Cryptococcaemia in a Jamaican Cohort

Author

Camille-Ann Thoms-Rodriguez

Co-Authors

Kristen Facey Holligan - kristen.facey@yahoo.com
Jessica Edwards - jae_baham@yahoo.com
Nicoy Downie - downienicoy@gmail.com
Alison Nicholson - alison.nicholson@uwimona.edu.jm
Racquel Wright – racq.wright@gmail.com
Orville Heslop - orville.heslop@uwimona.edu.jm
Glendee Reynolds-Campbell - glendee.reynoldscampbell02@uwimona.edu.jm

Affiliations

The University of the West Indies, Mona

Corresponding Author:

Camille-Ann Thoms-Rodriguez
The University Hospital of the West Indies, Mona, Jamaica
Email: camille.thomsrodriguez@uwimona.edu.jm

DOAJ: 36f03f3e4da745cc94a773f286b72d32

DOI:

Abstract

Background:
The study objective was to describe the clinical features associated with cryptococcaemia in a Jamaican cohort and determine associated risk factors and outcomes.

Materials and Methods:
All blood samples collected at the Microbiology Laboratory at a Tertiary Care Hospital in Jamaica between April 2015 and March 2017 were analysed. De-duplicated patients with cryptococcaemia were selected and their clinical information retrospectively analysed.

Results:
Six patients (three males, three females) ages 22-55 years, were diagnosed with cryptococcaemia. One patient had diabetes and the remaining five were infected with HIV. Over 70% of these patients were non-compliant with anti-retroviral medication and 40% had an associated viral load of >10,000 RNA copies/ml with low CD4 counts. Fifty percent of these patients were diagnosed with sepsis with 33.3% having had meningitis. At least two patients had a lower respiratory tract infection but none were formally diagnosed with pulmonary cryptococcosis. No information was available on environmental sources. All were treated with antifungals but only one had completed the full course of therapy with amphotericin B and fluconazole and was the only patient to survive.

Conclusion:
HIV remains a major risk factor for cryptococcaemia. These research findings suggest that there may be a need to have a high index of suspicion for cryptococcosis in these patients as >50% did not show signs of typical underlying cryptococcal infections. The mortality rate was high in these patients, which would suggest that,that further studies may be needed in order to determine whether the presence of cryptococcaemia is a possible predictor for a poor outcome.

Keywords: cryptococcaemia, fungi, HIV, AIDS, *cryptococcus*

Introduction:

Cryptococcal yeasts are encapsulated environmental saprophytes measuring 4-10 µm in diameter. The genus is subdivided into 4 serotypes A (*var grubii*), D (*var neoformans*), both belonging to the *C. neoformans* species and serotypes B & C from the *C gattii* species. [1] Hybrids also exist between the two species. The organisms are commonly transmitted to humans post inhalation of aerosolized particles from the environment. Zoonotic transmission has never been reported and direct human transmission has thus far only been observed with organ transplantation.[2]

C. gattii infections though rare, predominantly affects the immunocompetent in tropical and subtropical regions where it is commonly associated with several species of eucalyptus trees.[3] Cases have also been reported in temperate regions e.g., British Colombia, and western and southeastern regions of the United States. It causes both CNS and focal pulmonary masses, and though most affected patients tend to be afebrile, their symptomatology tends to have a long duration.

C. neoformans, the most isolated of the two species, has worldwide prevalence. These saprophytic commensals of the gastrointestinal tract of birds such as pigeons and chickens as well as bats, are often isolated from soil contaminated with bird excreta/ guano and contaminated fruits. As for their human hosts, these organisms commonly cause disease of the CNS and lungs of the immunocompromised, the majority being those suffering from the acquired immunodeficiency syndrome (AIDS) with CD4 count <100. These patients account for 80-90% of cryptococcal infections. Non-HIV patients with immunosuppressive conditions such as glucocorticoid treatment, organ transplantation, malignancy, chronic organ failure, rheumatological disease, liver disease, lymphoproliferative disorders, and sarcoidosis, account for the majority of the remaining cases .[4-5]. There exists a small percentage with unidentified risk/predisposing factors. In short, the incidence of cryptococcal infections has been increasing over the last 30 years, primarily due to the HIV epidemic as well as increased use of immunosuppressants.[5]

