

Prevalence of Cryptococcal Antigen and Outcomes in People With Human Immunodeficiency Virus in Honduras: A Cohort Study

Julio C. Zuniga-Moya,¹ Luis Enrique Romero-Reyes,² Emilio Barrueto Saavedra,² Sandra Montoya,³ Diana Varela,³ Mitchel Borjas,³ Alicia Cerna,³ Suyapa Bejarano,¹ Paola Martinez,¹ Karen Lujan,⁴ Karen Erazo,⁴ Isis Lainez,² Luisamaria Pineda,² David Yanes,¹ Jane A. O'Halloran,⁵ and Andrej Spec^{5,6}

¹Universidad Catolica de Honduras Campus San Pedro Sula Pablo, San Pedro Sula, Honduras, ²Hospital Dr. Mario Catarino Rivas, San Pedro Sula, Honduras, ³Hospital Escuela Universitario, Tegucigalpa, Honduras, ⁴Clinica de Servicios de Atencion Integral, Tegucigalpa, Honduras, ⁵Department of Medicine, Division of Infection, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA

Background. Cryptococcal meningitis is a major cause of death among people with human immunodeficiency virus (PWH). Cryptococcal antigen (CrAg) testing of asymptomatic patients is an important public health measure to reduce mortality in high-incidence areas. However, limited data exist on CrAg prevalence in Central America.

Methods. We conducted a prospective cohort study at the 2 largest human immunodeficiency virus (HIV) clinics and hospitals in Honduras. Cryptococcal antigen in serum and cerebrospinal fluid was performed in individuals with HIV who had CD4 ≤ 100 cells/mm³ between 2017 and 2018. After CrAg testing, individuals were observed for 12 months to assess mortality using adjusted Cox proportional hazard models.

Results. A total of 220 PWH were tested for CrAg, 12.7% (n = 28) of which tested positive. Cryptococcal antigen prevalence was higher among hospitalized individuals in 40% (n = 10 of 25) of the cases. The proportion (35.8%) of individuals taking *antiretroviral therapy* was significantly ($P < .01$) lower among those who tested positive for CrAg. Overall mortality among the cohort was 11.4% (n = 25 of 220) by 12 months. Cryptococcal antigen-positive cases were at a significantly higher risk of death (adjusted hazard ratio, 2.69; 95% confidence interval, 1.07–6.84) compared with CrAg-negative participants.

Conclusions. Cryptococcal antigen prevalence in Honduras was high among PWH. Moreover, individuals who tested positive for CrAg testing were at a higher risk of death. Systemic CrAg of PWH with a CD4 ≤ 100 cells/mm³ should be routinely performed in Central America.

Keywords. cryptococcal antigen; Honduras; Latin America; PWH.

Cryptococcal meningitis (CM) remains one of the leading causes of death among people with human immunodeficiency virus (PWH) worldwide, with as many as 15% of the deaths in PWH being attributed to CM [1]. With the development of the cryptococcal antigen (CrAg) lateral-flow assay, an inexpensive point-of-care test, diagnoses are now accessible in low-income countries [2–4], allowing clinicians to screen for asymptomatic antigenemia. This approach has been proven to be cost-effective [5] and to decrease the number of deaths attributed to CM [6].

Despite the availability of screening and diagnostic tools for cryptococcal antigenemia in low-income nations, little is known about cryptococcal antigenemia and its burden in Central American countries. Although recent evidence suggests high burden of CM in Latin America, Central America was not represented, because no studies exist from this region [1]. Efforts to address this gap have been mostly undertaken in South American countries [7–9] rather than in Central America or the Caribbean, where the burden of human immunodeficiency virus (HIV) is high [10] and the epidemic is worsening [11]. Honduras has recently reported high mortality rates associated with CM in individuals with HIV [12]. The burden of this diseases remains unknown in Honduras or any other part of Central America. The World Health Organization (WHO) recommends screening for CrAg in PWH with a CD4 ≤ 100 cells/mm³ [13].

