

Short Research Article

Cryptococcosis in HIV – infected hospitalized patients in Latvia.

ABSTRACT

Aims: to determine the prevalence of cryptococcal infection among HIV hospitalized patients, to evaluate clinical characteristics and outcomes in Latvia.

Study design: Prospective study.

Place and Duration of Study: Riga Eastern Clinical University Hospital, Latvian Center of Infectology, between January 2014 and February 2017.

Methodology: We conducted the study reporting demographics, epidemiological (age, sex, clinical aspects, paraclinical results (cryptococcal antigen in cerebrospinal fluid, serum, urine, cryptococcal DNA, HIV RNA and lymphocyte T CD4+ count), treatment and outcome aspects. We included 69 patients (71% men, 29% women) with HIV infection and cryptococcosis.

Results: 69 cases of cryptococcosis were confirmed for 699 HIV infected hospitalized patients tested, giving a prevalence of 9,9 %. 38 % (n=26/69) of patients were with clinical signs of infection with the central nervous system involvement, 19% (13/69) patients had pulmonary involvement. Other 43 % (n=30/69) of patients had disseminated non-CNS disease (elevated serum cryptococcal Ag or DNA). Most patients had advanced HIV disease (Median lymphocyte T CD4+ count=48,5 cells/uL, (1-1041), the average was 112,9 cells/ uL (SD 184.98). 87 % (n=59/68) of patients had lymphocyte T CD4+ cell count < 200cells/μL Only 25 % (n=14) of the patients known to have HIV infection (n=56/69) were receiving antiretroviral therapy at the time of presentation. Overall mortality rate was 25% (n=41/69).

Conclusion: prevalence of cryptococcal antigenemia was 9,9 %, indicating that the prevalence of cryptococcal infection among HIV patients in Latvia may be high enough to consider targeted screening. HIV positive patients have high mortality (35%) following cryptococcal infection which persists beyond their initial hospitalization. Follow-up studies of late mortality would be beneficial.

Comment [WU1]: Your sample size was 699; so rephrase you abstract 'we included 69 patients'

Comment [WU2]: confirm statistic please

Comment [WU3]: Edit

Keywords: *Cryptococcal infection; cryptococcal antigenemia; invasive fungal infection; HIV; AIDS.*

1. INTRODUCTION

Cryptococcus neoformans infection is a systemic invasive fungal infection (IFI) and is seldom among people who have healthy immune system. However, *C. neoformans* is a major cause of illness in people living with Human Immunodeficiency Virus (HIV), with an estimated 220,000 cases of cryptococcal meningitis occurring among people with HIV worldwide each year, resulting in nearly 181,000 deaths [1, 2]. Before antiretroviral therapy (ART) was discovered, fungal and other opportunistic infections were an essential problem for people with advanced HIV/AIDS. Since then, the numbers of fungal infections and deaths due to fungal infections in people with advanced HIV/AIDS have decreased significantly in the developed countries [3, 4]. Although the widespread availability of ART in developed countries has helped improve the immune system of many HIV patients so that they don't become vulnerable to infection with *Cryptococcus*. Cryptococcal meningitis is still a great problem in resource-limited countries where HIV prevalence is high and access to healthcare is limited. Most cryptococcal meningitis cases occur in sub-Saharan Africa (estimated in 2014 in sub-Saharan Africa 162,500 cases, 43,200 cases in Asia and Pacific, 9,700 cases in North/South America and Caribbean, 3,300 cases in North Africa and Middle East) [5]. However, fungal diseases, especially cryptococcosis, are still a concern for people living with HIV in Europe, for instance, in 2014 4,400 cases were estimated [5] despite the widespread availability in Europe of ART.

Comment [WU4]: edit

30 Mainstay therapy includes an induction phase with amphotericin B (Amb), either the lipid or deoxycholate formulation,
31 combined with flucytosine (5-FC), followed by the consolidation and subsequent maintenance phases, where higher and
32 lower doses of fluconazole are used. Lipid soluble formulations of Amb are preferred over deoxycholate Amb due to their
33 better tolerability and lower nephrotoxicity. However, cost and availability of lipid formulations of Amb and 5-FC are major
34 limitations in resource-limited settings [6].

