Epidemiology and Mortality of Cryptococcal Disease in Guatemala: Two-Year Results of a Cryptococcal Antigen Screening Program

Narda Medina 1,†, Juan Luis Rodriguez-Tudela 2,†, Juan Carlos Pérez 3,†, Danicela Mercado 3,†, Oscar Bonilla 3,†, Eduardo Arathoon 1,3,† and Ana Alastruey-Izquierdo 2,4,*

1 Asociación de Salud Integral, Ciudad de Guatemala 01001, Guatemala; nardagab@gmail.com (N.M.); earathoon@hotmail.com (E.A.)
2 Global Action for Fungal Infections, 1208 Geneva, Switzerland; jlrodrigueztudela@gaffi.org
3 Clínica Familiar “Luis Ángel García” (Hospital General San Juan de Dios), Ciudad de Guatemala 01001, Guatemala; jcpsmh@gmail.com (J.C.P.); diagnosticо@cflag.gt (D.M.); oscar.bonilla@cflag.gt (O.B.)
4 Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, 28222 Madrid, Spain
* Correspondence: anaalastruey@isciii.es
† This work was stated by the authors on behalf of FungiRed.

Abstract: Cryptococcal disease is an important opportunistic infection among people living with HIV. The cryptococcal antigen (CrAg) can be detected before the clinical onset of meningitis and its screening is recommended. Here, we evaluated CrAg frequency, and describe the epidemiological characteristics and mortality at 180 days in a cohort of HIV patients from Guatemala. A total of 3457 patients were screened with a CrAg lateral flow assay in serum between January 2017 and December 2018. CrAg positivity was 11.5% in patients with \( \leq 100 \) CD4/mm\(^3\), 8.7% in patients with <200 CD4/mm\(^3\), and 6.3% in patients with <350 CD4/mm\(^3\). In Latin America, we estimated 9.2% CrAg positivity (IC95% 7.9–10.7%) in patients with \( \leq 100 \) CD4/mm\(^3\). Among patients newly diagnosed with HIV, we estimated 4416 incident cases per year in Latin America in those with <200 CD4/mm\(^3\) and 5289 in those with <350 CD4/mm\(^3\). In addition, we calculated the burden in people not on ARV or without viral suppression and found 28,672 cases. CrAg screening should be considered in patients who have a CD4 cell count < 350 cells/mm\(^3\). Cryptococcal meningitis was associated with 30.8% mortality in Guatemala. Global access to diagnosis as well as to liposomal amphotericin B and flucytosine is a priority.

Keywords: cryptococcal antigen; meningitis; Guatemala; Latin America; PLWHIV

1. Introduction

Cryptococcal disease remains a major cause of opportunistic infections that affect people living with HIV (PLWHIV). The World Health Organization (WHO) guidelines recommend screening for cryptococcal antigen (CrAg) followed by pre-emptive antifungal therapy among CrAg-positive people with a negative cerebrospinal fluid (CSF) test to prevent the development of cryptococcal meningitis and before initiating or reinitiating antiretroviral therapy (ART) [1]. These guidelines advise CrAg screening in patients with <200 CD4/mm\(^3\) [1]. In 2017, an update of global cryptococcosis estimated that there were 278,000 people positive for cryptococcal antigen in people with <100 CD4/mm\(^3\) [2]. Cryptococcal meningitis causes \( \approx 15\% \) of HIV-related deaths [2]. Thus, rapid and accurate laboratory diagnosis is critical to initiate antifungal treatment in a timely manner, which is correlated with improved survival.

The cryptococcus lateral flow assay (CrAg LFA) has a sensitivity and specificity of >98% [3–5]. Minimal laboratory infrastructure is required to perform this test and results are available after 10 min. Several countries have implemented projects to screen for
cryptococcal disease among PLWHIV. The overall prevalence of CrAg in HIV patients with <100 CD4/mm$^3$ has been reported at 6.0% (95% CI 5.8–6.2) [2]. However, much of the data are based on studies performed outside of the Latin America region. Epidemiological data of cryptococcal disease is essential for health systems. They help to organize where the diagnostic resources should be located, in primary care or in more specialized settings, as well as the size and cost of the intervention required. In this study, we analyzed the results of a two-year CrAg screening program in Guatemala and compare them with those of previously published studies. Further, we analyzed the epidemiological characteristics of patients and 6-month mortality rate.