Cryptococcus neoformans possesses virulence factors that enable survival and replication within human hosts. These factors are enhanced in the face of compromised T-cell immunity. The capacity to grow at 37 ˚C, its antiphagocytic capsule, which diminishes cellular and humoral immune responses when shed in host cells, and the presence of laccase and melanin which disrupt phagocytic oxidative killing and allow for intracellular viability of the organism. Protease, oxidase and the presence of the capsule are therefore essential virulence factors as non-capsular strains do not cause disease.[6-7] Host sites with an abundance of carbon dioxide favour and potentiate capsule formation and therefore virulence.[8] Poor prognostic factors associated with infections such as cryptococcal meningoencephalitis include hyponatremia, visual disturbances, and an initially, positive India Ink test result.[9]. Approximately 10 -30% of patients with cryptococcal infections get cryptococcaemia. Given the limited availability of data on the clinical significance of cryptococcaemia, our research objective was to describe some of the clinical features associated with cryptococcaemia in a Jamaican cohort and determine the associated risk factors and outcome of infection.

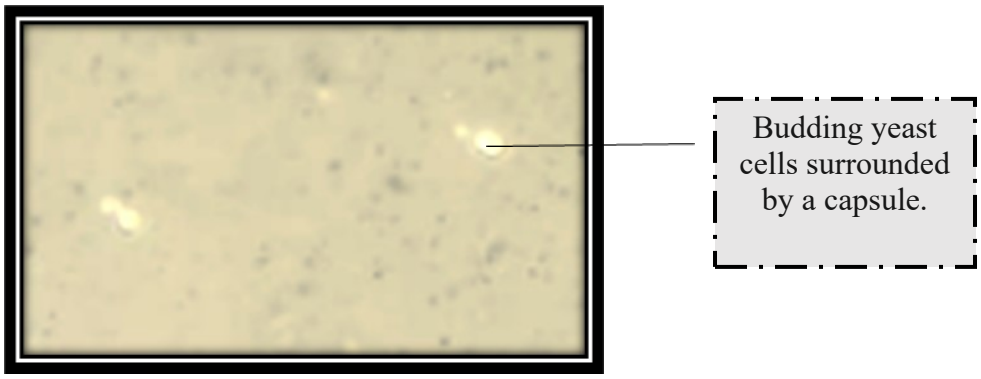
Materials and Methods:

After obtaining ethical approval from the University of the West Indies (UWI) Mona Campus Research Ethics Committee, all blood samples (n =7632) collected over a two (2) year period (April 2015 to March 2017) were analysed for this descriptive study. The study was conducted at the microbiology laboratory of a tertiary care hospital in Jamaica, the University Hospital of the West Indies (UHWI). This hospital is a ~500 bed university-affiliated medical centre which provides tertiary referral care, including recently, a renal transplantation service. The clinical mycology laboratory affiliated with this institution is the only one serving the country and is therefore often referred samples from patients across the island for processing. Standard microbiological methods were used for the culture and identification of

Cryptococcus spp.

An incubation period of four weeks was routinely used for all blood culture submitted for fungal diagnosis before they are reported as being negative. Majority of fungal blood cultures were processed using the automated BacT/ALERT® (bioMérieux) blood culture system. Positive cultures were evaluated using India Ink staining (Figure 1), looking for budding yeasts with a halo. The samples were plated on Sabouraud and Mycobiotic agar (Difco™) and incubated aerobically at 25-30 ˚C. Pathogen identification was confirmed by analysing growth patterns

Figure 1: *Cryptococcus neoformans* India Ink Preparation



and interpreting the results of biochemical tests such as the dalmau test and tests for urease. De-duplicated patient samples that were found to have cryptococcaemia were selected and their demographic and clinical information were retrospectively analysed. The study was primarily descriptive. Data extracted using a data extraction Microsoft Excel™ spreadsheet included age, gender, ward, diagnosis, CD4 counts, viral load and clinical outcomes.

Results:

Six patients, three males and three females, ages 28-63 years, were diagnosed with cryptococcaemia during the study period (Table 1). All patients were reported as having *Cryptococcus neoformans.* At the time of diagnosis most patients were on medical wards (50%). Among the other three cases, two patients were diagnosed at the time of presentation in the Accident and Emergency Department and one was diagnosed after admission to the Intensive Care Unit. For 50% of the cases *Cryptococcus* was detected in the blood after 4-5 days of incubation.