35 Screening of cryptococcal antigenemia in patients at risk allows early identification of asymptomatic cases. Cryptococcal
36 antigenemia in the absence of meningitis can represent early-stage cryptococcosis during which antifungal treatment
37 might improve outcomes. However, patients without meningitis are seldom tested for cryptococcal infection [7].
38

39 1.1 Objective

40 A prospective analysis of patients with HIV and cryptococcal infection was conducted to evaluate clinical characteristics
41 and outcomes in Latvia. Until today, there was no research in Latvia about prevalence of cryptococcal infection and
42 associated factors, characteristics of infection among HIV adults hospitalized patients.
43

44 2. MATERIAL AND METHODS

45 2.1 Study population

46 We conducted a prospective study of hospitalized patients older than 18 years with HIV infection and cryptococcosis of a
47 3-year period (from January 1, 2014 to February 1, 2017) at Riga Eastern Clinical University Hospital, Latvian Center of
48 Infectology (LCI) located in the capital city of Latvia - Riga.
49

50 An anonymity number was given to each patient to preserve the confidentiality. Personal data from participant and all
51 diagnostic results was kept strictly confidential.

52 IFIs were classified according the European Organisation for Research and Treatment of Cancer/Invasive Fungal
53 Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group
54 (EORTC/MSG) criteria [8]. The diagnosis of cryptococcosis was made according to EORTC/MSG criteria; additionally we
55 used EQUAL Cryptococcus Score 2018: A European Confederation of Medical Mycology Score Derived from Current
56 Guidelines to Measure QUALity of Clinical Cryptococcosis Management [6].

Comment [WU5]: Full meaning first, previous
abbreviation is IFI

57 2.2 Data Collection

58 Data included demographics, epidemiological (age, sex, clinical aspects, paraclinical results (cryptococcal antigen (CrAG)
59 in cerebrospinal fluid (CSF), serum, urine, cryptococcal DNA in serum and CSF, HIV RNA and Lymphocytes T CD4+ (LT
60 CD4+) count), treatment aspects were collected from the patient folders and LCI hospital HIV database.
61

Comment [WU6]: Ful meaning

62 Death date was obtained from the LCI hospital HIV database and country system's Medical Informatics database and
63 Population Register.

64 The mycological diagnosis of cryptococcosis was done in the Eastern Clinical University Hospital, LCI reference
65 laboratory. CrAG was detected using a latex agglutination test (PASTOREXTM CRYPTO PLUS) following the
66 manufacturer's instructions. CSF and urine was examined for research of antigen with the same technique. Cryptococcus
67 DNA was detected with polymerase chain reaction.

Comment [WU7]: Provide manufacturers name

68 2.3. Statistical Analysis

69 Statistical analysis was performed using SPSS.16 (California, USA, 2007) [9]. Means, medians and frequencies (%) were
70 used to describe patients' characteristics. Fisher's exact test was used to compare categorical variables where
71 appropriate. A p-value ≤ 0.05 was chosen as a level of statistical significance.

72 2.4 Country's Profile

73 According to the Latvian Central Statistical Administration data in 2018, the Latvia total population was estimated to be 1
74 million 934 thousands, with 54 % females. Of the general population, 15.8 % were younger than 15 years and 20.1 %

were above 65 years of age [10]. According to the Centre for Disease Prevention and Control of Latvia report, in 2018, Latvia had 326 new HIV infections including 89 AIDS-related deaths. There were 7669 people living with HIV on January 1, 2019, including 2036 in AIDS stage and 1121 AIDS-related death. An estimated number of children infected with HIV was 80 due to mother-to-child transmission in January 2019, and 5 in 2018 [11]. Also in 2017 Latvia reported the highest number of new HIV cases in the EU and European Economic Area (EEA). The HIV/AIDS Surveillance Report of the World Health Organization and the European Center for Disease Prevention and Control (ECDC) about the situation in Europe 2018 (2017 data) suggests that last year there were 18.8 new HIV cases per 100,000 residents in Latvia, which is higher than average of 6.2 cases in other countries [12,13].

Comment [WU8]: State the number of persons

Comment [WU9]: What does your study seek to achieve

3. RESULTS AND DISCUSSION

3.1 Patient characteristics

During the study period, a total of 699 patients infected by HIV were admitted in the hospital. A CrAG test in serum and urine and DNA assay in serum was performed for all patients. Of 69 hospitalized HIV positive adult patients all were diagnosed positive Cryptococcus Ag or DNA, meaning a prevalence of 9,9%. They were included in this study. The mean follow-up was 24.6 months (SD 24, 63; Median 16, 5 (0-62)).

