RESEARCH ARTICLE

Poor long-term outcomes despite improved hospital survival for patients with cryptococcal meningitis in rural, Northern Uganda

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Abstract

Background

Cryptococcal meningitis (CM) remains a major cause of death among people living with HIV in rural sub-Saharan Africa. We previously reported that a CM diagnosis and treatment program (CM-DTP) improved hospital survival for CM patients in rural, northern Uganda. This study aimed to evaluate the impact on long-term survival.

Methods

We conducted a retrospective study at Lira Regional Referral Hospital in Uganda evaluating long-term survival ($\geq$1 year) of CM patients diagnosed after CM-DTP initiation (February 2017-September 2021). We compared with a baseline historical group of CM patients before CM-DTP implementation (January 2015-February 2017). Using Cox proportional hazards models, we assessed time-to-death in these groups, adjusting for confounders.

Results

We identified 318 CM patients, 105 in the Historical Group, and 213 in the CM-DTP Group. The Historical Group had a higher 30-day mortality of 78.5% compared to 42.2% in the CM-DTP Group. The overall survival rate for the CM-DTP group at three years was 25.6%. Attendance at follow-up visits (HR:0.13, 95% CI: [0.03–0.53], p <0.001), ART adherence (HR:0.27, 95% CI: [0.10–0.71], p = 0.008), and fluconazole adherence: (HR:0.03, 95% CI: [0.01–0.13], p <0.001), weight >50kg (HR:0.54, 95% CI: [0.35–0.84], p = 0.006), and performance of therapeutic lumbar punctures (HR:0.42, 95% CI: [0.24–0.71], p = 0.001), were

associated with lower risk of death. Altered mentation was associated with increased death risk (HR: 1.63, 95% CI: 1.10–2.42, $p = 0.016$).

Conclusion

Long-term survival of CM patients improved after the initiation of the CM-DTP. Despite this improved survival, long-term outcomes remained sub-optimal, suggesting that further work is needed to enhance long-term survival.

Introduction

Cryptococcal meningitis (CM) is a leading cause of death among people living with HIV, accounting for 15% of all AIDS-related deaths globally and 181,100 deaths in sub-Saharan Africa (SSA) in 2014 [1]. In 2020, the estimated global burden of HIV-associated cryptococcal infection accounted for 19% (13–24%) of AIDS-related mortality [2]. Despite increased antiretroviral therapy (ART) use during the "test and treat" era [3], the burden of CM remains unacceptably high in SSA [4, 5]. and accounts for 60% of all meningitis cases in Uganda [6]. Long-term survival of CM patients has been under-studied in routine care settings despite the high burden of CM in SSA [6].

Despite decreased CM prevalence globally, high mortality persists [6, 7]. In rural, northern Uganda, we reported high mortality, with 90.9% of CM patients being ART-experienced at admission [8]. Therefore, it is important to determine why high mortality persists among CM patients despite the improved availability of ART [9, 10]. During the pre-ART era few patients survived long-term [10, 11], as the median survival for CM was 26 days, and 14-day survival was 47% [12]. Currently, the long-term mortality of CM ranges from 13% to 78% globally [13], primarily reported from studies involving routine care or randomized control trials [6].

Risk factors associated with long-term survival from CM after hospital discharge remain largely uncharacterized outside of research settings. Previous studies identified factors linked to short-term survival, and these same factors were found to be predictive of long-term survival outcomes [13–15]. Those factors associated with improved long-term survival of CM patients include effective antifungal therapy use in the induction phase [14] and ART use [13, 14, 15]. Additionally, the performance of therapeutic lumbar punctures (LPs) during hospitalization was also associated with improved long-term survival [16]. Fluconazole as prophylaxis to prevent CM relapse is recommended by the World Health Organization (WHO) and the Ugandan Clinical Guidelines [17, 18] although, when to stop fluconazole in the long term remains unclear. Conversely, factors associated with poor long-term survival of CM patients include high cerebrospinal fluid (CSF) antigen titers, immune reconstitution inflammatory syndrome (IRIS) [19–21], altered mental status, and seizures [22], all of which largely occur during hospitalization. Despite several efforts to improve hospitalization outcomes among CM patients, less effort has been made toward improving outcomes following hospital discharge.

In February of 2017, a standard of care CM diagnosis and treatment program (CM-DTP) began at Lira Regional Referral Hospital (LRRH) in rural, northern Uganda (9) based on the WHO and Uganda Clinical Guidelines [17, 18]. Prior to initiation of the CM-DTP at LRRH, diagnosis, and treatment of CM patients was based on the clinicians' judgment due to a lack of laboratory diagnostic and monitoring capabilities as well as the unavailability of antifungal agents such as Amphotericin B, all of which are common challenges to CM care in non-research centers in SSA [23]. Because of the inaccessibility and high cost of Amphotericin B,

implementation of CM treatment guidelines at LRRH was challenging prior to initiation of the CM-DTP, and outcomes for CM patients were poor with >75% of deaths occurring during hospitalization. This information was the impetus for the CM-DTP at LRRH. Although the current study is focused on rural, northern Uganda, this project provides a true representation of many other rural settings in SSA where resources are limited, to determine if following standard-of-care protocols could lead to improved outcomes. We found that the CM-DTP led to increased diagnosis of CM, improved treatment, and improved hospital outcomes for patients with CM and other forms of meningitis [9]. Here, we report on the impact of this standard-of-care program on the long-term survival of CM patients and the factors associated with long-term survival.