To address a public health concern associated with cryptococcosis as an opportunistic infection in PWH in Central America, and to address the knowledge gap surrounding cryptococcal disease burden, we conducted a prospective cohort study to determine CrAg prevalence and outcomes associated at the 2 largest clinics serving PWH in Honduras.

Received 2 September 2020; editorial decision 3 November 2020; accepted 6 November 2020.

Correspondence: Julio C. Zuniga-Moya, MD, Universidad Catolica de Honduras Campus San Pedro, San Pablo, Blvd Mario C. Rivas 21101, San Pedro Sula, Honduras (jczm1991@gmail.com).

Open Forum Infectious Diseases® 2021

© The Author(s) 2020. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
DOI: 10.1093/ofid/ofaa557

METHODS

Participants

Between April 2017 and December 2018, we conducted a prospective cohort study at 2 outpatient HIV clinics in San Pedro Sula and Tegucigalpa, Honduras as well as at the inpatient Infectious Disease Units of Hospital “Escuela Universitario” and “Mario Catarino Rivas” in both cities. Medical records and laboratory reports were used to identify eligible participants. This study was nested on a URC-Centers for Disease Control and Prevention (CDC) mycotic infections surveillance on PWH. We included PWH with a $CD4 \leq 100$ cells/mm³ who presented to either HIV clinic or the inpatient units during the study period regardless of age. Participants were excluded if they had a previous diagnosis of cryptococcosis. All participants who complied with the study criteria and attended either the ambulatory clinic or hospitals were approached, and all who consented were included in a sequential manner.

Enrollment and Procedures

Using the HIV outpatient clinics records and laboratory reports, we were able to identify eligible individuals for CrAg screening. At enrollment, clinical and demographic data were collected using URC-CDC *Cryptococcosis* and *Histoplasmosis* [14] case surveillance forms. In addition, a single whole blood sample was collected on each participant to test for CrAg using the Lateral Flow Assay (IMMY, Norman, OK) in serum. Participants with a positive serum CrAg were contacted 24 hours after the test was performed in serum and assessed for CM with a lumbar puncture (LP) to rule out CM. Individuals who presented with clinical findings that suggested central nervous system (CNS) compromise such as seizures, headache, and stiffness of neck had a cerebrospinal fluid (CSF) CrAg performed only.

Due to the lack of availability of flucytosine in Honduras, participants with positive CSF CrAg received therapy with 0.5 mg/kg amphotericin B deoxycholate and 800 mg of intravenous fluconazole daily for 14 days followed by 400 mg of oral fluconazole for 8 weeks and 200 mg afterwards. When amphotericin B deoxycholate was not available, 1200 mg of fluconazole was given daily for 14 days. Individuals who tested positive for CrAg in serum and negative in CSF received ambulatory therapy with 800 mg of oral fluconazole daily for 2 weeks followed by 200 mg daily for an additional 8 weeks [13].

The follow-up period conducted by the research team was composed of 4 visits within 12 months, where clinical examination was performed to assess potential symptoms of cryptococcal disease or recurrence for those individuals who tested positive for CrAg. Participants with a negative serum CrAg underwent routine follow up by their HIV clinic physicians, and potential cryptococcal disease after clinical examination was reported to the research team. Mortality was confirmed during the 12-month follow-up period using national registries

and hospital registries at both HIV clinics to minimize loss to follow up.

Statistical Analysis

For the statistical analysis, the cohort was divided in CrAg-positive and CrAg-negative groups. Descriptive statistics were performed, and *P* values were calculated using Fisher's exact test, χ^2 test, or Wilcoxon rank-sum test as needed. To gauge risk of mortality after 12-month follow up, Cox proportional hazard models were used adjusting for age and *antiretroviral therapy* (ART). Data were analyzed using R version 3.6.1 (available at <https://www.R-project.org/>) using the package “survival”.

Patient Consent Statement

The institutional review boards at Universidad Catolica de Honduras and Hospital Escuela Universitario approved the research protocol. Written informed consent was obtained from PWH 18 years or older and from parents for children <12 years old; assent was obtained from children ≥ 12 years old, as per the guidance of the review boards.