Comment [WU10]: But your sample size is 699

Comment [WU11]: Is this different from the general study?. Differentiate between the two in your methodology

The different characteristics of our study population and groups of patients depending on the result of antigenemia are described (Table 1).

Table 1. Characteristics of hospitalized HIV patients screened for cryptococcal antigen and DNA in Riga, Latvia.

Characteristics	Cryptococcal Ag Positive Total n (%)
Gender: n (%)	
Male	49 (71)
Female	20 (29)
Job: n (%)	
Work	16 (23,19)
Disability	2 (2,90)
Not working	50 (72,46)
Prisoner	1 (1,45)
Mean age (SD)	38,2 (8,18)
Min	23
Max	57
Age groups: n (%)	
15-30	11 (16)
31-45	44 (64)
>45	14 (20)
HIV serotype: n (%)	
HIV -1	69 (100)
HIV 2	00
LT CD4+ (cells/mm ³): n (%)	
0-199	59 (87)
200-499	6 (9)
>500	3 (4)
Unspecified	1
Mean HIV RNS (cop/ml) (SD)	671520,7 (1682950)
Median	55950
Min	40,8
Max	9800000
Antiretroviral therapy n (%):	
Yes	14 (20)
No	43 (62)

Discontinued due to the lack of compliance	12 (18)
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95 **3.1.1 Epidemiological aspects**

96 Of the 69 HIV-seropositive patients who were included in this study, the majority were male (n= 49/69, 71%). The
 97 predominance of adult patients was noted in our study population. The average age was 38, 2 years and patients ranged
 98 from 23 to 57 years. There were 57/69 patients with information on a possible HIV transmission route. 72% (n=41/57)
 99 were intravenous drug users.

Comment [WU12]: Not clear;what do you mean?

100 **3.1.2 Clinical aspects**

101 More patients (81%) were known to have HIV infection at the time of their presentation, average 6, 2 years (SD 4, 98; min
 102 – less than one year; max – 16 years). The remaining 19% (n=13/69) of patients had HIV infection diagnosed during this
 103 hospitalization. 59 % (n=41/69) of patients virus hepatitis were the associated underlying diseases. 40/41 of patients were
 104 hepatitis C virus co-infected, 1/41 - was hepatitis B virus co-infected. 38 % (n=26/69) of patients were with clinical signs of
 105 infection with the central nervous system (CNS) involvement, and 4 of them were CrAG in serum negative. 19%
 106 (n=13/69) patients have pulmonary involvement. Only two of them were CrAG in serum positive, others were CrAG in
 107 serum negative (respectively had localized pulmonary disease). Other 43 % (n=30/69) of patients had disseminated non-
 108 CNS disease (elevated serum CrAG or DNA). The prevalence of neuromeningeal signs was 22 % (n=15/69). They
 109 predominated in patients with positive cryptococcal antigenemia. 13% (n=9/69) of patients had concurrent final diagnoses.
 110 Final diagnoses included Pneumocystis jirovecii pneumonia (n=7/9) and Pneumocystis jirovecii colonization (n=2/9). 54 %
 111 (n=37/68) patients with medical charts available did not have evidence of any OI. Other 46% of patients (n=31/68) had
 112 past OI, half of them (n=15/31) had tuberculosis (TBC). 20% (n=14/69) of patients had a prior hospitalization coded for
 113 cryptococcal disease.

