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Cryptococcosis today: It is not all about HIV infection

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Abstract

Purpose of the review—Cryptococcal disease is most often thought of in the context of HIV infection. Much of our knowledge of the disease originates from its management in the HIV-positive population over the last 30 years. While the majority of cases globally continue to occur in the setting of advanced HIV, *Cryptococcus species* is increasingly responsible for disease in HIV-negative populations including those considered normal hosts and these HIV-negative populations will be the focus of this review

Recent findings—Currently available data indicated that significant differences exist in epidemiology, clinical presentation, management and outcomes of cryptococcal disease in HIV-negative populations when compared to those living with HIV.

Summary—Further research is required to improve our knowledge of cryptococcal disease in particular in HIV-negative cohorts so as to optimise management of the disease in the future.

Keywords

Cryptococcosis; non-HIV; transplant

Introduction

Cryptococcal disease (CD) or cryptococcosis refers to infection caused by pathogens from the genus *Cryptococcus*(1). Although more than 30 species of *Cryptococcus* are known, only two species are commonly pathogenic in humans, *Cryptococcus neoformans* and *Cryptococcus gatti*(2). *C. neoformans* was originally described as a human pathogen in 1894, although up until the late 1970's a limited number of cases of cryptococcosis were reported in the literature(3) but with the onset of the HIV epidemic, the incidence of cryptococcal infection increased dramatically(4, 5). Therefore, much of what we have learned about cryptococcosis over the last 30 year comes from studies in the HIV-infected population with limited data available on cryptococcosis in the HIV-negative patient population. However, important differences exist.

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Globally, CD remains a huge challenge particularly in resource limited settings(6, 7). In 2006 there were one million cases of cryptococcal meningitis and 625,000 deaths attributable to the disease worldwide in people living with HIV (PLWH)(8). Similarly, data from the United States demonstrates that even in the HAART era, CD remains problematic with an estimated 2-7 cases of cryptococcal meningitis per 1,000 person years in PLWH and mortality rates reported between 12-25%(9-12). However, with the earlier detection of HIV infection and the introduction of HAART, the global incidence of cryptococcosis in PLWH has decreased, most dramatically in North America (13, 14).As the rate of cryptococcosis in PLWH has decreased(15), the incidence of CD in the HIV-negative population has not decreased resulting in an increase in the proportion of CD cases in the HIV-negative population(11, 16). Recently, for most major academic centers, the HIV-negative population accounts for the majority of new diagnoses of CD (10, 12, 17).

Differences in clinical manifestations, treatment response and mortality of CD in PLWH compared to their HIV-negative counterparts have been reported. Recent data from a large cohort study of 3,728 patients with CD identified from the Health Care Cost and Utilisation Project's State Inpatient Databases of Florida (2006-2012) and California (2004-2010) highlighted several of these differences. In this study, 2,091 (56%) patients were HIV-positive and 1,637 (43.9%) were HIV-negative with the proportion of CD in HIV-patients diagnosed annually over the course of the study decreasing markedly(11). HIV-negative patients were significantly older as has been previously shown(10, 12, 17). In this and other studies, lower rates of cryptococcal meningitis have been identified in HIV-negative patients compared to the HIV-positive patients(10-12). This finding, however, did not translate to lower rates of overall disseminated disease. Another important difference highlighted in these studies is the significantly higher mortality rates observed in HIV-negative patients with CD(10-12). Some of the independent risk factors for death from CD reported in HIV-negative patients include advancing age, liver disease, renal failure, diabetes and haematological malignancies with receipt of chemotherapy(11, 18).

Frequently, HIV-negative patients with CD disease are discussed collectively in the literature. However, this group of patients is actually very heterogeneous with varying degrees and types of immunosuppression ranging from those post-organ transplant to those with rheumatological conditions and diabetes mellitus to even include those with apparently normal immune function. In fact 20% of non-HIV infected patients with CD have no identifiable immune-deficiency(3). Interestingly, recent data suggest that some CD may be a complication of a previously undiagnosed secondary immunodeficiency including the development of auto-antibodies directed at interferon or granulocyte-macrophage colony-stimulating factor as well as isolated cases of CD4 lymphopenias(19).