RESULTS

Population Baseline Characteristics

We enrolled 195 individuals in the outpatient clinics (screening) and 25 at the inpatient infectious diseases' units in both hospitals (Figure 1). Overall, the median age was 38 years (interquartile range [IQR], 30–46 years), 64.7% (*n* = 140) were males, and 93.6% (*n* = 206) were from urban areas. The median CD4 was 46 cells/mm³ (IQR, 25–72 cells/mm³), and 67% (*n* = 147) were receiving ART at the time of antigen testing (Table 1). The median duration of time since HIV diagnosis was 21 months (IQR, 1–111 months) among the cohort.

Cryptococcal Antigen Prevalence

Of the 220 enrolled, 12.7% (*n* = 28) of the individuals had a positive CrAg in either the CSF or serum. Furthermore, among participants who attended the outpatient clinics, 9.2% (*n* = 18) tested positive for CrAg. Forty percent (*n* = 10) of the participants who were hospitalized tested positive for CrAg.

Overall, 9.2% (*n* = 18) of the outpatient participants tested positive for CrAg in serum. All participants who tested positive for CrAg in serum at baseline consented to be tested for CrAg in CSF through LP. Fifty percent (*n* = 9) of the participants who tested CrAg positive in serum also tested positive for CrAg in CSF after LP. Furthermore, of 25 individuals who were only tested in CSF due to their clinical manifestations compatible with CNS compromise, 64% (*n* = 16) tested negative.

Antiretroviral therapy receipt at baseline was significantly lower among individuals who tested positive for CrAg compared with those who tested negative (*P* = .03). Furthermore, the proportion of individuals taking fluconazole for *Candida* prophylaxis was significantly lower within those who tested

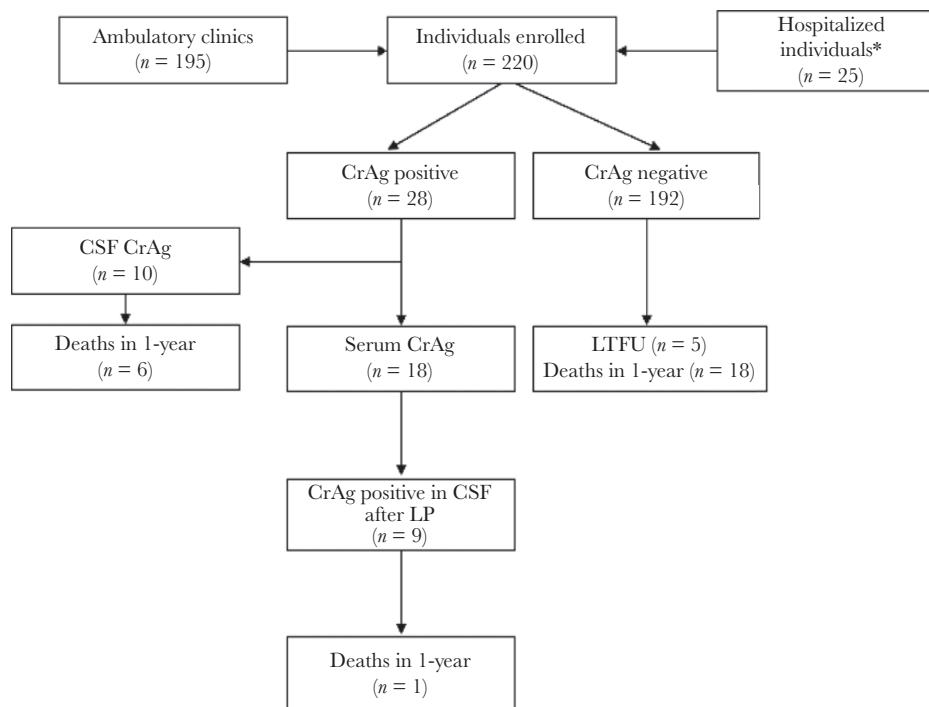


Figure 1. Flowchart of enrollment at human immunodeficiency virus clinics and hospitals in Honduras. *, Hospitalized individuals were only tested in cerebrospinal fluid (CSF). CrAg, cryptococcal antigen; LTFU, lost to follow-up.

positive for CrAg ($P = .04$) as was the time since diagnosis of HIV, which was significantly lower ($P < .01$) among those who tested positive for CrAg in the cohort (Table 1).