Table 1: Demographic Characteristics of Patients with Cryptococcaemia

Patient No.	Sex	Age	Comorbidities associated with Immunocompromise	Evidence of Sepsis	Evidence of Meningitis	Appropriateness of Antifungal Treatment	Patient Status
1	M	55	HIV	No	Yes	Sub-Optimal Course	Deceased
2	M	28	HIV	No	No	Sub-Optimal Course	Deceased
3	M	43	HIV	Yes	Yes	Complete & Appropriate Course	Survived
4	F	29	HIV	Yes	No	Sub-Optimal Course	Deceased
5	F	63	Diabetes	Yes	No	Sub-Optimal Course	Deceased
6	F	36	HIV	No	No	Sub-Optimal Course	Deceased

Figure 2: Antiretroviral Compliance

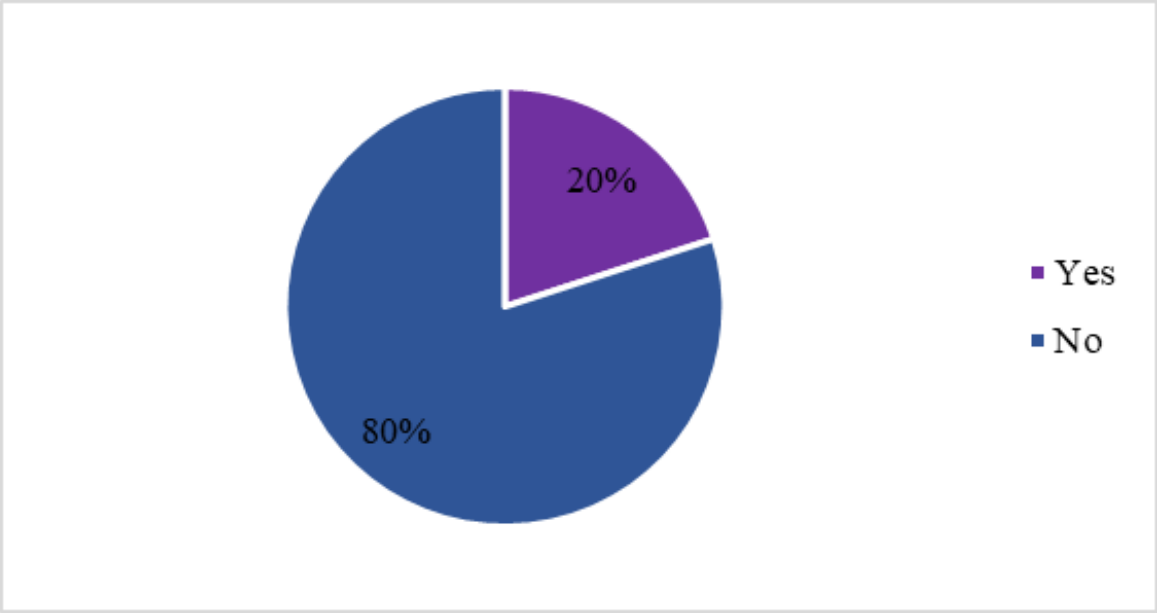


Table 2: CD4 Counts and Viral Loads of HIV-Positive Patients with Cryptococcaemia

Patient No.	+CD4 Count	++Viral Load (copies/ml)
1	63	28
2	10	*
3	11	2, 516,069
4	**	**
5	N/A	N/A
6	99	671,445

+ CD4 counts (via Flow Cytometry) at the time of culture; ++viral loads (via qPCR) within 35 days of sample collection; * not available at the time of culture; **missing data; N/A- not applicable

One patient had diabetes mellitus while the remaining five were infected with HIV (Table 1). Greater than 70% of these HIV positive patients were non-compliant with anti-retroviral medication (Figure 2) and at least 40% had an associated viral load of >10,000 RNA copies/ml with low CD4 counts (Table 2).

Fifty percent (50%) of these patients were diagnosed with sepsis with two having had meningitis. At least two patients (33.3%) had a lower respiratory tract infection, but none were formally diagnosed with pulmonary cryptococcosis. Of note, only two of these HIV seropositive patients were diagnosed with suspected disseminated cryptococcal disease/ cryptococcaemia.