2. Materials and Methods

2.1. Setting and Study Design

The opportunistic infection program implemented in Guatemala officially started in January 2017. This program encompasses data from 13 health care facilities that provide services for PLWHIV by means of a Diagnostic Laboratory Hub. Specific details of the program have been published [6,7]. Briefly, three groups of patients were enrolled: (i) patients newly diagnosed with HIV; (ii) patients who have not been receiving antiretroviral treatment (ART) for >90 days but who returned to care (Return/Restart); and (iii) patients on ART with symptoms of opportunistic infections (on ARV treatment). Patients were screened for tuberculosis (TB), non-tuberculous mycobacteria (NTM), histoplasmosis, and cryptococcal disease, regardless of their CD4 cell count and symptomatology. CrAg LFA screening was performed on serum samples at the health care facilities whilst other determinations were performed at the Diagnostic Laboratory Hub. Demographic data were collected using a standard electronic form. An audit and a quality control program were set up to ascertain the results provided by the health care facilities. One in every five serum samples was tested with a CrAg LFA at the Diagnostic Laboratory Hub for results confirmation.

2.2. Literature Review

A literature search was performed in May 2022 using PubMed, Scielo, and GoogleScholar to identify published studies in Latin America in which the CrAg frequency in serum was reported. Keywords included “cryptococcal antigen”, “cryptococcal infection”, “cryptococcal disease”, “HIV patients”, “people living with HIV”, “Latin America”, “Central America”, and “South America”. Other studies were identified using snowballing. According to these studies, and our results, we estimated the frequency of cryptococcal antigenemia in the region.

To calculate the burden of cryptococcal disease in Latin America, we used data published by UNAIDS [8]. To estimate the number of patients at high risk of cryptococcal disease in Latin America, we proposed the following scenario: (i) all patients were screened regardless of symptoms and CD4 counts, and we compared the % CrAg positivity in Guatemala to that of every country with data provided by UNAIDS for patients newly diagnosed with Advanced HIV Disease (AHD), defined as <200 CD4/mm$^3$. (ii) We calculated that 55% of patients have <350 CD4/mm$^3$ in the region and we applied the % CrAg positivity that we found in the Guatemalan cohort [7]. (iii) We also used UNAIDS data to calculate the number of PLWHIV who are not on ART and those who are not virally suppressed per country in 2020 [8]. Then, we found that 20% of patients in these two groups would have AHD, which is a more conservative figure than the average found in patients newly diagnosed with HIV (≈30%) (see Supplementary Material).

2.3. Statistical Analysis

The statistical analysis was performed using SPSS (IBM Iberica, Madrid, Spain). Baseline characteristics were compared with the chi-square test or Fisher’s exact test for categorical variables. Frequencies were calculated from patients tested globally and for each group of patients using percentages. Mortality among cryptococcal cases was analyzed with the Kaplan–Meier test at 180-day follow-up. A p value of <0.05 was considered statistically significant.
3. Results

We screened a total of 3457 patients with a CrAg LFA in serum between January 2017 and December 2018. These patients were enrolled as part of an opportunistic infection program implemented in Guatemala as described above. Among cryptococcal cases, the median age at screening was 38 years [IQR: 32–44 years], 71.3% (n = 117) were males, and 48.8% (n = 80) were from urban areas. Cryptococcosis was associated with men ($p = 0.0364$). Patients with cryptococcosis were slightly younger than patients without the disease (median 34 vs. 38 years old, $p < 0.0001$). Cryptococcal cases had significantly lower CD4 counts than non-cryptococcal cases (median CD4, 47 cells/mm$^3$ vs. 213 cells/mm$^3$; $p < 0.0001$). Multiple opportunistic infections were diagnosed in thirty-two patients (19.5%). The most frequent combinations were cryptococcosis plus histoplasmosis 15 (46.8%) and cryptococcosis plus tuberculosis 11 (34.2%). Table 1 summarizes patients’ characteristics.

Table 1. Baseline characteristics of patients screened using a CrAg LFA.