Cryptococcosis in solid organ transplant recipients

Cryptococcal disease is the third most common invasive fungal infection (IFI) in solid organ transplant (SOT) recipients, second only to *Candida* and *Aspergillus* and in one large prospective multi-center study it accounted for approximately 7% of IFIs(20, 21). Similar findings were also reported by the Transplant-Associated Infection Surveillance Network

(TRANSNET), a consortium of 23 US transplant centers, estimating the cumulative lifetime and 12-month risk of CD in SOT recipients at 1-2% and 0.2%, respectively(22).

SOT recipients who present with CD do so in one of four ways. Most cases arise in patients who either acquire a new primary fungal infection from recent exposure or reactivate a latent infection that has been dormant because of immunosuppression during the post-transplant period and tend to occur late after transplant(22, 23). Occasionally, patients acquire CD from the donor organ (24), the risk of which may be increased if the donor had disseminated infection prior to death(25). Less frequently, pre-existing unidentified CD will be identified post-transplant. Although this is rarely observed, it appears more common in patients receiving liver transplants. When diagnosed in SOT patients, CD usually is a late complication; infection in the early post-transplant period is likely to reflect donor-derived infection or undiagnosed pre-existing infection(26).

A further dilemma surrounding CD and transplant recipients arises in those patients with end-stage organ disease awaiting transplant that are diagnosed with CD. There is limited data available to address this question in the setting of end-stage liver disease. An observational study of 112 cirrhotic patients with CD demonstrated lower mortality in the group that underwent transplant compared to those who did not. However, it is hard to determine if this differential outcome is because healthier patients were chosen for transplantation and does not examine how patients with CD fared compared to those without(27). It is important to note that treatment for CD with fluconazole will complicate the management of transplantation through drug-drug interactions with anti-rejection medications.

Cryptococcal pulmonary infection can present as a variety of clinical manifestations ranging from subclinical granulomatous infections of the respiratory tract with an isolated pulmonary nodule (frequently detected on routine chest radiology) to acute respiratory failure that can be rapidly fatal(28). From 25-54% of transplant recipients with cryptococcosis have pulmonary disease and 6-33% these cases are limited to the lungs (29-31). Cryptococcal infection of the lungs is frequently asymptomatic in immunocompromised patients but in immunocompromised patients can rapidly progress to acute respiratory failure(1). Subclinical granulomatous infection of the respiratory tract with *Cryptococcus species* has been demonstrated to occur with increased frequency in lung transplant recipients when compared to HIV-positive subjects or controls (32). Similarly, pulmonary cryptococcosis is a more common manifestation of the disease in all SOT recipients than in their HIV-positive counterparts (17, 33).

In contrast to what was observed with pulmonary cryptococcosis, cryptococcal meningitis occurs less frequently in SOT recipients with CD when compared to their HIV-positive counterparts(10). CNS involvement was estimated in a recent study at 86% within the HIV-positive patient group (n = 86) compared to 43% amongst the 42 transplant recipients included in the study. 11% of HIV-positive subjects had pulmonary cryptococcosis compared with 57% of the transplant recipients(17). This pattern has been seen in several different studies(10-12). The use of tacrolimus in SOT recipients has been suggested as a potential explanation for the lower rate observed(34). This may not be sufficient to explain

the disparity, as it also occurs in HIV-negative patients without a transplant. Cryptococcal CNS disease may present as meningitis, brain abscess formation (cryptococcoma) or hydrocephalus. Symptoms range from headache, fever and fatigue to altered mental status and cranial neuropathies. Onset of symptoms tends to be subacute over several weeks but in severely immunocompromised hosts onset of symptoms may be acute. Patients receiving ibrutinib may have a rapid onset cryptococcal meningitis after initiation of this new therapy(35).