Methods

Design, study population, and setting

To assess long-term survival, we conducted a retrospective review of two groups of CM patients. One group included 213 CM patients living with HIV who were hospitalized at LRRH from February 2017 through September 2021 and enrolled in the CM-DTP (CM-DTP Group). This is part of an ongoing Meningitis Treatment Program we previously reported (May 2022) to have improved hospital outcomes [9]. Between September 2022 to January 2023, we accessed the data to evaluate the long-term survival of CM patients [data] [24]. To provide a historical context on CM care prior to the initiation of the CM-DTP, we also evaluated a historical group of 105 CM patients treated at LRRH between February 2015 and February 2017 (Historical Group). In the Historical Group, doctors relied on clinical judgment, and available tests including rapid lateral flow assays for CM diagnosis, gram stain and culture, and blood chemistry for monitoring CM patients were usually not performed because they were unavailable in the hospital or were too expensive at outside commercial laboratories for patients to afford. We included all adult CM patients (aged ≥18 years) with hospital records, within the study periods. We excluded patients without complete hospital records or known hospital dispositions. Patients were considered to have confirmed CM if they had laboratory-confirmed diagnoses of CM. We identified 176 of 213 patients with confirmed CM and 37 patients were presumed CM in the CM-DTP Group. In the Historical Group, 48 out of 105 patients were confirmed to have CM. Additionally, patients were considered to have presumed CM if they were treated for CM based on clinical judgment by the treating doctors. Long-term survival was defined as survival of 12 months or longer from the first day of hospitalization. The CM-DTP Group underwent follow-up throughout hospitalization, with additional data subsequently collected from medical records during scheduled clinic visits at LRRH. Post-discharge follow-up was based on records from follow-up appointments at LRRH and phone calls to patients, family members, and/or other health centers.

This study was conducted at LRRH in northern Uganda, a 350-bed government-owned public hospital located 340 kilometers from Uganda's capital, Kampala [25]. This hospital serves a population of 2.2 million Ugandans and is a high-volume hospital with 232,014 outpatient visits and 12,203 inpatient admissions yearly (as of 2022) [25].

CM care in the CM-DTP group

Confirmed CM was diagnosed using a cryptococcal antigen (CrAg) lateral flow assay (IMMY, Norman, Oklahoma) on cerebrospinal fluid (CSF) after a lumbar puncture, a CrAg latex agglutination assay, India ink, CSF culture, positive Biofire (PCR-based test) or CSF gram stain showing yeast as previously reported [9].

Patients enrolled in the CM-DTP received standard-of-care CM treatment based on WHO and Ugandan guidelines [17, 18], using deoxycholate amphotericin B and oral fluconazole as described previously [9]. Flucytosine was not used because it was unavailable during this study period [26]. The CM-DTP protocol included: Amphotericin B deoxycholate (0.7–1.0 mg/kg/day) which was administered for 7–10 days, and extended to 14 days for inadequate response. Fluconazole was initiated at 800–1200 mg/day during induction, tapered to 400 mg/day in consolidation, and maintained at 200 mg/day. Each Amphotericin B dose was flanked by 1.5 L normal saline infusions. Magnesium and potassium chloride supplementation were routine components of the CM treatment regimen. The specific doses depended on the severity of the deficiency and other factors like renal function. In addition, pre-, intra-, and post-treatment monitoring included: creatinine, electrolytes, and hemoglobin levels, detailed HIV and ART status at CM diagnosis (CD4 count, viral load, and ART use and adherence, prior CM history). ART adherence was assessed using standard pill counts, patient self-reported adherence, or information from ART cards issued by HIV clinics which also included duration, and type of ART. CM-DTP patients had follow-up at LRRH after discharge where active assessments could be performed during their monthly return visits for drug refills (ART and Fluconazole).

CM care in the Historical Group (prior CM-DTP)

Patients with CM in the Historical Group received treatment based on the clinician's judgment at the time they presented to the hospital. Supportive treatment was often inadequately provided or in some cases not available. Medical supplies were often lacking, with delays in diagnosis and initiation of treatment being a challenge [23, 27]. In addition, a majority of medical workers had insufficient competence to handle CM patients. These challenges are a common situation in a majority of health centers in Uganda [23, 27]. Antifungal therapy given to patients was not uniform, and induction antifungal therapy was comprised of Amphotericin B, intravenous fluconazole, or oral fluconazole depending on availability and patient's ability to pay. Supplementation with magnesium and potassium chloride was not a routine part of the CM therapy due to the lack of access and availability in this rural resource-poor setting. Patients were sent back to their primary HIV clinics after hospital discharge, although a subset was followed in the HIV Clinic at LRRH.

Outcomes: All-cause mortality among CM patients (CM-DTP)

The primary outcome in this study was all-cause mortality among CM patients who were treated using standard-of-care (CM-DTP Group). We also assessed the factors associated with long-term survival among CM patients who participated in the CM-DTP, including the impact of adherence to ART and the maintenance phase of fluconazole therapy (secondary prophylaxis). Records of CM deaths were from patient files and the follow-up registry. We also made phone calls to patients, next of kin, and/or their primary HIV clinics if the patients were unreachable. At the analysis time, we inquired if the patient was alive or deceased, physical status, comorbidities, and complications (including neurological deficits such as blindness). If the patient died, we recorded details surrounding their death such as date of death, location of death, and cause.