Clinical Features and Outcomes

Fever (64.5% of cases), weight loss (56.8% of cases), and headache (50.5% of cases) were the 3 most frequent clinical

features among the cohort. This finding was consistent in both CrAg-positive and CrAg-negative participants. Of all the participants, 9.1% ($n = 19$) had no signs or symptoms at baseline and thus were considered asymptomatic (Table 2). Thirty-six percent ($n = 9$ of 25) of the hospitalized individuals had clinical manifestations of CM that was confirmed with a CrAg-positive test in CSF. Median age of individuals

Table 1. Baseline Characteristics of Patients Enrolled for Cryptococcal Antigen Testing in Honduras

Baseline Characteristics	Total n = 220 (%)	CrAg Positive n = 28 (%)	CrAg Negative n = 192 (%)	P^a
Age years (median [IQR])	38 [30–45.5]	36 [28.5–46.5]	38 [31–45.3]	.86
Male sex	140 (63.7)	17 (60.7)	123 (64.0)	.83
Urban residence	206 (93.6)	25 (89.3)	181 (94.3)	.39
Taking ART	147 (66.8)	10 (35.8)	137 (71.4)	<.01
CD4 count cells/mm ³ (median [IQR])	46 [25–72]	40 [23.75–54]	48 [25–72]	.20
Time since HIV diagnosis in months (median [IQR])	21 [1–111]	1 [1–51]	36 [1–120]	<.01
History of previous mycosis	47 (21.4)	5 (17.9)	42 (21.9)	.80
Fluconazole prophylaxis	98 (44.5)	7 (25.0)	91 (47.4)	.04
Hemoglobin g/dL (median [IQR])	11.3 [9.5–12.72]	11.0 [8.85–12.7]	11.3 [9.50–12.7]	.52
Hospitalized participants	25 (11.4)	9 (32.1)	16 (8.3)	<.01

Abbreviations: ART, antiretroviral therapy; CrAg, cryptococcal antigen; HIV, human immunodeficiency virus; IQR, interquartile range.

^a χ^2 , Fisher's exact test, or Wilcoxon rank-sum test as appropriate to compare CrAg-positive and CrAg-negative groups.

Table 2. Baseline Clinical Features of 220 PWH in Honduras by CrAg Status

Total n = 220	CrAg Negative n = 192 (%)	CrAg Positive n = 28 (%)	P^a
Clinical Features			
Weight loss	118 (61.5)	17 (60.7)	.93
Fever	117 (60.9)	25 (89.3)	<.01
Headache	93 (48.4)	18 (64.3)	.11
Cough	78 (40.6)	10 (35.7)	.62
Diarrhea	74 (38.5)	7 (25.0)	.16
Oral lesions	56 (29.2)	9 (32.1)	.74
Nausea	54 (28.1)	17 (60.7)	<.01
Wasting syndrome	46 (23.9)	12 (42.9)	.03
Dyspnea	42 (21.9)	6 (21.4)	.95
Mental status impairment	27 (14.1)	11 (39.3)	<.01
Cranial nerve compromise	18 (9.4)	5 (17.9)	.09
Seizures	10 (5.2)	3 (10.7)	.25
Nape rigidity	6 (3.13)	7 (25.9)	<.01

Abbreviations: CrAg, cryptococcal antigen; PWH, people with human immunodeficiency virus.