No information was available on potential environmental sources (e.g. pigeon droppings). All were treated with antifungals however one had completed the full course of therapy and was the only patient to survive. This patient was treated for underlying cryptococcal meningitis with amphotericin B and fluconazole.

Discussion:

The average age range for cryptococcal infections is 30 to 40 years old.[10] (10). There was a greater incidence in males than females. [11-12](11-12). We did not have a similar observation in our study, as the age range for the

study cohort in Jamaica was 28-63 years with an equivocal 50% affected males and 50% affected females. The incidence of cryptococcal cases have tended to be higher in the HIV positive or stable transplant group.[13] (13). In the U.S., while the frequency of cryptococcosis did not change in the last two decades, there is a decreasing trend of cryptococcal infections in HIV seropositive persons which can be attributed to the introduction of HAART. The consistency in frequency is owed to the increase in the HIV-negative/non-transplant patient cases. In other words, there are fewer stable transplant or HIV positive cryptococcal cases and more cases with neither. [13](13). Our observations suggest that cryptococcaemia still has prevalence in our HIV positive populations (83%) which may be a result of poor HAART compliance as previously reported. Non-compliance may stem from issues we face in Jamaica such as stigmatization/discrimination, poor health literacy, poor retention in care, and late or no access to treatment. As this was a retrospective study with only one case surviving treatment, it would be difficult to gather more information on the effect of the above listed factors. In the HIV negative population (16%), we observe a case of a patient with diabetes mellitus with no record of immunosuppressive therapies, underlying malignancy, or commonly associated comorbidities such as rheumatologic diseases. Patients with type two diabetes or obesity suffer alterations in their immune system that lead to dysregulation of innate and adaptive immunity. [14](14). The proliferation of T cells and macrophages is altered and the function of B and NK cells is impaired. These abnormalities in immunity may play a role in this patient's presentation. Interestingly, T cell defects (T cell defect, leukopenia) have been strongly associated and correlated with cryptococcal meningitis, which was suspected in this patient.[15] (15). As the number of chronic illnesses such as rheumatoid arthritis, continues to rise, and as we continue to rely more heavily on immunosuppressants for their treatment, this group will likely increase since there are no prophylactic guidelines.

Treatment consists of induction therapy with 2 weeks of intravenous amphotericin B (AmB) and flucytosine, followed by fluconazole for consolidation (8 weeks) and maintenance therapy (≥ 1 year) [16-17](16-17). Flucytosine is often unavailable in disease burdened areas, and issues with cost and the side effect profile of the drug have limited its use in resource-limited settings, as such it is not commonly used in our setting. Fluconazole however is readily available and associated with much fewer adverse events. It penetrates the CSF well and this along with the fact that it is affordable make it a suitable alternative to flucytosine for use in combination with amphotericin B and this combination has been put forward in reputable guidelines.[18] (18). In our cohort evidence of induction therapy was noted as previously stated (16%). Locally, patients may alternatively be given Fluconazole 1200 mg monotherapy, as Amphotericin B may also be unavailable, but this has been shown to be ineffective.[18] (18). This is further supported by the data in our study where fifty percent of patients treated with monotherapy yielded poor outcomes.

Primary prophylaxis is not widely recommended in preventing cryptococcal infections. Factors such as the uncommon nature of cryptococcal disease incidences, limited effect of prophylaxis on overall mortality, possibility of antimicrobial resistance, likelihood of noncompliance are often cited in favour of this recommendation. [18] (18). However, in patients who are HIV positive with CD4 counts below counts <50 cells/μL, it has been demonstrated that itraconazole and fluconazole when used is able to decrease the occurrence of cryptococcosis and itraconazole have been shown to reduce frequency of primary cryptococcal disease in places such as sub-Saharan Africa[19-20] (19-20).

Limited availability of HAART, prevalent HIV-drug resistance, and high incidences and burden of cryptococcal disease, favors the recommendation of cryptococcal prophylaxis. Prophylaxis in conjunction with serum cryptococcal antigen screening in patients with CD4 count <100 prior to initiation of ART has proven to be beneficial as it is highly effective for identifying those at risk of cryptococcal meningitis and death and thus allows for targeted preventative treatment .[21] (21).