Study data and covariates

Covariate data were extracted from hospital records and clinical record forms (CRFs) and included: age, sex, clinical presentations, category of CM diagnoses (new, relapse, or Immune reconstitution Syndrome, symptom duration, CD4, HIV viral load, lumbar punctures, medicines utilized, ART history, ART and fluconazole adherence, laboratory results, diagnoses, CM

management, and outcomes. After hospital discharge, information was obtained from outpatient and follow-up records. Lastly, routine clinic review notes in the patient's medical record notebooks provided additional information on adherence.

Patient consent statement

This study protocol was approved by the Gulu University Research Ethics Committee (GUREC-066-19), the Mbarara University of Science and Technology Research Ethics Committee (MUSTREC—Reference Number: 1/7), the Uganda National Council of Science and Technology (UNCST: HS 2675), and the University of Minnesota IRB (STUDY00011386). The study met the international human subject protection standards. We sought written informed consent from the patients and no information used could identify them.

Statistical analyses

Descriptive statistics utilized univariate analysis; categorical variables were compared using the Chi-Square test or Fisher's Exact test. Continuous variables were reported as means (standard deviation), medians (interquartile ranges), and proportions or percentages. We performed Chi-Square tests to describe mortality in the CM-DTP group. We performed multivariate regression analysis using the Cox Proportional Hazard regression model. The outcome variable was survival time ("time-to-death") for the survival status. Missing data on outcome (missing at random, 6.6%) were not included in the analyses. We conducted unadjusted and adjusted Cox regression analyses, modeling the survival time with the covariates. We regressed secondary exposures (Fluconazole adherence, ART status, and follow-up adherence) with the outcome. We accounted for both time-varying factors (such as weight) and time-fixed variables (such as sex) in our regression models. We adjusted for confounding variables using regression models based on the directed acyclic graph (DAG) and compared CM mortality outcomes between groups using Kaplan-Meier analyses. Historical data limitations (non-comparative treatment, poor 60-day survival [<15%], low long-term survival, and poor follow-up information) restricted analyses to descriptive statistics for the Historical Group. We reported effect estimates and risk (hazard ratio) with 95% CI at 0.05 two-sided alpha level of significance. We used Stata version 17 (StataCorp, College Station, TX, USA), and R statistical software (Version 4.2.3, Vienna, Austria, The R Foundation of Statistical Computing) for data analyses.

Results

Characteristics of the study population

We identified 318 CM patients: 105 patients in the Historical Group, and 213 patients in the CM-DTP Group. The majority were male (54.7%) with a median age of 35 years (IQR: 30–44), and the median weight was 53 Kg (IQR: 46–58.0). (Table 1). All patients had confirmed HIV infection, 31 (11%) were ART naïve, and 217 (77.2%) patients were on ART and considered to be adherent. We found a significant difference in the proportion of ART experience between Historical Group (22.3% were ART naïve) and the CM-DTP Group (4.5% were ART naïve). Most patients, 247/291 (84.9%) presented with an index episode of CM. The median CD4 count was 63 cells/mm^3 (IQR: 22–149) and the median HIV viral load was 3.9 x 10^4 copies/mL (IQR: 0.4–15.5). CD4 counts were performed in only 104 of 318 (32.7%) patients and viral loads were performed in 28 of 318 (8.8%) patients, primarily because of lack of availability. Most CM-DTP Group patients, 181 of 213 (85%) underwent LP compared to 43 of 105 (41%) patients in the Historical Group.

Table 1. Characteristics of the study population.

	ALL Patients	Historical Group	CM-DTP Group
	N = 318	n = 105	n = 213
Clinical Characteristics			
Gender, females, n (%)	144 (45.3)	40 (38.1)	104 (48.8)
Median age (IQR) [b], years	35 (30–44)	35 (29–44)	36 (30–44)
Median weight (IQR) [b], Kg	53 (46–58)	49 (29–58)	53 (46–58)
CM Diagnosis category, n (%)	291 (91.5) [b]	90 (30.9)	201 (69.1)
New, n (%)	247 (84.9)	83 (92.2)	164 (81.6)
IRIS, n (%)	23 (7.9)	7 (7.8)	16 (8.0)
Relapse, n (%)	21 (7.2)	0	21 (10.4)
TB treatment current, yes, n (%) [b]	34 (37.0)	2 (18.2)	32 (39.5)
History of TB, yes, n (%) [b]	23 (34.3)	3 (42.9)	20 (33.3)
CM Symptom duration, n (%)	280 (88.1) [b]	89 (31.8)	191 (68.2)
Median (IQR) days	14 (7–28)	7 (5–21)	14 (7–29)
<14 days, n (%)	105 (37.5)	46 (51.7)	59 (30.9)
≥14 days, n (%)	175 (62.5)	43 (48.3)	132 (69.1)
Altered mentation, yes, n (%)	119 (48.8)	46 (38.7)	73 (61.3)
Laboratory Characteristics			
CD4 cell count, n (%)	104 (32.7) [b]	41 (39.4)	63 (60.6)
Median CD4 (IQR) cells/mm^3	63 (22,149)	56 (15–98)	74 (31–193)
<200 cells/mm^3	86 (82.7)	39 (95.1)	47 (74.6)
≥200 cells/mm^3	18 (17.3)	2 (4.9)	16 (25.4)
HIV Viral Load, n (%)	28 (8.8)	5 (17.9)	23 (82.1)
Median VL (IQR) X10^4 copies/mL	3.9 (0.4–15.5)	3.2 (0.2–50)	4.2 (0.5–11.7)
Lumbar punctures done, n (%)	224 (70.4) [b]	43 (41.0)	181 (85.0)
0–1, n (%)	92 (41.1)	35 (81.4)	57 (31.5)
2–3, n (%)	100 (44.6)	7 (16.3)	93 (51.4)
≥4, n (%)	32 (14.3)	1 (2.3)	31 (17.1)
Average (Min, Max)	2.1 (0,5)	1.2 (0,5)	2.4 (0,5)
ART status, n (%)	281 (88.4) [b]	103 (36.7)	178 (63.3)
ART Naive	31 (11.0)	23 (22.3)	8 (4.5)
ART uninterrupted	217 (77.2)	77 (74.8)	140 (78.7)
Defaulted	12 (4.3)	2 (1.9)	10 (5.6)
Stopped but restarted	21 (7.5)	1 (1.0)	20 (11.2)
Mortality, n (%)	250 (78.6)	65	185
At 30 days	129 (51.6)	51 (78.5)	78 (42.2)
≤ 60 days	156 (62.4)	56 (86.2)	100 (54.1)
Unable to be traced, n (%)	54 (16.9)	40 (38.1)	14 (6.6)