^a χ^2 test/Fisher's exact test as appropriate.

with CM was 40 years (IQR, 33–43), and male sex was predominant with 66.7% ($n = 6$) of the cases. Furthermore, median CD4 was 40 cells/mm³ (IQR, 28–58 cells/mm³). Sixty-six percent ($n = 6$) of individuals with CM were taking ART at the time of the diagnosis.

Eleven percent ($n = 25$) of study participants died during the 12-month follow-up period. The proportion of deaths was significantly higher among those who tested CrAg positive (25% vs 9.4%; $P = .01$). Thirty-six percent ($n = 9$ of 25) of the overall deaths occurred during the first 90 days. Forty-two percent ($n = 3$) of the 7 overall deaths in the CrAg-positive group occurred during the first 90 days of follow up. Of 9 individuals who tested positive in serum but negative in CSF, no deaths were reported after a 12-month follow-up period. Furthermore, none of these individuals developed CM during the same follow-up period.

Among those individuals who received fluconazole-based therapy for CNS involvement, 57.1% (4 of 7) died during their hospitalization. In contrast, among individuals who received combined therapy with fluconazole and amphotericin B deoxycholate, 25.0% (3 of 12) died during their hospitalization.

Individuals who tested positive for CrAg were at a higher risk of death after adjusting for sex and use of ART at baseline (hazard ratio, 2.69; 95% confidence interval, 1.07–6.84) (Figure 2). Three percent ($n = 5$) of the participants in the CrAg-negative group were lost to follow up. Loss to follow up was not reported in the CrAg-positive group.

DISCUSSION

To our knowledge, this the first study to assess CrAg prevalence and outcomes in Central America, demonstrating a high prevalence of 12.7% among PWH whose CD4 was ≤ 100 cells/mm³. Although previous models have estimated that the burden of cryptococcal disease is high in Latin America [1], these models were built in absence of any data from Central America. This study allows for updates for the world estimates of cryptococcosis and informs the need for CrAg screening of PWH with a CD4 ≤ 100 cells/mm³ in Central America. Furthermore, this study describes the risk of death among individuals tested for CrAg during a 12-month follow-up period in an unexplored HIV cohort.

The overall prevalence of CrAg among the Honduran cohort was 12.7%. This finding is particularly high when compared with other African low-income settings where CrAg prevalence ranges from 4% to almost 10% [15–17]. Furthermore, when gauging the characteristics of those participants who tested positive for CrAg, our findings are comparable to reports elsewhere [15–17] where young males are more likely to test positive for CrAg.

Although little is known about the prevalence of cryptococcal antigenemia in Central American countries, some reports have estimated its prevalence in South American countries. Our findings suggest that cryptococcal antigenemia prevalence is potentially higher than most of the studies in South America [8, 9, 18]. However, testing among these cohorts was performed on individuals with CD4 < 200 cell/mm³ [7, 8], and thus this