The Caribbean is second only to sub Saharansub–Saharan Africa in HIV disease burden and prevalence. In Jamaica alone, HIV is the 4th leading cause of death amongst individuals aged 15-49 years old. Despite this, our prophylactic guidelines mirror that of the USA, therefore none of our patients receive cryptococcal antigen (CrAg) screening or prophylaxis prior to initiation of ART. Of note, antifungal prophylaxis is recommended even in the absence of CrAg screening provided CD4 counts are appropriately low. [22] (22) . Though Jamaica is not burdened per se with

cryptococcaemia cases, it is not an uncommon occurrence either. Additionally, the UHWI, Mona is the only fungi laboratory of its kind in the island which suggests that a greater number of cryptococcaemia cases or cryptococcal-related deaths in both HIV seropositive and seronegative cases may largely be unaccounted for. This is a possible limitation of this study. The small sample size of positive patients for whom some clinical data could not be accessed further limits the available information that can be analyzed and perhaps a 10 year10-year review is likely to provide more information and can be contemplated for future studies. Regardless, given the high mortality rate of cryptococcaemia patients, the pervasiveness of HIV and its role as a major risk factor for cryptococcaemia it would perhaps serve us well to consider adopting similar prophylactic protocols against cryptococcus. This would have to be optimized by addressing noncompliance and health literacy issues within the population. Regions that do not fall under this category may use fluconazole as secondary prophylaxis in patients with a history of cryptococcal meningitis (when CD4 count is <100/µl).[17] (17).

Conclusions:

HIV remains a major risk factor for cryptococcaemia. These research findings suggest that there may be a need to have a high index of suspicion for cryptococcosis in these patients as >50% did not show signs of typical underlying cryptococcal infections, particularly meningitis. The mortality rate was high in these patients, which would suggest that,that further studies may be needed in order to determine whether the presence of cryptococcaemia is a possible predictor for a poor outcome.

Acknowledgements: We would like to acknowledge Steven Stone for assistance with formatting and Jenene Cameron for assistance with data collection.

Conflicts of Interest: There are no competing interests to disclose. No significant funding was received for this research.

Ethical Considerations: Ethical approval was obtained from the UWI Mona Campus Research Ethics Committee.

Author Contributions: Camille-Ann Thoms-Rodriguez – conceptualized the study, was involved in data analysis, drafting and finalizing the manuscript.

Kristen Facey Holligan – involved in data collection, data analysis and drafting of the manuscript

Jessica Edwards – involved in data collection, analysis and drafting of the manuscript.

Nicoy Downie – involved in data analysis/interpretation as well as drafting the manuscript

Alison Nicholson – revised the manuscript critically for intellectual content

Racquel Wright – involved in data collection and analysis

Orville Heslop – revised the manuscript critically for intellectual content

Glendee Reynolds-Campbell –assisted with data collection and revised the manuscript critically for intellectual content as Head of Mycology department at UHWI

All authors approved the final version of the manuscript and agree to be accountable for the work.

References

1. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans var. grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol.* 1999 37(3):838-40. doi: 10.1128/JCM.37.3.838-840.1999. PMID: 9986871; PMCID: PMC84578
2. Baddley JW, Schain DC, Gupte AA, Lodhi SA, Kayler LK, Frade JP, Lockhart SR, Chiller T, Bynon JS Jr, Bower WA. Transmission of *Cryptococcus neoformans* by Organ Transplantation. *Clin Infect Dis.* 2011 Feb 15;52(4):e94-8. doi: 10.1093/cid/ciq216. Epub 2011 Jan 10. PMID: 21220771.
3. Bicanic T and Harrison T. Cryptococcal Meningitis. *Brit Med Bull*. 2004; 72 (1):99–118, https://doi.org/10.1093/bmb/ldh043
4. Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ, Henderson H, Kauffman CA, Haas DW, Saccente M, Hamill RJ, Holloway MS, Warren RM, Dismukes WE. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis.* 2001 Sep 1;33 (5):690-9. doi: 10.1086/322597. Epub 2001 Jul 26. PMID: 11477526.

5. Casadevall A and Perfect J. Cryptococcus neoformans. ASM Press. 1998; 37 (5):371

6. Kwon-Chung KJ, Rhodes JC. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. *Infect Immun.* 1986;51(1):218-23. doi: 10.1128/IAI.51.1.218-223.1986. PMID: 3079732; PMCID: PMC261090..