SD—Standard Deviation, CD—cluster of differentiation, IQR -Interquartile Range, CSF–Cerebrospinal

fluid, TB—Tuberculosis, IRIS—immune reconstitution inflammatory syndrome, VL—Viral Load.

() [b] the number of patients with outcomes, is different from the total number of patients due to missing data.

We assessed how often fluconazole was taken by patients following hospital discharge. Although fluconazole treatment was recommended for all patients after hospital discharge, the majority in the Historical Group, 49/54 (90.7%) had no record of continued fluconazole use following discharge compared to 94/207 (45.4%) in the CM-DTP Group.

Survival of CM patients in the Historical Group was poor

Patients in the Historical Group did not receive uniform CM care before the CM-DTP implementation and, most (78.5%) died within 30 days of hospitalization. (Table 1). The 60-day survival rate remained poor at 13.8%. Further long-term survival analyses for this Historical Group were not feasible because 60-day survival was less than 15%, non-comparable treatments were given, and lack of long-term follow-up information, with 38.1% of patients not traceable. Nevertheless, the poor 60-day survival in the Historical Group provided background information to suggest that the CM-DTP did improve long-term survival as described below.

Long-term survival improved in the CM-DTP group

We assessed the long-term survival of patients in the CM-DTP Group using univariate Chi-Square tests and Kaplan-Meier survival curves (Table 2 and Fig 1). Survival status was available for 199 of 213 patients (93.4%) from hospitalization through the follow-up period after hospital discharge. Patients who could not be traced were lost to follow-up and were not included in survival analyses (14/213, 6.6% of CM patients). Overall, the follow-up person-days contributed by 199 patients was 37,876 days (103.8-person years), and a mortality rate of 143 deaths/100-person years. The proportion of CM deaths at 3 years was 148/199 (74.4%). (Table 2). More females (80.2%) died compared to males (69.4%), but no age differences were found in the proportion of CM deaths. The overall long-term survival among all patients was poor, with of 25.6% survival rate at three years. The mean survival time was 256 days (SD: 401) and the median was 39 days (IQR: 11–367). Thus, although in-hospital mortality improved after initiation of the CM-DTP, [9] long-term survival remained poor.

ART Adherence and prolonged fluconazole use improved long-term survival

Follow-up care and adherence to fluconazole, ART, and follow-up clinic attendance were evaluated to determine the association with the long-term survival of patients (Table 3). We found that patients who adhered to treatment guidelines had better survival compared to those who did not. Those who adhered to fluconazole after hospital discharge had a 98% decreased hazard of mortality compared to those who did not continue fluconazole (HR: 0.02, 95% CI: (0.01, 0.04), p-value <0.001). (Table 3). In addition, patients who adhered to ART as per the Ugandan guidelines prior to hospital admission had better survival than those who did not. Patients who adhered to ART had a 65% lower relative hazard of mortality compared to ART naïve patients, (HR: 0.35, 95% CI: (0.21, 0.59) p-value <0.001). This beneficial effect of ART adherence remained at 41% in the adjusted analyses (HR: 0.59, 95% CI: (0.34, 1.01), p-value 0.054). (Table 3). Patients who adhered to routine outpatient follow-up visits had a 0.02 relative hazard of mortality compared to those who had no follow-up, (HR: 0.02, 95% CI: (0.01, 0.05), p-value <0.001). Survival benefits were observed, even among those with incomplete follow-up, compared to those who never participated in follow-up, (HR: 0.12, 95% CI: (0.08, 0.18), p-value <0.001). (Table 3).

Predictors of long-term survival for the CM-DTP group

We conducted a subgroup analysis of patients to identify additional predictors of long-term survival in those who participated in the CM-DTP. Adherence to fluconazole predicted favorable long-term survival among patients (Table 4). Those who adhered had a 97% decreased risk of mortality compared to those who did not take maintenance fluconazole (HR: 0.03, 95% CI: (0.01, 0.13), p-value <0.001). In addition, patients who adhered to ART before

Table 2. Mortality of CM patients in the CM-DTP group.