Cutaneous infection is the third most common clinical manifestation of CD and usually reflects disseminated disease although cases of primary cutaneous cryptococcosis have occurred rarely(36). Any cutaneous disease should prompt an evaluation for other sites of infection, including lung and meninges, and if the serum antigen is positive at 1:8 or more, the patient should be treated for disseminated disease even in the absence of other sites of infection. Cutaneous CD presents in a variety of manners including solitary nodules, ulcers, abscesses and cellulitis (figure 1). Interestingly, SOT recipients on tacrolimus have increased rates of cryptococcal skin disease and lower rates of meningitis possibly related to the temperature dependent effect of tacrolimus on calcineurin function in *Cryptococcus* (1, 37).

Given its propensity to disseminate, *Cryptococcus* can involve virtually any body site including prostate, bone and ocular infections with atypical presentations also reported in transplant recipients(38). Therefore, the threshold for including *Cryptococcus* in a differential diagnosis should be low in immunocompromised patients and particularly, in the highly endemic areas of the southeastern United States.

Cryptococcal disease in non-HIV non-transplant patients

Although often considered to be less immunosuppressed or even immune competent, data suggests that the non-HIV/non-transplant (NHNT) population experiences poorer outcomes including increased mortality. In a single center study that examined outcomes of 207 patients including 86 HIV-positive, 42 transplant recipients and 79 NHNT, the attributable mortality in the NHNT population with severe disease was nearly two-times higher compared to HIV-positive and transplant recipients(17). Similarly, NHNT have higher odds of mortality when compared to HIV-positive patients (OR 2.17, 95% CI 0.86-5.26) and SOT recipients (OR 2.17, 95% CI 0.95-4.76) (10). Furthermore, a multi-center study of 86 patient with cryptococcal meningitis classified as HIV-positive, immunosuppressed (including patients in receipt of immunosuppressive agents such as corticosteroids or cancer chemotherapy; hematopoietic stem cell or solid organ transplantation patients and patients with liver cirrhosis) or non-immunosuppressed, the highest mortality was noted in the non-immunosuppressed group (46%) compared to those immunosuppressed (19%) and HIV-positive (15%) patient groups(39).

Morbidity, particularly neurological sequelae may also be more prominent in the NHNT patient population(3, 40). This is supported by recent data from the *Cryptococcus* Infection Network in non-HIV Cohort (CINCH) study which reported outcomes on 150 HIV-negative patients with CD, 75% of whom were non-transplant patient. This study demonstrated that cumulative neurologic morbidity was significant as measured with serial assessment of

Montreal Cognitive Assessment (MoCA) scores in these patients the cohort of patients who were enrolled with impaired cognition (78%) were slow to improve, and some did not return to normal, even after a year of therapy(41). There has been speculation that an overly robust immune response in the presumably immunocompetent group may be the cause of this increased morbidity.

Host factors contributing to poorer outcomes in non-HIV, non-transplant patients

Several factors are thought to contribute to the poorer clinical outcomes observed in NHNT. Multiple studies have demonstrated a significantly longer time to diagnosis when compared with their HIV-positive counterparts. One study reported a mean time to diagnosis among NHNT patients of 68 days compared with 22 days for the HIV-positive group and 26 days in SOT recipients ($p<0.0001$)(10), and this finding has been replicated by others (12).