| Characteristics         | CrAg + ve | CrAg − ve | Patients Screened |
|-------------------------|-----------|-----------|-------------------|
|                         | N         | %         | n                 | %         | n         | %         |
| Gender                  |           |           |                   |           |           |           |
| Male                    | 117       | 71.3%     | 2082              | 63.2%     | 2199      | 63.6%     |
| Female                  | 46        | 28.0%     | 1175              | 35.7%     | 1221      | 35.3%     |
| Transsexual             | 1         | 0.6%      | 36                | 1.1%      | 37        | 1.1%      |
| Sexual orientation      |           |           |                   |           |           |           |
| Heterosexual            | 129       | 78.7%     | 2427              | 73.7%     | 2556      | 73.9%     |
| Homosexual              | 16        | 9.8%      | 599               | 18.2%     | 615       | 17.8%     |
| Bisexual                | 7         | 4.3%      | 205               | 6.2%      | 212       | 6.1%      |
| Ethnic group            |           |           |                   |           |           |           |
| Ladino                  | 126       | 76.8%     | 2404              | 73.0%     | 2530      | 73.2%     |
| Mayan                   | 12        | 7.3%      | 480               | 14.6%     | 492       | 14.2%     |
| Other                   | 2         | 1.2%      | 19                | 0.6%      | 21        | 0.6%      |
| Unknown                 | 24        | 14.6%     | 390               | 11.8%     | 414       | 12.0%     |
| Rural                   |           |           |                   |           |           |           |
| Yes                     | 74        | 45.1%     | 1565              | 47.5%     | 1639      | 47.4%     |
| CD4 cell count          |           |           |                   |           |           |           |
| Unknown                 | 39        | 23.8%     | 829               | 25.2%     | 868       | 25.1%     |
| <50                     | 66        | 40.2%     | 397               | 12.1%     | 463       | 13.4%     |
| 50–99                   | 21        | 12.8%     | 265               | 8.0%      | 286       | 8.3%      |
| 100–199                 | 22        | 13.4%     | 486               | 14.8%     | 508       | 14.7%     |
| 200–349                 | 10        | 6.1%      | 612               | 18.6%     | 622       | 18.0%     |
| ≥350                    | 6         | 3.7%      | 704               | 21.4%     | 710       | 20.5%     |

At enrollment, CD4 cell counts were available for 2589 patients (74.9%). The incidence of CrAg in patients with $\leq 100$, $<200$ and $<350$ cells/mm$^3$ was 11.5%, 8.7%, and 6.3%, respectively (Table 2). Patients newly diagnosed with HIV had the highest CrAg incidence in patients with $\leq 100$ cells/mm$^3$ compared to patients on ARV and those who return to care (Table 2) but this result was not statistically significant ($p = 0.5427$).

In other Latin American countries, published studies showed that CrAg positivity ranged from 8.1% to 12.7% in patients with $<100$ cells/mm$^3$ and from 7.9% to 11.2% in patients with AHD (references) (Figure 1). For more information about the reporting criteria in each country, see Supplementary Data (Supplementary Material). Considering all regional publications, four reported the frequency of CrAg in patients with $\leq 100$ cells/mm$^3$ [9–12] and three in patients with $<200$ cells/mm$^3$ [9,13,14]. Using these results and ours, the estimated overall CrAg frequency in the region for PLWHIV with $<100$ CD4 cells/mm$^3$ was 9.2% (IC95% 7.9–10.7%) and 8.1% in those with AHD (IC95% 7.9–10.7%).
Table 2. Incidence of CrAg in serum according to the CD4 count in Guatemala.

| Characteristics           | CD4 Threshold (Cells/mm\(^3\)) | Overall Incidence |
|---------------------------|---------------------------------|-------------------|
|                           | ≤100   | <200  | <350  | ≥350  |                  |
| Newly diagnosed with HIV  | 12.3%  | 8.8%  | 6.4%  | 0.3%  | 5.0%             |
| On ARV                    | 10.1%  | 8.8%  | 6.8%  | 1.3%  | 4.6%             |
| Return to the ARV         | 10.5%  | 8.2%  | 5.7%  | 1.6%  | 4.6%             |
| Total                     | 11.5%  | 8.7%  | 6.3%  | 0.8%  | 4.8%             |

In other Latin American countries, published studies showed that CrAg positivity ranged from 8.1% to 12.7% in patients with ≤100 cells/mm\(^3\) and from 7.9% to 11.2% in patients with AHD (references) (Figure 1). For more information about the reporting criteria in each country, see Supplementary Data (Supplementary Material). Considering all regional publications, four reported the frequency of CrAg in patients with ≤100 cells/mm\(^3\) [9–12] and three in patients with <200 cells/mm\(^3\) [9,13,14]. Using these results and ours, the estimated overall CrAg frequency in the region for PLWHIV with ≤100 CD4 cells/mm\(^3\) was 9.2% (IC95% 7.9–10.7%) and 8.1% in those with AHD (IC95% 7.9–10.7%).

Figure 1. Frequencies of cryptococcal antigenemia in LATAM among patients with ≤100 cells/mm\(^3\) and those with AHD (<200 CD4/mm\(^3\)) [9–14].

According to UNAIDS, in 2020, 2.1 million of people were living with HIV in Latin America and 109,030 cases were newly diagnosed with HIV [8]. Considering the burden of AHD per country (Supplementary Material), 2828 incident cases per year are expected; however, if we take into account that 55% had <350 CD4 cells/mm\(^3\), the yearly incidence in the region would rise to 3778 cases. This means that if we only screen AHD patients, 950 cases will be missed every year. For other at-risk groups, such as those who abandon ART or those on ART, we considered UNAIDS data on the number of patients without ART treatment as well as those who are not virally suppressed. Then, we considered that 20% of them would have AHD (Supplementary Material), which means that 13,205 and 15,466 cases of cryptococcosis could be expected in those at-risk groups, respectively.