Comment [WU13]: Tables can help

114 **3.1.3. Paraclinical aspects**

115 HIV-1, the predominant serotype, was found in all of cases. In none of samples Cryptococcus neoformans was detected
 116 by fungal culture. CrAG screening in serum was performed in all 69 patients. In 32% (n=22/69) of cases CrAG was
 117 negative, including 4 patients with CNS involvement and CrAG positive in CSF, 11 patients with pulmonary disease, and 7
 118 patients with positive Cryptococcus DNA in serum. A lumbar puncture (LP) was performed in individuals who had CNS
 119 symptoms (headache, neck stiffness, confusion, ataxia, vomiting, photophobia), and if the patient had focal neurological
 120 symptoms. LP was performed in 46% (n=32/69) of patients. CrAG or DNA screening in the CSF was positive in 26 cases.
 121 90% CSF characterised with lymphocytic pleocytosis with the average 60, 5 cells/μL (SD 99, 58; Median 19, 5 (2-373)).
 122 Patients with pulmonary involvement (infiltrates or nodule on imaging, respiratory symptoms) underwent diagnostic
 123 bronchoscopy (n=18/69). 13 patients' bronchoalveolar lavage fluid (BAL) samples were positive in 'Pastorex Crypto-Plus'
 124 test (Bio-Rad). CrAG screening in urine was performed in all 69 patients. 7% (n=5/69) of patients had positive CrAG in
 125 urine. LT CD4 count was available for 68/69 participants. Most patients had advanced HIV disease (Median LT CD4+
 126 count=48, 5 cells/uL, (1-1041) .The average LT CD4 + was 112, 9 cells/ uL (SD 184.98) 87 % (n=59/68) of patients had
 127 CD4+ cell count < 200cells/μL. Mean Log viral load was 5.

Comment [WU14]: You may use tables

128 **3.2 Therapeutic and outcome aspects**

129 Despite that most patients had advanced HIV disease, only 25 % (n=14) of the patients known to have HIV infection
 130 (n=56/69) were receiving antiretroviral therapy at the time of presentation. Antifungal therapy of cryptococcosis with
 131 Fluconazole (800mg/day per os or intravenous) was administered to all 69 cases during hospitalization. And 22%
 132 (n=15/69) received combination therapy with Lipid soluble formulations of Amb due to severe illness. 20% (n=14/69) of
 133 patients in additions received corticosteroids.

134 Overall mortality rate was 59% (n=41/69). 37% died within 90 days of cryptococcal diagnosis (early mortality), and 63 %
 135 died after 90 days (late mortality). In hospital mortality rate was 25% (n=17/69), with mean days at hospital – 16, 1 days
 136 (SD 8, 72). 41 % (n=7/17) died in Intensive Care Unit. After hospitalization mortality rate was 35% (n=24/69).

137 In 88% (n=15/17) patients who died in hospital, the cause of death was IFI – cryptococcosis. In 12% (n=2/17) the cause of
 138 death was other reasons (1- renal insufficiency glomerulonephritis due, 1- hepatic insufficiency).The cause of death after
 139 hospitalization was IFI (including cryptococcosis) in 21% (n=5/24) of cases. In 46% (n=11/24) of cases the cause of death
 140 were other reasons, from them 4 cases – TBC,3- hepatic insufficiency,2 – bacterial meningoencefalitis , 2 – lymphoma.
 141 And in the remaining 33% (n=8/24) the cause of death was unknown.

142
143 **4. DISCUSSION**
144

145 Advanced HIV disease remains an essential challenge. Despite major progress over the last decade in expanding access
146 to ART and reducing HIV-related deaths, up to half of people living with HIV present to care with advanced disease, and
147 many continue to die from HIV-related opportunistic infections. We need to find better ways to identify and manage
148 advanced HIV disease, in order to reach the global goal to reduce HIV deaths by 50% by 2020. Cryptococcal meningitis is
149 a serious opportunistic infection and a major cause of morbidity and mortality among HIV positive people with advanced
150 disease. Most people dying of cryptococcal meningitis live in low-income countries. Often people are not diagnosed early
151 enough because rapid diagnostic tests and lumbar puncture are unavailable. The first-line antifungal drugs that are used
152 for treatment are costly and often not available to save the lives of people infected with cryptococcal meningitis. In Europe
153 important death factors are antifungal drug toxicity, intracranial pressure and immune raised reconstitution inflammatory
154 syndrome [14].

155
156 In this prospective study we report a prevalence rate 9, 9 % of cryptococcosis among hospitalized patients with advanced
157 HIV infection. Other published research data, shows a different situation to Latvia in other European countries. For
158 instance, in Germany Cryptococcal antigenaemia was found in 1, 6% of patients with LT CD4 <100 cells/uL [15]. The data
159 about the burden of cryptococcal disease in the Baltic States is scarce.
160

161 According to the Centre for Disease Prevention and Control of Latvia report, in 2018, Latvia had 326 new HIV infections,
162 including 99 AIDS stage and 89 AIDS-related deaths [10]. According to these findings, one quarter of individuals were
163 recently diagnosed at such advanced stage. Late HIV diagnosis remains a problem in Latvia. This could be one of the
164 reasons why the disease is more common. In our study 19% of patients had HIV infection diagnosed during the
165 hospitalization with an existing opportunistic AIDS disease – cryptococcosis.
166