In NHNT patients underlying medical conditions may also act as contributing factors with certain patient sub-groups having particularly poor outcomes. One such group of patients are those with end-stage liver disease (ESLD)(27, 42). ESLD patients have a 5-fold higher rate of dissemination compared to other HIV-negative patients with CD(43). Mortality in this group has been reported at 80% although the underlying mechanism driving this increased mortality remains uncertain(12). In this group *Cryptococcus* can be frequently isolated from the peritoneal fluid(12), and this has often been termed spontaneous fungal peritonitis (44-47). This is poor terminology, as the mechanism is not likely translocation from the bowel such as for spontaneous bacterial peritonitis, but rather dissemination from the lungs and it also happens to be identified in the peritoneum. These patients should be treated aggressively, as mortality is exceedingly high(12). Paradoxical immune response in NHNT patients has also been suggested as a potential contributing factor to the high mortality in this group. It has been likened to cryptococcal immune reconstitution syndrome that can occur post-ART initiation in HIV-positive patients(48). However, in NHNT patient's one obvious difference is that this process occurs without the introduction of ART. Although the clinical presentation is similar in both HIV-positive patient with IRIS and NHNT patients that experience a paradoxical immune response the underlying mechanism differs. In contrast to HIV infection where deficient T-cell immunity plays a major role, a recent CSF immune phenotyping study of 17 apparently immune competent patients with severe cryptococcal meningitis identified a highly-activated antigen-presenting dendritic cell population within the CSF, accompanied by a highly active T-lymphocyte population with potentially damaging inflammatory cytokine responses(49). Understanding the underlying mechanisms driving the reactive immune reconstitution response, in the absence of HIV may assist in targeted control with treatment of this patient population.

Differences in cryptococcal species infection amongst various risk groups

With its worldwide distribution, *C. neoformans* is responsible for over 95% of case of cryptococcosis. However in recent years the incidence of *C. gattii* infection has increased(1). Historically, *C. gattii* was restricted to tropical and subtropical regions (50) but has now been identified in temperate climates. (51). *C. neoformans* is commonly associated with pigeon excreta as well as multiple species of trees while *C. gattii* was classically associated with

eucalyptus trees although with its changing geographical distribution, it has been associated with a wider variety of trees specific to temperate habitats(52).

Aside from these ecological differences, studies have indicated that clinical presentation also differs between *C. neoformans* and *C. gattii* with the later having a predilection for immuneocompetent hosts(53, 54). A large, prospective, population based study performed in the southern hemisphere examined 312 episodes of cryptococcal disease, 265 cases caused by *C. neoformans* and 47 by *C. gattii*. There were 98% of the cases caused by *C. neoformans* occurred in immunocompromised patients, whereas 89% of *C. gattii* infections occurred in immuneocompetent hosts(55). However, this is in contrast with data on *C. gattii* infection from the Vancouver Island outbreak where rates of immuneocompromise were estimated closer to 40% (56). It is possible that some of the differences in rates may relate to how the authors defined immuneocompromise but also raises the question whether specific molecular types of *C. gattii* may be more virulent.

In general, *C. gattii* expresses the same major virulence factors found in *C. neoformans* namely the ability to grow at 37°C, the presence of a polysaccharide capsule and laccase activity necessary for the production of melanin(57). Interestingly, over-expression of the laccase gene, LAC1, was found in the R265 strain of the *C. gattii* subtype, VGIIa, which was the subtype responsible for 85% of cases in the Vancouver outbreak(58, 59). It should be noted, that a more recent study examining longitudinal clinical findings and outcomes of *C. gattii* infection in British Columbia reported 20.7% of patients were immunocompromised. This is a figure that is in keeping with the Australian experience although patients in the study with a cancer diagnosis were not included in the immunocompromised group(59).

Rarely, other species of *Cryptococcus* including *C. laurentii*, *C. albidus* and *C. uniguttulatus* have been reported to cause disease in humans (60-63). However, they appear to lack the virulence of the other two species and will need to be isolated from sterile body sites to ensure potential disease causation.

Diagnostic approach in HIV-negative patients with cryptococcal disease

The recommended approach to the diagnosis of CD does not vary between HIV-positive patients or their HIV-negative counterparts. Diagnostic modalities including direct microscopic examination of clinical samples, culture, histopathology and antigen detection tests. In histopathology, the mucicarmine and Fontana-Masson are very useful at differentiating CD from other mycoses as other medically important yeasts do not produce in tissue a large capsule or detectable melanin, respectively(64).