In Guatemala, only 91 patients (55.4%) with a positive serum CrAg result had a lumbar puncture to rule out meningeal involvement. Sixty (65.9%) had cryptococcal meningitis. In a short survey, the health care facilities participating in the program stated that the main reasons for not performing a lumbar puncture were: patients’ refusal, lack of medical supplies, and clinical contraindications to performing a lumbar puncture.

One hundred and fifty-six cryptococcal cases were followed up at 180 days in this study; of these, 48 deaths (30.8%) were reported. Thirty-six (75%) of these deaths occurred in the first 30 days. Figure 2 shows the overall probability of death in patients with
cryptococcal antigenemia and those with cryptococcal meningitis per group of patients. Among patients with positive serum antigenemia, the highest mortality was found in patients newly diagnosed with HIV, with 36.4% (32 out of 88). In patients who return to care and those on ART, mortality was 22.6% and 24.3%, respectively. AHD patients had a higher mortality than non-AHD patients (31.8% vs. 20%, \( p = 0.322 \)). Regarding cryptococcal meningitis, overall mortality at 180 days of follow-up was 38.3% (23 out of 60). Fifty percent (5 out of 10) of patients on ART died, whilst mortality was 28.6% and 38.9% in patients who return to care and among patients newly diagnosed with HIV, respectively. In patients who had a positive serum antigen but did not have a lumbar puncture, overall mortality was 30.3%. On the other hand, mortality in those with a negative CrAg lumbar puncture was 16.7% (5 out of 30, \( p = 0.150 \)). Among these cases, three had coinfections, one had tuberculosis and two had histoplasmosis. It is important to take into account that although liposomal amphotericin B and flucytosine are recommended as antifungal treatments, none were available in Guatemala during the study period. Amphotericin B deoxycholate and fluconazole were the drugs used for treatment.

![Figure 2. (A) Overall mortality among patients with a positive CrAg serum. (B) Overall mortality among patients with a positive CrAg serum and cryptococcal meningitis.](image)

4. Discussion

Among Latin American people with HIV, we estimated that CrAg positivity is 9.2% (IC95% 7.9–10.7%) in patients with <100 CD4/mm\(^3\) [9–14]. This number is 3.2% higher than the global estimation by Rajasingham et al. in asymptomatic patients [2], which is an uncertain denomination considering the deep immunosuppressed status of such HIV patients. In published articles from Brazil, Argentina and Honduras, the criteria to establish the burden of cryptococcal disease were similar, with CD4 thresholds of <100 or <200 cells/mm\(^3\) [10,11,13]. However, in Guatemala, we screened patients regardless of the CD4 cell count, symptoms, or hospitalized status, with an overall incidence of 4.8% (Table 2), which is a real measurement of the burden of disease in PLWHIV. The burden of disease is key to planning how and where health resources should be allocated depending on the frequency of the disease and the complexity of diagnosis. Therefore, it is important to analyze the study criteria to avoid underestimations of the burden of disease. In at high-risk populations, such as patients with AHD, screening based on symptoms can be biased due to the noise [15] that different clinicians with different training and judgment can introduce in patient selection. Evaluating the absence or the presence of symptoms in highly immunosuppressed populations is a risky exercise. For instance, only asymptomatic
hospitalized patients were included in the study by Vidal et al. [13], where only a 3.1% incidence was ascertained, and this may mean that patients had another diseases that caused hospitalization and cryptococcal cases diagnosed were comorbidities more than a burden of this disease in asymptomatic people at risk. Thus, to establish the burden of disease, we can choose an objective entry criterion, such as CD4 threshold. However, to calculate the real burden of disease, studies analyzing the whole population at risk of the disease must be performed. In this case, for HIV, we stratified by risk factors (e.g., CD4 threshold). In Table 2, we show the number of CrAg-positive patients according to CD4 counts, based on which we can decide whether to introduce screening strategies and at what threshold.

One of the most striking results of this analysis is the burden of cryptococcosis disease in patients who return to ARV therapy and those on ARV but with unsuppressed viral loads. The estimation of the burden of cryptococcal disease in PLWHIV and those with AHD could reach 28,000 cases. Considering the mortality rate observed in Guatemala at 180 days, we estimate that ≈8600 patients will die in Latin America due to a life-threatening disease which is easy to diagnose with a CrAg LFA. In Botswana, despite the wide availability of HIV testing and treatment services, a study showed that there was a substantial burden of cryptococcal meningitis due to a failure to effectively engage or retain patients in care [16]. Several studies have described the barriers that face patients who return to care after loss to follow up [17,18]. As cryptococcal disease will not go away without treatment and can lead to meningitis and eventually death, it is possible that many such patients die before diagnosis.