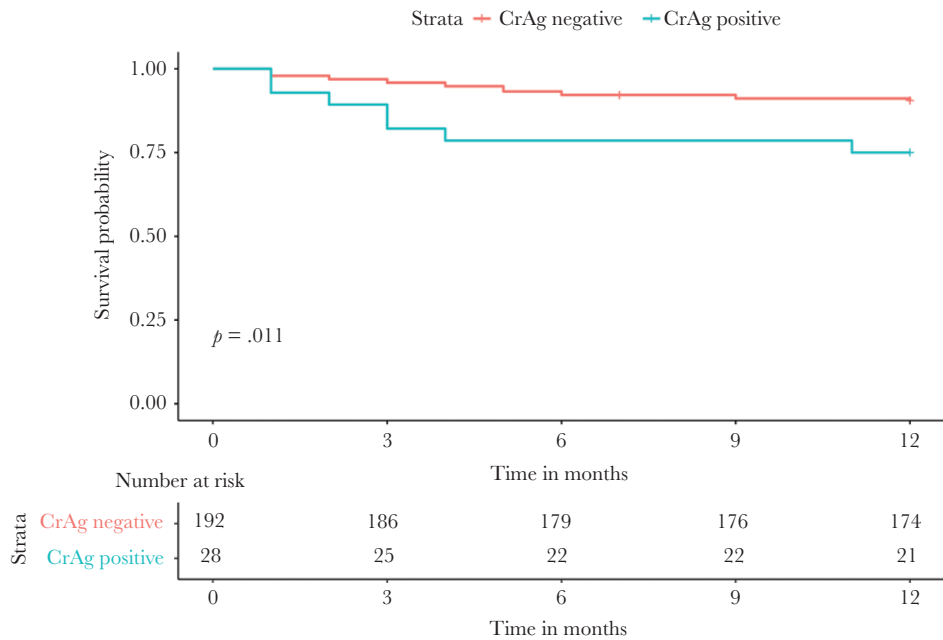


Figure 2. Kaplan-Meier 12-month survival analysis comparing cryptococcal antigen (CrAg)-positive individuals with CrAg-negative.

could explain potential differences of cryptococcal antigenemia prevalence with our study. Moreover, a Brazilian cohort also showed a prevalence of CrAg in CSF of 8.9% [8], comparable to our findings where prevalence in CSF was almost 8%. However, unlike the Honduran cohort, this study included only inpatient participants. Likewise, a recent Argentinian cohort of HIV participants with $CD4 \leq 100$ cells/mm³ reported to have a prevalence of 8.1% [9], which is also lower than what is reported in our study. Thus, our finding suggests that the burden of cryptococcal antigenemia in Central America might be the highest or among the highest in Latin America and therefore urges the need of CrAg testing among individuals with HIV with $CD4 \leq 100$ cells/mm³.

Latin America ranks third in HIV prevalence worldwide below Africa and Southeast Asia [19]. Likewise, when assessing the prevalence of CM, Latin America also ranks third [1]. However, models that state that the burden of CM is high in African countries include several studies performed at this region, where CrAg testing is more frequent than in Latin America. Therefore, Latin America might have a lower reported burden compared with Africa due to lack of access to tools to diagnose this disease or lack of public health programs that require testing for this disease. This is why our estimates in this study become notable because with a significantly smaller number of HIV cases, prevalence of cryptococcal antigenemia might be similar [15, 20, 21] or even higher [4, 17, 22–24] than in some African countries with a higher burden of HIV. Therefore, our findings suggest that actual models of cryptococcal infections worldwide might not be accurate when assessing the burden of this disease in Central America.

The proportion of fatalities in our study was 11.4% among the whole cohort. A recent report undertaken at a Brazilian HIV clinic showed that overall mortality was 27%. However, the Brazilian cohort performed CrAg testing in participants with $CD4 < 200$ cell/mm³ [8]. Nevertheless, our estimates show that CrAg-positive individuals had a higher risk of mortality compared with those who tested negative. This concurs with what has been reported recently, where the risk of death is almost 3 times higher among those individuals who tested positive for CrAg [16, 25]. Likewise, this study in a South African cohort of PWH with $CD4 \leq 100$ cells/mm³ reported an overall mortality among CrAg-positive participants of 25% after 12 months follow up [16], which was the same as our estimate. Furthermore, our results reinforce previous findings that mortality is still high among this particular set of patients even after implementing preemptive therapy [16, 25].

Almost 50% of those individuals who tested negative for CrAg were taking fluconazole for *Candida* infection prevention compared with only 25% among those who tested positive. These differences could be explained by the fact that individuals who were under fluconazole therapy in the

CrAg-negative group had also a higher median amount of time taking ART. Recent findings show that enhanced prophylaxis with fluconazole reduces the frequency of cryptococcosis among PWH [26]. Although prophylaxis for cryptococcal disease is not recommended in contexts in which the prevalence is low [27], this could potentially be reconsidered in the setting of a low-resource country where the prevalence of CrAg is potentially high, which is consistent with our study that reports one of the highest CrAg prevalence estimates in Latin American and even worldwide.