7. Brueske CH. Proteolytic activity of a clinical isolate of *Cryptococcus neoformans*. *J Clin Microbiol.* 1986 Mar;23(3):631-3. doi: 10.1128/JCM.23.3.631-633.1986. PMID: 3514663; PMCID: PMC268711.

8. Granger D, Perfect J and Durak D. Virulence of *Cryptococcus neoformans*. Regulation of capsule synthesis by carbon dioxide. *J Clin Invest.* 1985;76(2):508-516. https://doi.org/10.1172/JCI112000.

9. Subramanian S and Mathai D. Clinical Manifestations and Management of Cryptococcal Infection. *J Postgrad Med* 2005; 51(50:21-26

10. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis.* 1995 Jul;21(1):28-34; discussion 35-6. doi: 10.1093/clinids/21.1.28. PMID: 7578756.

11. Mitchell DH, Sorrell TC, Allworth AM, Heath CH, McGregor AR, Papanaoum K, Richards MJ, Gottlieb T. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis*. 1995 Mar;20(3):611-6. doi: 10.1093/clinids/20.3.611. PMID: 7756484.

12. Shih CC, Chen YC, Chang SC, Luh KT, Hsieh WC. Cryptococcal meningitis in non-HIV-infected patients, *QJM: Int. J. Med.* 2000; 93(4):245–251, https://doi.org/10.1093/qjmed/93.4.245.

13. Bratton EW, El Husseini N, Chastain CA, Lee MS, Poole C, et al. . Comparison and Temporal Trends of Three Groups with Cryptococcosis: HIV-Infected, Solid Organ Transplant, and HIV-Negative/Non-Transplant. *PLoS One.* 2012; 7(8).

14. Zhou TH, Hu Z, Yang S, Sun L, Yu Z, and Wang G. Role of Adaptive and Innate Immunity in Type 2 Diabetes Mellitus. *J. Diabetes Res.* 2018.

15. Zonios DI, Falloon J, Huang CY, Chaitt D, Bennett JE. Cryptococcosis and idiopathic CD4 lymphocytopenia. *Medicine (Baltimore).* 2007; 86(2):78-92. doi: 10.1097/md.0b013e31803b52f5. PMID: 17435588..

16. Jackson A, Van Der Horst C. New Insights in the Prevention, Diagnosis, and Treatment of Cryptococcal Meningitis. *Curr HIV/AIDS Rep.* 2012 Sep;9(3):267-77. doi: 10.1007/s11904-012-0127-7. PMID: 22763808.

17. Kaplan JE, Benson C, et al. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-infected Adults and Adolescents: Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *CDC*, Atlanta, Georgia, 2009.

18. Perfect JR, Dismukes WE., et al. Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2010; 50(3):291-322.

19. Powderly WG, Finkelstein D, Feinberg J, Frame P, He W, van der Horst C, Koletar SL, Eyster ME, Carey J, Waskin H, et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. NIAID AIDS Clinical Trials Group. *N Engl J Med.* 1995; 16;332(11):700-5. doi: 10.1056/NEJM199503163321102. PMID: 7854376.

20. McKinsey DS, Joseph WL., et al. Itraconazole Prophylaxis for Fungal Infections in Patients with Advanced Human Immunodeficiency Virus Infection: Randomized, Placebo-Controlled, Double-Blind Study. *Clin Infect Dis.* 1999; 28(5):1049-1056.

21. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis.* 2009; 1;48 (7):856-62. doi: 10.1086/597262. PMID: 19222372; PMCID: PMC2875173.Jarvis JN, Lawn SD, Vogt M, et al. Screening for Cryptococcal Antigenemia in Patients Accessing an Antiretroviral Treatment Program in South Africa. *Clin Infect Dis.* 2009 Apr 1;48(7):856-62. doi: 10.1086/597262. PMID: 19222372; PMCID: PMC2875173.

22. Awotiwon AA, Johnson S, Rutherford GW, Meintjes G, Eshun-Wilson I. Primary antifungal prophylaxis for

cryptococcal disease in HIV-positive people. *Cochrane Database Syst Rev.* 2018;8(8):CD004773. Published 2018 Aug 29. doi:10.1002/14651858.CD004773.pub3.