The CrAg LFA has a sensitivity and specificity of >98% [5]. In Guatemala, a comparative performance analysis found that 97% of cryptococcal cases were diagnosed by this assay [19]. This test has been included in the WHO essential diagnostics list since 2017 [20]. However, in many low- and middle-income countries, this test is not yet available. Concerning when to screen for CrAg, our study has an advantage in that the test was performed regardless of the CD4 count. Screening patients for AHD disease is unrealistic unless the clinician has the CD4 count as soon as the patient arrives. It is clear and straightforward that quickly knowing the CD4 count reduces the bias and noise of different clinical criteria. However, in many places, it is difficult to obtain this result. In Guatemala, only 74.9% of patients had their CD4 count available at evaluation. In addition, most of the studies and guidelines have focused on the benefit of CrAg screening at CD4 cell counts <100 or on AHD. However, the results of the Guatemala screening shows that 95% of the cryptococcal cases were diagnosed at <350 CD4/mm$^3$, which is 8% higher than those diagnosed at <200 CD4/mm$^3$. If we extrapolate our results for patients newly diagnosed with HIV in Latin America, we estimate that using a threshold of 200 CD4/mm$^3$, 950 cases would have been missed in comparison to using a threshold of <350 CD4/mm$^3$. Overlooked detection of positive cases has severe consequences because CrAg positivity is an independent predictor of meningitis and death [5]. In Guatemala, in 2021, each CrAg test cost USD 7 and USD 2 per pre-emptive treatment with fluconazole. However, in Africa, the total cost associated with one case of cryptococcal meningitis is estimated at USD 2125 [21]. According to our results (Table 2), we recommend performing CrAg screening for all patients with <350 cells/mm$^3$. Of course the clinician’s judgment must prevail when the CD4 count is not available at evaluation but taking into account that cryptococcosis is a life-threatening disease, a CrAg LFA is cheap, easy to perform and provides a result within ten minutes, which allows making health interventions based on evidence.

Concerning lumbar puncture, the WHO guidelines recommend performing a lumbar puncture to rule out meningitis in any CrAg LFA-positive patient [1]. However, in some settings, access to lumbar punctures may be difficult, in addition to the fact that some patients will refuse this procedure. Here, only 55.4% of patients with a CrAg-positive result received a lumbar puncture, of whom 65% had meningitis. Thus, it is certain that some meningeal cases were not diagnosed. The use of a CrAg LFA needs to be reinforced because of the high mortality rate of cryptococcal meningitis. In the future, the use of a
Microorganisms 2022, 10, 1388

Semiquantitative test and artificial intelligence algorithms for CrAg interpretation may help to identify those at high risk of meningitis and death [22–24]. Early cryptococcal diagnosis is key to decreasing mortality rates. In this study, the 180-day probability of death in patients with a CrAg-positive result was 30.8%. Three-quarters of those deaths occurred within 30 days of diagnosis. As expected, mortality in patients with cryptococcal meningitis was higher than in non-meningeal cases (38% vs. 16.7%); however, although mortality in non-meningeal cases was substantial, the co-occurrence of other infections may contribute to it. Three out of five (60%) patients had coinfections—one tuberculosis and two histoplasmosis—which highlights the importance of screening programs that provide a rapid diagnosis for the most frequent opportunistic infections.

The current recommended treatment for cryptococcal meningitis includes amphotericin B deoxycholate and flucytosine, followed by fluconazole [1]. It is important to highlight that liposomal amphotericin B is preferred since it has demonstrated equivalent efficacy and better safety and tolerability than deoxycholate formulation. The principal barriers for this liposomal amphotericin B plus flucytosine regimen are its availability and cost. Data from the Global Action for Fungal Infections (GAFFI) showed that only three countries in the region had flucytosine [25]. Additionally, the daily price of flucytosine varied from <USD 1 to USD 31 [26]. Despite new approaches such as the use of a single high dose of liposomal amphotericin B plus flucytosine and fluconazole showing a non-inferior response and fewer adverse events [27], access to these treatments is mandatory.

