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## Cryptococcosis infection among HIV patients

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## ABSTRACT

Cryptococcosis is commonly known as a central nervous system infection due to *Cryptococcus neoformans*. It is one of the most frequent infections in AIDS patients. Disseminated cryptococcosis appears in almost one third of these patients. In this review, we will discuss the clinical presentation of cryptococcal infections among HIV patients and various methods of diagnosis, such as India ink, latex agglutination test and culture.

## 1. Introduction

Cryptococcosis is known as a central nervous system infection which is caused by *Cryptococcus neoformans* (*C. neoformans*). The contamination is made by inhaling the fungi, then it produces the first lesion in the lung tissue. This phase is non symptomatic. In debilitated people, the infection disseminates and affects the meninges. The AIDS patients are the most affected group, and the dissemination of fungus occurs in almost one third of this group of people[1].

The unique spice proving to be pathogenic is represented by *C. neoformans*. It looks like an encapsulated yeast, having a round or oval form reproduced just by budding[2].

Depending on the capsular polysaccharide and glucuronoxylomannan, we can classify the yeast into four

stereotypes. Capsular types A to D represent the variants of *C. neoformans*, namely, *Cryptococcus neoformans* var. *grubii* (A); *Cryptococcus neoformans* var. *gattii* (B and C); *Cryptococcus neoformans* var. *neoformans* (D) and *C. neoformans* AD hybrids[3]. Currently, *Cryptococcus gattii* has become classified as a different species, as the indicated analysis has revealed it to be genetically distinct from C[4].

*Cryptococcus neoformans* var. *neoformans* is observed throughout the world and seems to be linked to pigeons or other bird droppings, or just ground infected with these droppings. It generally attacks the immuno-compromised host[5]. *Cryptococcus neoformans* var. *gattii* is endemic in certain regions, especially tropical and subtropical areas such as Southern California and Australia. This variety is related to *Eucalyptus camaldulensis*[6]. It basically affects people without immunosuppression and is uncommon in patients with HIV infections[5].

Since *Cryptococcus* reaches the lungs by means of inhalation, the immune system could eliminate it without any medical intervention. The infection may remain located in the lungs. Otherwise, it may be transmitted by blood and extended in all parts of the body. Concerning healthy people having normal functioning immune

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systems, the disease is often self-controlled, and the person affected does not show any symptoms. In case the body does not heal itself, the fungus starts to grow in the lungs and may expand to some different parts of the body, especially the brain and also the central nervous system, leading to cryptococcal meningitis[7]. Cryptococcal meningitis in the AIDS patients caused by *C. neoformans* is considered as an opportunistic infection having a high level of mortality, even in developed countries[8].

Concerning seropositive HIV patients, almost all occurrences of cryptococcal meningitis probably indicate the reactivation of a latent infection that could have been obtained several years before. HIV-associated cryptococcal meningitis generally presents like a subacute meningoencephalitis in subjects with profound immunosuppression (CD4 cell counts < 100 cells/ $\mu$ L)[9].

*C. neoformans* may be localized apart from the meninges. The determined pulmonary disease was well illustrated[10]. It is generally presented as a solitary nodule in the lack of other symptoms. Cryptococcal pneumonia also has been reported[11,12]. Cutaneous involvement is frequent, which indicates disseminated diseases. The most common skin involvement is comparable to that of molluscum contagiosum[13-15].

## 2. Diagnosis

The diagnosis of cryptococcal meningitis is made after lumbar puncture that usually reveals the increased opening pressure, significant protein level, augmented white cell count, and no more than one-third have hypoglycorrhachia. Approximately 20% have more than 20 white blood cells per cubic millimeter for cerebrospinal fluid (CSF)[16].

The diagnosis of cryptococcal meningitis may be confirmed by India ink stain in > 50% HIV-negative patients having cryptococcal meningitis and in > 90% of patients having AIDS[17]. Prior to every wet mount preparation, the India ink needs to be well shaken. Excessive stain can make the background very dark, and the stain must be regularly verified for quality control and contamination simply by checking the stain under a microscope[17]. Centrifuging the CSF at 500 r/min for at least 10 min and making India ink stain on the pellet may increase the sensitivity of the test. India ink stain of the CSF is positive, revealing encapsulated yeasts[17].

The cryptococcosis diagnosis is mainly done by the latex agglutination test for capsular polysaccharide antigen. The detection of antigen could be done in CSF or serum, and when it is found in CSF, the antigen is around 90% sensitive and specific for the diagnosis of cryptococcal meningitis[18].

The cryptococcal antigen titer is generally reduced after treatment. However, it may stay at reduced titers for an extended time, even after a powerful therapy[17]. Antigen testing needs to be treated in parallel with culture when low levels of antigen titre continue to

persist for too long, even if the organism is free from CSF. Rare false positive reaction is also observed, especially in the existence of rheumatoid factor as well as infections with *Trichosporon* spp. Pronase treatment is needed when serum is tested as opposed to CSF.

A positive fungal culture seems to be the gold standard regarding the diagnosis of cryptococcal infection, while CSF samples reveal fungal growth in the majority of the cases.

Cryptococcus grows on almost all the mycological media at 25 °C and 35 °C. The identification is established by colony morphology after 48–72 h of cultivation on fungal media such as Sabouraud's at 25 °C and brain-heart infusion agar at 35 °C by isolation of yeast colonies with a white mucoid aspect and by microscopy morphology, showing spherical to oval encapsulated yeast cells, budding on a narrow base ranging from 2 to 20  $\mu$ m in diameter but generally measuring 4–10  $\mu$ m in diameter[19,20].

The *C. neoformans* is additionally detected by urease production. This particular test is a rapid method to make evidence for urease activity by urea hydrolysis and production of ammonia. This useful screening test for the presumptive diagnosis of *C. neoformans* is simple and unchanged by pH value and needs 15 min to be executed. No false negatives have been recognized by this method[21].

In recent times, the produced point-of-care cryptococcal antigen tests (CrAg) have been used to check cryptococcosis in HIV-positive persons. Such a tool identifies the same cryptococcal polysaccharide capsule glucuronoxylomannan like the latex agglutination test[22]. The innovative CrAg lateral flow assay was cleared by US Food and Drug Administration to be used in serum or CSF specimens with the consideration of its close correlation (> 99%) with the latex agglutination and enzyme immunoassay[23,24]. This semi-quantitative test seems to offer many advantages over the other serologic methods. In addition to its high sensitivity and specificity in serum (> 98%) and urine (sensitivity of 85%), it is a quick test (approximately 15 min) with low cost and a minimal requirement for laboratory infrastructure, stability at room temperature and wider capture of *Cryptococcus gattii* polysaccharides[25,26].

## 3. Conclusion

Cryptococcal infection is a major cause for morbidity and mortality in seriously immuno-compromised population, especially in people with AIDS-associated cryptococcal meningitis. The diagnosis can be made by different methods, but the gold standard to acquire diagnosis is a positive culture of CSF, especially with other tests such as CSF India ink. The new developed test CrAg lateral flow assay seems to be an attractive point-of-care testing in both available resources and a limited setting because of its excellent performances and low cost.

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## Conflict of interest statement

We declare that we have no conflict of interest.

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