167 Although access to HIV diagnosis, and immediate start to ART (regardless of the stage or LT CD4 counts) constitutes a
168 major medical advancement in the clinical management of HIV in Latvia, its success depends on strict adherence to
169 prescribed regimen. 18 % of our patients with cryptococcosis were on ART, with poor adherence to medical treatment.
170

171 In our study in 43% of cases occurs occult cryptococcal antigenemia among hospitalized HIV-seropositive patients. Health
172 care providers should evaluate HIV-infected patients for cryptococcal antigenemia, even in the absence of meningitis.
173 Based on World Health Organization and European AIDS clinical society guidelines, it is recommended to screen all HIV-
174 infected people with LT CD4 <100 cells/uL for CrAg, and based on World Health Organization guidelines it may be
175 considered at a higher LT CD4 cell count threshold of <200 cells/uL [16,17]. Routine screening might identify
176 asymptomatic meningitis, too. Symptom-based diagnosis is not a reliable predictor of the central nervous system
177 involvement [18].

Comment [WU15]: You have not discuss all your findings e.g Therapeutic and outcome aspects

178
179 **5. CONCLUSION**
180

181 Cryptococcal disease often has an insidious presentation and can be difficult to recognize. However, delayed diagnosis
182 can lead to increased morbidity and mortality. In our study the prevalence of cryptococcal antigenemia was 9, 9 %,
183 indicating that the prevalence of cryptococcal infection among HIV patients in Latvia may be high enough to consider
184 targeted screening.

185 HIV+ patients have high mortality (35%) following cryptococcal infection which persists beyond their initial hospitalization.
186 Identifying patients at higher risk for mortality is critical for successful treatment and outcomes. Follow-up studies of late
187 mortality would be beneficial.

188 Our research data again highlights that there is a need for a broader body of society, educating the public to discover HIV
189 infection more quickly and reduce the number of patients at high risk of opportunistic infection (OI) developing; already
190 known HIV positive patients should improve their adherence. This will improve the health and survival of a particular
191 individual and reduce the public's financial costs of OI treating.
192

193 **COMPETING INTERESTS**

194
195 The authors have declared that no competing interests exist.
196
197

198 **CONSENT**

199
200 All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication
201 of this case report and accompanying images. A copy of the written consent is available for review by the Editorial
202 office/Chief Editor/Editorial Board members of this journal.
203

204 **ETHICAL APPROVAL**

205
206 All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee
207 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of
208 Helsinki.
209

210 **REFERENCES**

211
212 1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-
213 associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis.* 2017; 2017 Aug; 17(8):873-881. DOI:
214 10.1016/S1473-3099(17)30243-8.

215 2. Centers for Disease Control and Prevention [homepage on the Internet]. C.neoformans infection statistics.
216 Available: <https://www.cdc.gov/fungal/diseases/cryptococcosis-neoformans/statistics.html>. (Page last reviewed: October
217 9, 2018).

218 3. Mirza SA, Phelan M, Rimland D, Graviss E, Hamill R, Brandt ME, et al. The changing epidemiology of
219 cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000. *Clin Infect*
220 *Dis.* 2003 Mar 15;36(6):789-94. DOI: 10.1086/368091. PMID: 12627365.

221 4. Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, et al. Epidemiology of human
222 immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral
223 therapy. *Clin Infect Dis.* 2000 Apr;30 Suppl 1:S5-14. DOI: 10.1086/313843. PMID: 10770911.

224 5. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM et al. Global burden of disease of HIV-
225 associated cryptococcal meningitis : an updated analysis. *Lancet Infect Dis.* 2017 Aug;17(8):873-881. DOI:
226 10.1016/S1473-3099(17)30243-8. PMID: 28483415 PMCID: PMC5818156.

227 6. Andrej Spec, Carlos Mejia-Chew, William G Powderly, Oliver A Cornely. EQUAL Cryptococcus Score 2018: A
228 European Confederation of Medical Mycology Score Derived From Current Guidelines to Measure QUALity of Clinical
229 Cryptococcosis Management. *Open Forum Infect Dis.* 2018 Nov; 5(11): ofy299. DOI:10.1093/ofid/ofy299.