This study has limitations. The relatively low number of studies in the region and the lack of estimates concerning different groups of people living with HIV who are at high risk of cryptococcal disease could introduce changes in our estimates. Lack of detailed information regarding symptoms and ART adherence, which might influence outcomes, is not available. Furthermore, we define deaths in the cryptococcal disease group as attributable to this pathogen; however, patients with HIV could have additional comorbidities. Despite these limitations, this study shows a substantial burden of cryptococcal disease among people living with HIV in Latin America in addition to a high mortality rate, especially in those with cryptococcal meningitis and those without a lumbar puncture. Estimations by Rajasingham et al. (2) suggest a burden of 7000 (3600–11,100) cases for Latin America in PLWHIV with <100 CD4/mm$^3$. Our analysis shows that the burden of cryptococcal disease among those with AHD in the region is substantially higher, reaching a prevalence of 31,503 cases. Although we calculated the numbers of cases for PLWHIV with AHD, the incidence of cryptococcal disease in Latin America for those with <100 CD4/mm$^3$ is 3.2% higher than the global estimation, meaning that an underestimation of the burden of this life-threatening disease in this region is highly probable. There is very robust data for the Guatemalan cohort because of the number of patients included and the prevalence of cryptococcal disease in patients with <100 CD4/mm$^3$ is 11.5%, almost 2-fold that reported for the global population (2), which further supports the estimations for this region. Optimal treatment of cryptococcal meningitis requires the integration of lumbar puncture as well as the availability of liposomal amphotericin B and flucytosine to reduce AIDS-related deaths.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms10071388/s1, Table S1. Cryptococcal antigenemia prevalence studies in LATAM; Table S2. Estimates of CrAg in people living with HIV in Latin America.

Author Contributions: Conceptualization, N.M., A.A.-I. and J.L.R.-T.; data curation and resources, O.B., D.M. and J.C.P.; writing—original draft, N.M., A.A.-I. and J.L.R.-T.; writing—review and editing N.M., A.A.-I., J.L.R.-T. and E.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Global Action Fund for Fungal Infections and JYLAG, a charity foundation based in Switzerland (E.A. received this funding under the proposal: “Minimising HIV deaths through rapid fungal diagnosis and better care in Guatemala”). Other contributions came from Intrahealth International and the Ministry of health in Guatemala (MSPAS).
Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Fungired Group: (i) Oscar Eduardo López Pérez, Hospital La Amistad Japón-Guatemala, Izabal; (ii) Brenan Ortiz Barrientos, Hospital General San Juan de Dios, Guatemala city; (iii) Vilma Alejandrina Reyes Muñoz, Hospital Nacional “Jorge Vides Molina” Huehuetenango; (iv) Gladys Sajché Aguilar, Hospital Nacional “Juan José Ortega” Coatepeque, Quetzaltenango; (v) Aura Marina Méndez Andrade, Hospital Nacional de Escuintla, Escuintla; (vi) Luis Roberto Santa Marina de León, Hospital Nacional de Malacatán, San Marcos; (vii) Ana Lucía Gómez Alcázar, Hospital Nacional de Occidente, Quetzaltenango; (viii) Eduardo Celada González, Hospital Nacional de Retalhuleu, Retalhuleu; (ix) Gustavo A. Quiñónez M., Hospital Nacional Infantil “Elisa Martínez” Izabal; (x) Germán Orlando Cuyuch Sontay and Marco Paez, Hospital Regional “Hellen Lossi de Laugerud” Alta Verapaz; (xi) Alba Virtud Contreras Marín, Hospital Regional de Cuiapa, Santa Rosa; (xii) Maria de Lourdes Fong Araujo, Hospital Regional de San Benito, Petén; (xiii) Claudia Mazariogies L., Hospital Regional de Zacapa, Zacapa; (xiv) Brenda Guzmán, Diagnostic Laboratory Hub, Asociación de Salud Integral, Guatemala City.

Conflicts of Interest: In the last three years, A.A-I. has received honoraria as a speaker from Gilead Sciences and Pfizer outside the submitted work. E.A. has received honoraria from GILEAD for educational conferences and participation in advisory board meetings. All other authors declare no conflicts of interest.