ALL CM patients		Numbers N = 213 n [d]	Deaths n (%)	Chi-Squared, X^2 P-value
Gender		199	148 (74.4)	0.083
	Female	91	73 (80.2)	
	Male	108	75 (69.4)	
Age, years		199	148 (74.4)	0.632
	≤35 years	86	62 (72.1)	
	>35 years	113	86 (76.1)	
Altered mentation		166		
	No	104	75 (72.1)	0.420
	Yes	62	49 (79.0)	
ART status		160	116 (72.5)	0.281
	ART Naïve	6	6 (100)	
	Adherent	125	89 (71.2)	
	Defaulted	9	5 (55.6)	
	Restarted	20	16 (80.0)	
Weight, kgs		128	98 (76.6)	**0.011**
	< = 50 kg	51	45 (88.2)	
	>50 kg	77	53 (68.8)	
Fluconazole adherence		192	144 (73.4)	**<0.001**
	Not used	92	92 (100)	
	Adhered	51	9 (17.7)	
	Defaulted	49	40 (81.6)	
History of CM		181	136(75.1)	0.216
	Index	146	113(77.4)	
	IRIS	14	8(57.1)	
	Relapse	21	15(71.4)	
Follow-up adherence		197	148 (74.4)	**<0.001**
	No follow-up returns	92	92 (100)	
	Adhered	51	9 (17.6)	
	Defaulted	49	40 (81.6)	
CD4 Category		58	41 (70.7)	0.944
	<200 cells/mm^3	44	31 (70.5)	
	≥200 cells/mm^3	14	10 (71.4)	
Lumbar Punctures		168	126 (75.0)	0.080
	0–1	49	42 (85.7)	
	2–3	88	64 (72.7)	
	≥4	31	20 (64.5)	

[d] Numbers of patients with outcomes excluding missing values,

IRIS–Immune Reconstitution Inflammatory Syndrome, Kg–Kilogram(s), X^2 –Chi-Squared

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hospitalization had a 73% reduced risk of mortality compared to ART-naïve CM patients, (HR: 0.27, 95% CI: (0.10, 0.71), *p*-value 0.008). Patients who attended follow-up return visits had an 87% reduced risk of mortality compared to those who did not attend, (HR: 0.13, 95% CI: (0.03–0.53), *p*-value <0.001). Patients who had a weight greater than 50 kgs had a 46% decreased risk of death compared to those less than 50 kgs, (HR: 0.54, 95% CI: (0.38, 0.86), *p*-value 0.006) suggesting survival benefits in those with better nutritional status. When we evaluated the long-term survival benefits of in-hospital therapeutic LPs among patients, reduced
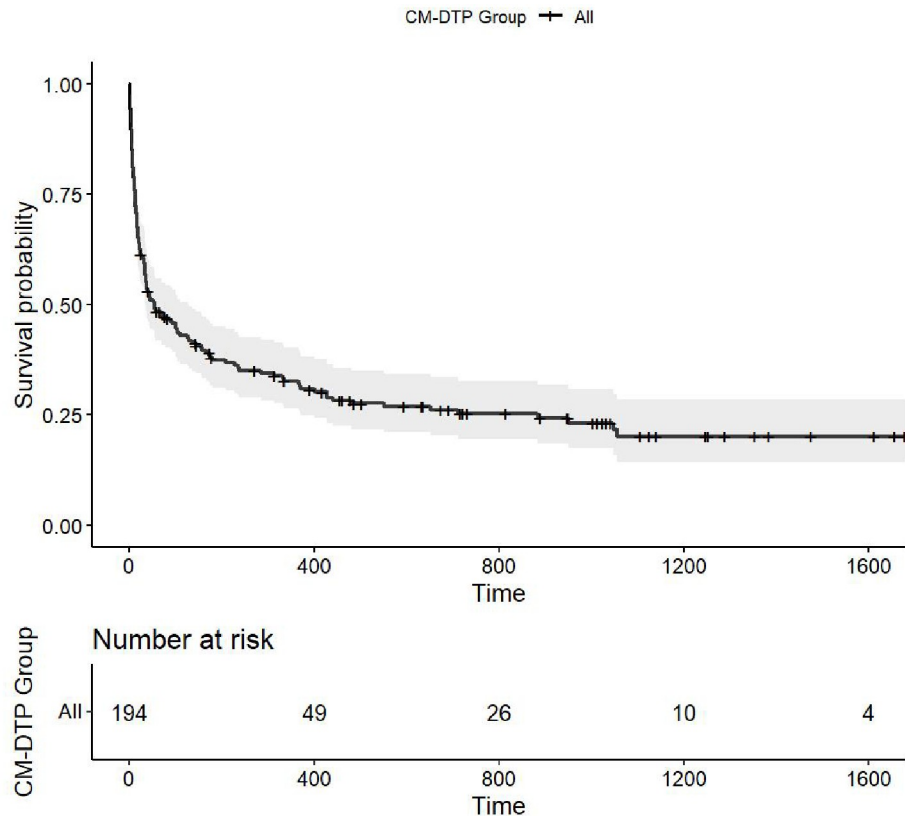
Fig 1. Survival probability over time (days) and number at risk for CM-DTP group. The cumulative survival probability curve for patients in the CM-DTP group expressed as a ratio (y-axis) from admission (day 0) is shown over time in days (x-axis).