Some differences in the sensitivity of diagnostic tests between these groups have been reported and may be related to the burden of disease. For example, India ink staining of the CSF for encapsulated yeast is 30-50% sensitive in non-HIV positive individuals where as in HIV-positive patients its sensitivity increases to 80%(1). Other differences in CSF analysis such as higher CSF cell counts have been reported in apparently normal hosts when compared to HIV-positive individuals.

Although culture remains the gold standard for diagnosis its clinical utility has long ago been amplified by the polysaccharide antigen test. In recent years, improvements in cryptococcal antigen (CrAg) testing with the introduction of lateral flow assays has been a significant development (65). A recent meta-analysis of 12 studies containing 4,622 samples reported the pooled sensitivity and specificity for serum samples at 97.6% and 98.1%, respectively and for CSF at 98.9% and 98.9%, respectively(66). Little data exists related specifically to differences in CrAg validity between different immune states. Although one study did report that immunocompetent hosts with CD were less likely to have a serum CRAG of greater than 1:512 compared HIV-positive individuals(3). It is likely that differences are related to the general burden of yeasts in the host.

Management consideration for CD in HIV-negative individuals

Current Infectious Diseases Society of America guidelines on management of CD identified three different risk categories including HIV-infected individuals, organ transplant recipients and NHNT patients (67). Although the agents recommended for induction, consolidation and maintenance of cryptococcal CNS infection are the same for all three groups, (amphotericin B and flucytosine, followed by fluconazole) the duration of therapy, dosing and suggested antifungal formulation varies depending on the risk category. For instance, in HIV-positive individuals, amphotericin B deoxycholate (0.7–1.0mg/kg daily) plus flucytosine (100 mg/kg per day orally in 4 divided dose) for at least 2 weeks, followed by fluconazole (400 mg daily) for a minimum of 8 weeks is recommended. Alternatively, lipid formulations of AmB, including liposomal AmB and AmB lipid complex for at least 2 weeks, could be substituted for AmBd in patients with underlying renal disease(67). For SOT recipients, liposomal AmB or AmB lipid complex plus flucytosine is recommended for at least 2 weeks for induction followed by fluconazole (400–800 mg daily) for 8 weeks. In these patients it is recommended that AmBd be avoided in contrast to recommendations in HIV positive individuals.

Finally, for NHNT patients, the current first line recommendation regimen is AmB plus flucytosine extended to at least 4 weeks and in fact, in patient with cryptococcal CNS disease with neurological complications it is recommended that consideration be given to extending the duration to 6 weeks and for those with a good prognosis, consideration of two week induction period like the other groups has been advocated.

However, in clinical practice, due to better toxicity data, liposomal or lipid complex formulations, where available, have all but replaced AmBd for disseminated cryptococcosis in the developed world. Many academic hospitals in the USA no longer keep AmBd on formulary.

Following induction with AmB and flucytosine, consolidation treatment with fluconazole (400 mg daily) for 8 weeks should then be initiated(67). For all groups maintenance therapy with fluconazole (200 mg daily) is recommended. In SOT recipients and NHNT patient the recommended duration of therapy is 6–12 months, and may be extended based on clinical response, and ongoing need for immunosuppression.

All azoles have clinical activity for CD, and can be used for maintenance therapy if there is a secondary indication for another azole, such as the use of posaconazole for neutropenic prophylaxis.

Aside from specific antifungal considerations, there are several other issues that require consideration for management of cryptococcal disease that may differ between risk groups. One obvious difference relates to the management of immune modulatory therapy. Regarding adjunctive steroid therapy, dexamethasone did not reduce mortality among patients with HIV-associated cryptococcal meningitis and in fact was associated with higher rates of adverse events and disability than placebo(68). However, some experts feel that in the HIV-negative population there are patients who would benefit from adjunctive steroids, and commonly prescribe them. Of note, there are also clinical situations in which corticosteroids are indicated including IRIS-like reactions and cerebral edema associated with a cryptococcoma.