References

1. World Health Organization. Guidelines for the Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children; World Health Organization: Geneva, Switzerland, 2018; ISBN 978-92-4-155027-7. [CrossRef]

2. Rajasingham, R.; Smith, R.M.; Park, B.J.; Jarvis, J.N.; Govender, N.P.; Chiller, T.M.; Denning, D.W.; Loyse, A.; Boulware, D.R. Global Burden of Disease of HIV-Associated Cryptococcal Meningitis: An Updated Analysis. Lancet Infect. Dis. 2017, 17, 873–881. [CrossRef]

3. Escandón, P.; Lizarazo, J.; Aguadelo, I.; Chiller, T.; Castañeda, E. Evaluation of a Rapid Lateral Flow Ow Immunoassay for the Detection of Cryptococcal Antigen for the Early Diagnosis of Cryptococcosis in HIV Patients in Colombia. Med. Mycol. 2013, 51, 765–768. [CrossRef]

4. Ford, N.; Shubber, Z.; Jarvis, J.N.; Chiller, T.; Greene, G.; Migone, C.; Vitoria, M.; Meintjes, G. CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-Analysis. Clin. Infect. Dis. 2018, 66, S152–S159. [CrossRef] [PubMed]

5. Rajasingham, R.; Wake, R.M.; Beyene, T.; Katende, A.; Letang, E.; Boulware, D.R. Cryptococcal Meningitis Diagnostics and Screening in the Era of Point-of-Care Laboratory Testing. J. Clin. Microbiol. 2019, 57, e01238-18. [CrossRef] [PubMed]

6. Samayoa, B.; Aguirre, L.; Bonilla, O.; Medina, N.; Lau-Bonilla, D.; Mercado, D.; Moller, A.; Perez, J.C.; Alastruey-Izquierdo, A.; Arathoon, E.; et al. The Diagnostic Laboratory Hub: A New Health Care System Reveals the Incidence and Mortality of Tuberculosis, Histoplasmosis, and Cryptococcosis of PWH in Guatemala. Open Forum Infect. Dis. 2020, 7, ofz534. [CrossRef] [PubMed]

7. Medina, N.; Alastruey-Izquierdo, A.; Bonilla, O.; Gamboa, O.; Mercado, D.; Pérez, J.C.; Salazar, L.R.; Arathoon, E.; Denning, D.W.; Luis Rodriguez-Tudela, J. A Rapid Screening Program for Histoplasmosis, Tuberculosis, and Cryptococcosis Reduces Mortality in HIV Patients from Guatemala. J. Fungi 2021, 7, 268. [CrossRef]

8. UNAIDS. UNAIDS Data 2021; UNAIDS: Geneva, Switzerland, 2021.

9. Borjes, M.A.S.B.; de Araújo Filho, J.A.; de Jesus Silva Oliveira, B.; Moreira, I.S.; de Paula, V.V.; de Bastos, A.L.; de Bastos Ascenço Soares, R.; Turchi, M.D. Prospective Cohort of AIDS Patients Screened for Cryptococcal Antigenemia, Pre-Emptively Treated and Followed in Brazil. PLoS ONE 2019, 14, e0219928. [CrossRef]

10. Frola, C.; Guelfand, L.; Blugerman, G.; Szyld, E.; Kaufman, S.; Cahn, P.; Sued, O.; Pérez, H. Prevalence of Cryptococcal Infection among Advanced HIV Patients in Argentina Using Lateral Flow Immunoassay. PLoS ONE 2017, 12, e0178721. [CrossRef]

11. Zumiga-Moya, J.C.; Romero-Reyes, L.E.; Barrueto Saavedra, E.; Montoya, S.; Varela, D.; Borjas, M.; Cerna, A.; Bejarano, S.; Martinez, P.; Lujan, K.; et al. Prevalence of Cryptococcal Antigen and Outcomes in People Living with HIV in Honduras: A Cohort Study. Open Forum Infect. Dis. 2020, 8, ofaa557. [CrossRef]

12. Vidal, J.E. Preemptive Therapy for Cryptococcal Meningitis: A Valid Strategy for Latin America? J. Fungi 2016, 2, 14. [CrossRef]

13. Ferreira, M.; Brito-Santos, F.; Trilles, L.; Almeida, M.; Wanke, B.; Veloso, V.; Nunes, E.; dos S. Lazerza, M. Cryptococcal Antigenemia Prevalence and Clinical Data in HIV-Infected Patients from the Reference Centre at INI-FIOCRUZ, Rio de Janeiro, Southeast of Brazil. Mycoses 2019, 63, 145–150. [CrossRef] [PubMed]

14. Vidal, J.E.; Tonio, C.; Paulino, A.; Colombo, A.; dos Anjos Martins, M.; da Silva Meira, C.; Lucia Pereira-Chioccola, V.; Figueiredo-Mello, C.; Barros, T.; Duarte, J.; et al. Asymptomatic Cryptococcal Antigen Prevalence Detected by Lateral Flow Assay in Hospitalised HIV-Infected Patients in Sao Paulo, Brazil. Trop. Med. Int. Health 2016, 21, 1539–1544. [CrossRef] [PubMed]
15. Kalehman, D. Noise: A Flaw in Human Judgment; Hachette Book Group USA: New York, NY, USA, 1377; pp. 68–70.