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long-term mortality was observed in those who had 4 or more therapeutic LPs (HR: 0.23, 95% CI: (0.09, 0.60), *p*-value of 0.003) or those who had 2 to 3 therapeutic LPs (HR: 0.46, 95% CI: (0.24–0.88), *p*-value 0.020) compared to those who had less than 2 LPs. Lastly, CM patients who experienced altered mentation during their initial hospitalization had 1.63 times the risk of long-term mortality compared to those without altered mentation (HR: 1.63, 95% CI: 1.10–2.42, *p*-value = 0.016). This suggests that CM patients who had altered mentation possibly had more severe disease which increased the risk of subsequent death post-hospitalization.

Discussion

We observed long-term survival of CM patients improved following the implementation of the standard-of-care (CM-DTP) for CM in rural Uganda. However, post-discharge care including attendance at follow-up visits and adherence to fluconazole prophylaxis and ART were often sub-optimal. Thus, implementation of this program during hospitalization had long-term benefits in a real-world resources-limited setting, but further work is needed to improve CM care after hospital discharge. Although we previously showed that implementation of this CM-DTP improved diagnosis, treatment, and in-hospital mortality among CM patients, [9] the current results show that long-term three-year survival remained poor at only 25.6%, suggesting that large numbers of patients die after hospital discharge. There was no

Table 3. Crude and an adjusted Hazard Ratios (HR) for association between long-term survival of CM patients and exposures at Cox proportional hazard regression.

Variable	CM Total (Deaths)	Crude Hazard Ratios			Adjusted Hazard Ratios		
		HR	95% CI	*P*-value	HR[v]	95% CI	*P*-value
Primary Exposure							
Overall Group [w]							
CM-DTP	199 (148)	0.36	(0.26, 0.48)	<0.001	0.35	(0.24, 0.49)	**<0.001**
Secondary Exposures							
Fluconazole Adherence [x]							
Not Used	139 (139)	1.00			1.00		
Adhered	52 (10)	0.02	(0.01, 0.04)	<0.001	0.02	(0.01, 0.04)	**<0.001**
Defaulted	52 (42)	0.13	(0.09, 0.20)	<0.001	0.13	(0.08, 0.20)	**<0.001**
ART Status [y]							
Naïve	17 (17)	1.00			1.00		
Adhered	174 (137)	0.35	(0.21, 0.59)	<0.001	0.59	(0.34, 1.01)	0.054
Defaulted	10 (6)	0.25	(0.10, 0.64)	0.004	0.59	(0.22, 1.58)	0.291
Restarted	21 (17)	0.27	(0.14, 0.54)	<0.001	0.50	(0.25, 1.03)	0.061
Follow-up Adherence [z]							
No follow-up returns	146 (146)	1.00			1.00		
Adhered	53 (13)	0.02	(0.01, 0.04)	<0.001	0.02	(0.01, 0.05)	**<0.001**
Defaulted	62 (50)	0.12	(0.08, 0.17)	<0.001	0.12	(0.08, 0.18)	**<0.001**

[v] Adjusted Hazard Ratio (HR), Confidence Interval (CI); Lumbar puncture (LP); *P* value level of significance <0.05 (indicating significant p values.

[w] Treatment Group adjusted for Age, Sex, and Antiretroviral use status

[x] Fluconazole adherence adjusted for Age, Sex, and Treatment Group

[y] Antiretroviral use status adjusted for Age, Sex, and Treatment Group

[z] Follow-up adherence adjusted for Age, Sex, and Treatment Group

difference in mortality between males and females, similar to an earlier study conducted in Uganda [28]. Even in the interventional CryptoDex trial in Uganda and Asia, a similar trend of sub-optimal long-term survival was observed, with mortality of 60% at 2 years among HIV patients with CM [29, 30]. Conversely, an earlier study in Uganda observed a 5-year survival rate of 42% among 189 CM patients which is higher compared to our study [11]. A systematic review report on HIV-associated meningitis in sub-Saharan Africa reported long-term mortality (6 months) among 614 cases of CM patients of 44% (95% CI: 36%, 52%, I^2 = 71%) [6]. However, these studies were conducted in research settings, whereas our study was conducted in a real-world rural setting where healthcare resources are limited. Overall, our study and others suggest that long-term outcomes for CM remain sub-optimal.

Factors associated with improved long-term survival included adherence to fluconazole and ART, attendance at follow-up return visits after hospital discharge, weight above 50Kgs, and receiving >2 LPs during hospitalization, all of which are consistent with systematic review reports [7, 6]. Inadequate follow-up has been reported to be responsible for the sub-optimal long-term outcomes in similar studies in resource-limited settings [31, 32]. Our results suggest, however, that follow-up visit attendance and adherence to ART and fluconazole in the outpatient setting remained sub-optimal even after initiation of the CM-DTP, perhaps because the CM-DTP focused primarily on improving treatment in the inpatient setting. In addition, high fluconazole resistance (24.1%) in relapse isolates might contribute to long-term mortality, despite the absent of routine testing [33]. Further work needs to be done to improve care after hospital discharge.

Table 4. Predictors of long-term survival of CM-DTP group patients at Cox proportional hazard regression at 3 years.