230 7. Joseph N Jarvis, Stephen D Lawn, Monica Vogt, Nonzwakazi Bangani, Robin Wood, Thomas S Harrison.
231 Screening for Cryptococcal Antigenaemia in Patients Accessing an Antiretroviral Treatment Program in South Africa. *Clin*
232 *Infect Dis.* 2009 Apr 1; 48(7): 856-862. DOI: 10.1086/597262.

233 8. De Pauw B., Walsh T.J., Donnelly J.P., Stevens D.A., Edwards J.E., Calandra T., Pappas P.G., Maertens J.,
234 Lortholary O., Kauffman C.A., et al. Revised definitions of invasive fungal disease from the european organization for
235 research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and
236 infectious diseases mycoses study group (EORTC/MSG) Consensus Group. *Clin Infect Dis.* 2008 Jun 15;46(12):1813-21.
237 DOI: 10.1086/588660. PMID: 18462102 PMCID: PMC2671227.

238 9. SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc. Available: [http://www-](http://www-01.ibm.com/support/docview.wss?uid=swg21476197)
239 [01.ibm.com/support/docview.wss?uid=swg21476197](http://www-01.ibm.com/support/docview.wss?uid=swg21476197).

240 10. Latvian Central Statistical Administration. Available: www.csb.gov.lv.

241 11. Centre for Disease Prevention and Control of Latvia. Available: www.spkc.gov.lv.

- 242 12. World Health Organization [homepage on the Internet]. Geneva: World Health Organization. HIV/ AIDS
243 Surveillance in Europe 2018. Available: [http://www.euro.who.int/en/health-topics/communicable-](http://www.euro.who.int/en/health-topics/communicable-diseases/hivaids/publications/2018/hivaids-surveillance-in-europe-2018-2018)
244 [diseases/hivaids/publications/2018/hivaids-surveillance-in-europe-2018-2018](http://www.euro.who.int/en/health-topics/communicable-diseases/hivaids/publications/2018/hivaids-surveillance-in-europe-2018-2018).
- 245 13. European Center for Disease Prevention and Control [homepage on the Internet]. HIV/ AIDS Surveillance in
246 Europe 2018. 2018, European Center for Disease Prevention and Control (ECDC).2018 Nov 28. Available:
247 <https://ecdc.europa.eu/en/publications-data/hivaids-surveillance-europe-2018-2017-data>.
- 248 14. World Health Organization [homepage on the Internet]. World Health Organization. Cryptococcal disease: what's
249 new and important. 2018 March. Available: [https://www.who.int/hiv/mediacentre/news/cryptococcal-disease-key-](https://www.who.int/hiv/mediacentre/news/cryptococcal-disease-key-messages/en/)
250 [messages/en/](https://www.who.int/hiv/mediacentre/news/cryptococcal-disease-key-messages/en/)
- 251 15. Katchanov J, Jefferys L, Tominski D, Wöstmann K, Slevogt H, Arastéh K, Stocker H . Cryptococcosis in HIV-
252 infected hospitalized patients in Germany: Evidence for routine antigen testing. J Infect. 2015 Jul;71(1):110-6. DOI:
253 [10.1016/j.jinf.2015.01.011](https://doi.org/10.1016/j.jinf.2015.01.011). PMID: 25644318.
- 254 16. World Health Organization [homepage on the Internet]. World Health Organization. Guidelines on the diagnosis,
255 prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children.2018 March.
256 [Adobe Acrobat document,62p.]. Available: <https://www.who.int/hiv/pub/guidelines/cryptococcal-disease/en/>.
- 257 17. European AIDS Clinical Society [homepage on the Internet]. EACS. European AIDS Clinical Society .European
258 AIDS Clinical Society guidelines. 2018 October.[Adobe Acrobat document].Available:
259 <http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html>.
- 260 18. Williams DA, Kiiza T, Kwizera R, Kiggundu R, Velamakanni S, Meya DB, et al. Evaluation of Fingerstick
261 Cryptococcal Antigen Lateral Flow Assay in HIV-Infected Persons: A Diagnostic Accuracy Study. Clin Infect Dis. 2015;
262 61(3):464–7. DOI: [10.1093/cid/civ263](https://doi.org/10.1093/cid/civ263). PMID: 25838287 PMCID: PMC4503809.

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