Overall, this study reinforces the need of implementing CrAg screening in Central America to determine the real burden of cryptococcal antigenemia among PWH. Honduras currently does not require CrAg testing as screening when HIV is newly diagnosed or among PWH with $CD4 \leq 100$ cells/mm³ as recommended by the WHO [13]. Furthermore, implementing CrAg screening has proven to be cost-effective in low-resource settings [2, 5, 6] and should therefore be an option in Honduras, taking into account this study where the prevalence of CrAg positivity appears to be high.

One of the major limitations associated to our mortality analysis is not being able to determine the amount of time individuals in the cohort were taking ART during the study period or previously limited our analysis. Furthermore, when assessing mortality, we were not able to determine the causes of death among the cohort. The methodology of testing individuals either in serum or CSF depending on clinical manifestations at presentation might have led to bias in the prevalence of CrAg in serum but not the overall CrAg prevalence. Finally, we were not able to determine (1) the compliance to ART among participants, (2) the rate of abandoning ART among those who had been taking ART for a longer time, and (3) how the result on CrAg testing would be affected by this situation.

CONCLUSIONS

In conclusion, our study findings suggest there is a high prevalence of cryptococcal antigenemia among PWH in Central American, even when compared with studies in Africa where the HIV burden is higher. Systematic screening for CrAg should be considered in the region based on our results to address this major public health concern.

Acknowledgments

We thank to Dr. Carlos Leitzelar, head of the microbiology department where the cryptococcal antigen samples were processed. We also thank Dr. Roberto Flores from URC-Centers for Disease Control and Prevention (CDC) for assistance with the logistics of undertaking this screening project.

Financial support. Cryptococcal antigen testing kits were donated by URC-CDC, through U.S. President's Emergency Plan for AIDS Relief (PEPFAR) funding.