Variable	Category	CM Total (Deaths)	HR [f]	95% CI	P-Value
				Adjusted Hazard Ratios	
Sex	Males	91 (73)	1.00		
	Females	108 (75)	1.15	(0.67, 1.95)	0.617
Age	< = 35 years	86 (62)	1.00		
	> 35 years	113 (86)	1.02	(0.63, 1.65)	0.926
Fluconazole Adherence [g]	Not Used	92 (92)	1.00		
	Adhered	51 (51)	0.03	(0.01, 0.13)	**<0.001**
	Defaulted	49 (40)	0.68	(0.18, 2.55)	0.565
ART Status [h]	Naïve	6 (6)	1.00		
	Adhered	125 (89)	0.27	(0.10, 0.71)	**0.008**
	Defaulted	9 (5)	0.41	(0.09, 1.78)	0.233
	Restarted	20 (16)	0.44	(0.15, 1.36)	0.155
Follow-up Adherence [k]	No follow-up return	92 (92)	1.00		
	Adhered	51 (9)	0.13	(0.03, 0.53)	**<0.001**
	Defaulted	49 (40)	0.05	(0.01, 0.19)	**<0.001**
Weight Category [m]	< = 50Kgs	51 (45)	1.00		
	> 50Kgs	77 (53)	0.54	(0.35, 0.84)	**0.006**
Altered mentation [p]	No	104 (75)	1.00		
	Yes	62 (49)	1.63	(1.10, 2.42)	**0.016**
Lumbar Punctures [m]	0–1 LP	49 (42)	1.00		
	2–3 LPs	88 (64)	0.65	(0.44, 0.97)	**0.033**
	> = 4 LPs	31 (20)	0.42	(0.24, 0.71)	**0.001**

HR [f], Hazard Ratio

[g] Adjusted for sex, age, ART status, follow-up adherence, weight, and LP

[h] Adjusted for sex, age, fluconazole adherence, follow-up adherence, weight, and LP

[k] Adjusted for sex, age, ART status, fluconazole adherence, weight, and LP

[p] Adjusted for Sex, Age, and lumber punctures

[m] Adjusted for sex and Age

The World Health Organization recommends that ART-experienced people who develop CM be evaluated for possible underlying ART treatment failure, potentially through HIV VL tests [26]. However, the challenge of accessing VL tests and ART resistance tests hampers the implementation of this recommendation in resource-limited settings [34], and most of the patients in this study did not receive testing because CD4 counts and viral load tests, which were not routinely available outside of centralized government laboratory processing available only at authorized HIV clinics. Increasing the utilization of these tests could identify patients with HIV treatment failure, and prompt reflex cryptococcal antigen screening for early diagnosis, intervention [34, 35], and possibly mortality benefits [36]. However, no overall survival benefits were observed through CrAg screening [37], except for patients with CrAg titers ≥1:160, who had 2.6-fold higher 6-month mortality compared to patients with titers <1:160 [35]. Other laboratory tests such as quantitative CSF yeast count and clearance, CSF cytokines, CSF white cell count, and antifungal susceptibility have been reported in several studies to affect hospital outcomes [38–41]. These factors were not evaluated in this study because they were not routinely available for clinical care in this setting. Previously, a study from Uganda observed that therapeutic LPs were associated with a 69% relative improvement in survival, regardless of initial intracranial pressure among admitted CM patients [16]. Similarly, we

observed long-term risk reduction of death in those who underwent more LPs among CM patients. This highlights the need for more frequent LPs during induction therapy, to optimize long-term survival.

Limitations to this study included the retrospective design and missing data because we were unable to contact some patients after discharge. Incomplete medication refill records and use of self-reported adherence likely resulted in inaccurate adherence evaluations and recall bias. This could have led to random misclassification of the exposure, biasing the effect estimates towards the null, causing an underestimation of the association. Also, VL testing could not be used to assess the validity of self-reported adherence because of the lack of availability. The Historical Group had a small sample size, and a greater number of patients without records because most of these patients were referred to their primary health units for follow-up care. Therefore, a complete assessment of post-hospitalization deaths was not feasible. Available outcome data for the Historical Group, however, demonstrated evidence of very high short-term mortality. Although the CM-DTP results clearly showed that attendance at clinic visits and medication adherence were factors associated with long-term survival, survivorship bias could have been possible because patients who had survived post-admission could have had factors (residual confounders) that made them survive up to the point in time where long-term follow-up was possible. Therefore, the survival benefits should be interpreted with caution and in the context of this resource-limited setting outside research centers. However, it is logical that attendance at follow-up visits and adherence to medications would be beneficial to survival.

Conclusion

Our findings suggest that the long-term survival of CM patients is achievable and can be improved through appropriate standard-of-care treatment and follow-up, even in resource-limited settings. Routine follow-up of CM patients (like all other hospitalized medical patients) is often done passively based on the patient's willingness to return, creating a situation that is sub-optimal for many CM patients because they do not return for follow-up care. We observed outpatient follow-up after hospital discharge and medication adherence are key components in CM care to improve long-term survival. During hospitalization, performing therapeutic LP improves long-term survival. In addition, identifying patients with risk factors such as altered mental status or low weight during hospitalization may allow closer monitoring or efforts to improve nutritional status, which could lead to improved outcomes. Our results highlight the need for improved follow-up care after hospital discharge to improve the long-term survival of patients with CM in rural SSA. Despite improved hospital survival, long-term outcomes remained sub-optimal, suggesting that further work is needed to improve follow-up care and enhance long-term survival.