16. Tenforde, M.W.; Mokomane, M.; Leeme, T.; Patel, R.K.K.; Lekwape, N.; Ramodimoosi, C.; Dube, B.; Williams, E.A.; Mokobela, K.O.; Tawanana, E.; et al. Advanced Human Immunodeficiency Virus Disease in Botswana Following Successful Antiretroviral Therapy Rollout: Incidence of and Temporal Trends in Cryptococcal Meningitis. Clin. Infect. Dis. 2017, 65, 779–786. [CrossRef] [PubMed]

17. Adelekan, B.; Andrew, N.; Nta, J.; Comwalk, A.; Ndemb, N.; Mensah, C.; Dakum, P.; Aliyu, A. Social Barriers in Accessing Care by Clients Who Returned to HIV Care after Transient Loss to Follow-Up. AIDS Res. Ther. 2019, 16, 1–7. [CrossRef] [PubMed]

18. Beres, L.K.; Schwartz, S.; Simbeza, S.; McGready, J.; Eshun-Wilson, I.; Mwamba, C.; Sikombe, K.; Topp, S.M.; Somwe, P.; Mody, A.; et al. Patterns and Predictors of Incident Return to HIV Care Among Traced, Disengaged Patients in Zambia: Analysis of a Prospective Cohort. J. Acquir. Immune Defic. Syndr. 2021, 86, 313. [CrossRef]

19. Medina, N.; Alastruey-izquierdo, A.; Mercado, D.; Aguirre, L.; Samayo, B.; Borilla, O.; Pérez, J.C.; Rodriguez-tudela, J.L. Comparative Performance of the Laboratory Assays Used by a Diagnostic Laboratory Hub for Opportunistic Infections in People Living with HIV. AIDS 2020, 34, 1625–1632. [CrossRef]

20. WHO. World Health Organization Model List of Essential In Vitro Diagnostics, 1st ed.; World Health Organization: Geneve, Switzerland, 2018.

21. Chen, T.; Mwenge, L.; Lakh, S.; Chanda, D.; Mwaba, P.; Molloy, S.F.; Gheorghe, A.; Griffiths, U.K.; Heyderman, R.S.; Kanyama, C.; et al. Healthcare Costs and Life-Years Gained From Treatments Within the Advanced Cryptococcal Meningitis Treatment for Africa (ACTA) Trial on Cryptococcal Meningitis: A Comparison of Antifungal Induction Strategies in Sub-Saharan Africa. Clin. Infect. Dis. 2019, 69, 588–595. [CrossRef]

22. Bermejo-Peláez, D.; Álamo, E.; Medina, N.; Soto, J.C.; Bravo, I.; Diez, N.; Dacal, E.; Luengo-Oroz, M.; Luis Rodriguez-Tudela, J.; Alastruey-Izquierdo, A. Artificial Intelligence Algorithm for Automatic and Objective Interpretation of the Semiquantitative Cryptococcal Antigen Lateral Flow Assay; ECCMID: Brussels, Belgium, 2022.

23. Wake, R.M.; Britz, E.; Sriruttan, C.; Rukasha, I.; Omar, T.; Spencer, D.C.; Nel, J.S.; Mashamaite, S.; Adelekan, A.; Chiller, T.M.; et al. High Cryptococcal Antigen Titers in Blood Are Predictive of Subclinical Cryptococcal Meningitis Among Human Immunodeficiency Virus-Infected Patients. Clin. Infect. Dis. 2018, 68, 66. [CrossRef]

24. Jarvis, J.N.; Tenforde, M.W.; Lechiile, K.; Milton, T.; Boose, A.; Leeme, T.B.; Tawe, L.; Muthoga, C.; Rukasha, I.; Mulenga, F.; et al. Evaluation of a Novel Semiquantitative Cryptococcal Antigen Lateral Flow Assay in Patients with Advanced HIV Disease. J. Clin. Microbiol. 2020, 58, e00441-20. [CrossRef]

25. Drugs Maps. Available online: https://antifungalsavailability.org/maps/map/flucytosine (accessed on 30 May 2022).

26. Kneale, M.; Bartholomew, J.S.; Davies, E.; Denning, D.W. Global Access to Antifungal Therapy and Its Variable Cost. J. Antimicrob. Chemother. 2016, 71, 3599–3606. [CrossRef]

27. Jarvis, J.N.; Lawrence, D.S.; Meya, D.B.; Kagimu, E.; Kasibante, J.; Mpoza, E.; Rutakingirwa, M.K.; Ssebambulidde, K.; Tugume, L.; Rhein, J.; et al. Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. N. Engl. J. Med. 2022, 386, 1109–1120. [CrossRef] [PubMed]