In transplant recipients and other patients on immunosuppressive therapy, the timing of reduction or discontinuation of immune modulating drugs including steroids and calcineurin inhibitors is also open for debate. For many years it has been standard practice to reduce immunosuppressive therapy in the setting of a severe opportunistic infection. However, in recent times increasing evidence exists to indicate that discontinuation of immunosuppressive therapy in the setting of CD may in fact be associated with worse outcomes by increased risk of immune reconstitution. In a study of 89 SOT recipients there was a 5-fold increased risk of immune reconstitution associated with discontinuation of calcineurin inhibitors(69). Therefore, further studies to examine strategies for management of immunosuppressive therapy in the setting of CD are required.

One over-riding factor in CD management is that irrespective of the risk group involved, the arsenal of treatment options available for management is limited, with no new class of antifungal agent with cryptococcal activity licenced in almost 30 years. Adding to this ongoing challenge of limited drugs are toxicities associated with current treatment regimens, as well as ever present concerns for the development of antimicrobial resistance as has been the case with other fungal infections.

The antifungal activity of several existing psychiatric medication has been under investigation lately. *In vitro* studies have demonstrated synergistic effects with the addition of sertraline or chlorpromazine to amphotericin B for *C. neoformans*(70). The use of sertraline as an adjunctive therapy for management of cryptococcal meningitis was examined in a recent open-labelled dose finding study. In this study, patients receiving sertraline may had faster cryptococcal CSF clearance and a decreased incidence of immune reconstitution syndrome reported (71). Therefore, further randomised trials to examine the overall effectiveness of sertraline as an adjunctive therapy for CD would be required before conclusions on its efficacy can be drawn. Recent *in vitro* data examining the activity of a novel fungal CYP51 inhibitor, VT-1129, demonstrated potent activity against *C. neoformans* and *C. gattii* with activity noted against isolates with reduced fluconazole susceptibility(72). Other novel non-pharmacological approaches to the management of cryptococcal meningitis are under investigation. In animal models, mechanical filtration of CSF substantially

substantial reduced the cryptococcal burden, at a rate much faster than pharmaceutical interventions(73). However, it remains to be seen if this will be associated with improved outcomes in patients.

Along with pharmacological therapy, effective management of elevated intracranial pressure in patients with cryptococcal meningitis is a critical factor in determining outcome and has been shown in the HIV-positive population to improve survival(74). Data in this regard is currently not available in the HIV-negative patient population. Current guidelines state that if cryptococcal meningitis is suspected, opening pressure during the initial lumbar puncture should be measured and in symptomatic patients repeated daily lumbar punctures performed until intracranial pressure normalises and symptoms stabilise(67). However, optimal management of elevated intracranial pressure does not always occur. This observation was highlighted in a study that examined the impact of infectious diseases consultation on CD outcomes in HIV-negative patients. Patients who received an infectious diseases consult were much more likely to receive an indicated lumbar puncture than those who did not (86% vs 32%, $p<0.001$). Furthermore, the same study also reported a lower 90-day mortality, despite higher fungal burden, in those who received an ID consultation (27% vs 45%, $p<0.001$ (18)).

Conclusion

There are still many outstanding challenges in the management of CD. Important nuances exist in the clinical manifestations, diagnosis and management of CD between HIV-negative and HIV-positive population. Currently, there is limited data available that focuses on CD in specific HIV-negative populations, particularly the NHNT cohort, which still has a substantial morbidity and mortality with present with therapies. The studies that are available are usually small and retrospective in nature and leave many important questions unanswered. Further collaborative efforts such as those seen with the CINCH cohort are required to improve the knowledge currently available in this area and the development of novel therapies such as VT-1129 may also play a pivotal role in the management of CD in the future.

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Figure 1.
(a) Extensive localised cryptococcal skin infection at time of diagnosis (b) skin lesion following 2 weeks of treatment with liposomal amphotericin B and flucytosine.