Potential conflict of interests. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* **2017**; 17:873–81.
2. Ramachandran A, Manabe Y, Rajasingham R, Shah M. Cost-effectiveness of CRAG-LFA screening for cryptococcal meningitis among people living with HIV in Uganda. *BMC Infect Dis* **2017**; 17:225.
3. Reepalu A, Balcha TT, Yitbarek T, et al. Screening for cryptococcal antigenemia using the lateral flow assay in antiretroviral therapy-naïve HIV-positive adults at an Ethiopian hospital clinic. *BMC Res Notes* **2015**; 8:8–10.
4. Letang E, Müller MC, Ntamatungiro AJ, et al. Cryptococcal antigenemia in immunocompromised human immunodeficiency virus patients in rural Tanzania: a preventable cause of early mortality. *Open Forum Infect Dis* **2015**; 2:ofv046.
5. Kimaro GD, Guinness L, Shiri T, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced human immunodeficiency virus infection starting antiretroviral therapy in Tanzania and Zambia: a cost-effectiveness analysis. *Clin Infect Dis* **2020**; 70:1652–7.
6. Rajasingham R, Meya DB, Greene GS, et al. Evaluation of a national cryptococcal antigen screening program for HIV-infected patients in Uganda: a cost-effectiveness modeling analysis. *PLoS One* **2019**; 14:1–17.
7. Borges MASB, de Araújo Filho JA, de Jesus Silva Oliveira B, et al. Prospective cohort of AIDS patients screened for cryptococcal antigenaemia, pre-emptively treated and followed in Brazil. *PLoS One* **2019**; 14:1–12.
8. Ferreira M de F, Brito-Santos F, Trilles L, et al. Cryptococcal antigenemia prevalence and clinical data in HIV-infected patients from the reference centre at INI-FIOCRUZ, Rio de Janeiro, Southeast of Brazil. *Mycoses* **2020**; 63:145–50.
9. Frola C, Guelfand L, Blugerman G, et al. Prevalence of cryptococcal infection among advanced HIV patients in Argentina using lateral flow immunoassay. *PLoS One* **2017**; 12:e0178721.
10. UNAIDS. Latin America and the Caribbean. Available at: <https://www.unaids.org/en/regionscountries/latinamerica>. Accessed 25 July 2020.
11. UNAIDS. New HIV infections rising in Latin America—key populations particularly affected. Available at: https://www.unaids.org/en/resources/presscentre/featurestories/2019/october/20191014_latin-america. Accessed 27 July 2020.
12. Crabtree Ramírez B, Caro Vega Y, Shepherd BE, et al. Outcomes of HIV-positive patients with cryptococcal meningitis in the Americas. *Int J Infect Dis* **2017**; 63:57–63.
13. World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children: supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Available at: <https://www.who.int/hiv/pub/guidelines/cryptococcal-disease/en/>. Accessed 22 August 2020.
14. University Research Co. URC current projects. Available at: [https://www.urc-chs.com/sites/default/files/URC Current Projects.pdf](https://www.urc-chs.com/sites/default/files/URC%20Current%20Projects.pdf). Accessed 22 August 2020.
15. Cassim N, Coetzee LM, Govender NP, Glencross DK. District and sub-district analysis of cryptococcal antigenaemia prevalence and specimen positivity in KwaZulu-Natal, South Africa. *Afr J Lab Med* **2018**; 7:757.
16. Longley N, Jarvis JN, Meintjes G, et al. Cryptococcal antigen screening in patients initiating ART in South Africa: a prospective cohort study. *Clin Infect Dis* **2016**; 62:581–7.
17. Hurt WJ, Tenforde MW, Molefi M, et al. Prevalence and sequelae of cryptococcal antigenemia in antiretroviral therapy-experienced populations: an evaluation of reflex cryptococcal antigen screening in Botswana [published online ahead of print April 1, 2020]. *Clin Infect Dis* **2020**. doi:10.1093/cid/ciaa356
18. Vidal JE, Toniolo C, Paulino A, et al. Asymptomatic cryptococcal antigen prevalence detected by lateral flow assay in hospitalised HIV-infected patients in São Paulo, Brazil. *Trop Med Int Health* **2016**; 21:1539–44.
19. World Health Organization. Number of people (all ages) living with HIV estimates by WHO region. Available at: <https://apps.who.int/gho/data/view.main.22100WHO?lang=en>. Accessed 27 July 2020.
20. Rick F, Niyibizi AA, Shroufi A, et al. Cryptococcal antigen screening by lay cadres using a rapid test at the point of care: a feasibility study in rural Lesotho. *PLoS One* **2017**; 12:14–22.
21. Alemu AS, Kempker RR, Tenna A, et al. High prevalence of cryptococcal antigenemia among HIV-infected patients receiving antiretroviral therapy in Ethiopia. *PLoS One* **2013**; 8:1–8.
22. Zuma P, Ramsamy Y, Mlisana K, Archary M. Serum cryptococcal antigen testing in immunosuppressed HIV-positive children and adolescents. *Pediatr Infect Dis J* **2020**; 39:217–20.
23. Sawadogo S, Makumbi B, Purfield A, et al. Estimated prevalence of cryptococcus antigenemia (CrAg) among HIV-infected adults with advanced immunosuppression in Namibia justifies routine screening and preemptive treatment. *PLoS One* **2016**; 11:1–9.
24. Lakoh S, Rickman H, Sesay M, et al. Prevalence and mortality of cryptococcal disease in adults with advanced HIV in an urban tertiary hospital in Sierra Leone: a prospective study. *BMC Infect Dis* **2020**; 20:141.
25. Wake RM, Govender NP, Omar T, et al. Cryptococcal-related mortality despite fluconazole preemptive treatment in a cryptococcal antigen screen-and-treat program. *Clin Infect Dis* **2020**; 70:1683–90.
26. Hakim J, Musiime V, Szubert AJ, et al. Enhanced prophylaxis plus antiretroviral therapy for advanced HIV infection in Africa. *N Engl J Med* **2018**; 377:233–245.
27. Centers for Disease Control and Prevention (CDC). Guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV recommendations from the Centers for Disease Control and Prevention. *MMWR Recomm Rep* **2019**; 424:H1–8.