely employed techniques such as Indian Ink, fungal cultures, and PCR were futile, thus conferring more clinical dilemma and indecision for initiating treatment in cryptococcal meningitis [36, 37].

Conclusions

A high index of suspicion and low threshold for treatment of cryptococcal meningitis should be traditionally endorsed in poorly controlled HIV patients. The inherent inability to detect cryptococcal antigen in the CSF in early meningoencephalitis of HIV patients might represent a technical error of antigen capture assays due to discordance of cryptococcal antigens relative to antibodies. Therefore, diagnostic ambiguity during early phase of cryptococcal meningoencephalitis should not preclude clinicians from initiating antifungal therapy as these cases might progress at a rapid phase with higher in-patient mortality. Accordingly, clinicians should lean on a battery of tests including CSF PCR, Fungitell β-D glucan (BDG) and next generation sequencing assays. Finally, further research is highly encouraged to decipher highly specific tests to confirm the diagnosis of cryptococcal meningitis.

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