Acknowledgments

Author Contributions

Conceptualization: Mark Okwir, Abigail Link, Betty Nabongo, Joshua Rhein, David Meya, Paul R. Bohjanen.

References

1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017; 17: 873–881. https://doi.org/10.1016/S1473-3099(17)30243-8 PMID: 28483415

2. Rajasingham R, Govender NP, Jordan A, Loyse A, Shroufi A, Denning DW, et al. The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet Infect Dis. 2022; 22: 1748–1755. https://doi.org/10.1016/S1473-3099(22)00499-6 PMID: 36049486

3. World Health Organization. Progress report 2016: prevent HIV, test, and treat all: WHO support for country impact. World Health Organization; 2016. Report No.: WHO/HIV/2016.24. https://apps.who.int/iris/handle/10665/251713

4. Jarvis JN, Boulle A, Loyse A, Bicanic T, Rebe K, Williams A, et al. High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. AIDS Lond Engl. 2009; 23: 1182–1183. https://doi.org/10.1097/QAD.0b013e32832be0fc PMID: 19451796

5. Beyene T, Woldeamanuel Y, Asrat D, Ayana G, Boulware DR. Comparison of Cryptococcal Antigenemia between Antiretroviral Naïve and Antiretroviral Experienced HIV Positive Patients at Two Hospitals in Ethiopia. PLoS ONE. 2013; 8: e75585. https://doi.org/10.1371/journal.pone.0075585 PMID: 24124498

6. Tenforde MW, Gertz AM, Lawrence DS, Wills NK, Guthrie BL, Farquhar C, et al. Mortality from HIV-associated meningitis in sub-Saharan Africa: a systematic review and meta-analysis. J Int AIDS Soc. 2020; 23: e25416. https://doi.org/10.1002/jia2.25416 PMID: 31957332

7. Muzazu SGY, Assefa DG, Phiri C, Getinet T, Solomon S, Yismaw G, et al. Prevalence of cryptococcal meningitis among people living with human immuno-deficiency virus and predictors of mortality in adults on induction therapy in Africa: A systematic review and meta-analysis. Front Med. 2022; 9. Available: https://www.frontiersin.org/articles/10.3389/fmed.2022.989265 PMID: 36160163

8. Okwir M, Link A, Rhein J, Obbo JS, Okello J, Nabongo B, et al. High Burden of Cryptococcal Meningitis Among Antiretroviral Therapy-Experienced Human Immunodeficiency Virus-Infected Patients in Northern Uganda in the Era of "Test and Treat": Implications for Cryptococcal Screening Programs. Open Forum Infect Dis. 2022; 9: ofac004. https://doi.org/10.1093/ofid/ofac004 PMID: 35111875

9. Link A, Okwir M, Meya D, Nabongo B, Okello J, Kasprzyk D, et al. Impact of a Cryptococcal meningitis diagnosis and treatment program at Lira Regional Referral Hospital in rural, Northern Uganda. PLOS Glob Public Health. 2022; 2: e0000254. https://doi.org/10.1371/journal.pgph.0000254 PMID: 36713975

10. Kambugu A, Meya DB, Rhein J, O'Brien M, Janoff EN, Ronald AR, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of HAART. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008; 46: 1694–1701. https://doi.org/10.1086/587667 PMID: 18433339

11. Butler EK, Boulware DR, Bohjanen PR, Meya DB. Long Term 5-Year Survival of Persons with Crypto-coccal Meningitis or Asymptomatic Subclinical Antigenemia in Uganda. PLoS ONE. 2012; 7: e51291. https://doi.org/10.1371/journal.pone.0051291 PMID: 23251485

12. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS. 2002; 16: 1031. https://doi.org/10.1097/00002030-200205030-00009 PMID: 11953469

13. Pasquier E, Kunda J, De Beaudrap P, Loyse A, Temfack E, Molloy SF, et al. Long-term Mortality and Disability in Cryptococcal Meningitis: A Systematic Literature Review. Clin Infect Dis Off Publ Infect Dis Soc Am. 2018; 66: 1122–1132. https://doi.org/10.1093/cid/cix870 PMID: 29028957

14. Kanyama C, Molloy SF, Chan AK, Lupiya D, Chawinga C, Adams J, et al. One-year Mortality Out-comes from the Advancing Cryptococcal Meningitis Treatment for Africa Trial of Cryptococcal Menin-gitis Treatment in Malawi. Clin Infect Dis. 2020; 70: 521–524. https://doi.org/10.1093/cid/ciz454 PMID: 31155650

15. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combina-tions for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med. 2018; 378: 1004–1017. https://doi.org/10.1056/NEJMoa1710922 PMID: 29539274

16. Rolfes MA, Hullsiek KH, Rhein J, Nabeta HW, Taseera K, Schutz C, et al. The Effect of Therapeutic Lumbar Punctures on Acute Mortality from Cryptococcal Meningitis. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014; 59: 1607–1614. https://doi.org/10.1093/cid/ciu596 PMID: 25057102

17. World Health Organization 2018. Guidelines for the diagnosis, prevention, and management of crypto-coccal disease in HIV-infected adults, adolescents, and children. [cited 16 May 2023]. https://www.who.int/publications-detail-redirect/9789241550277

18. Uganda MOH. Consolidated Guidelines for Prevention and Treatment of HIV in Uganda | MOH Knowl-edge Management Portal. 20 Dec 2016 [cited 16 May 2023]. http://library.health.go.ug/communicable-disease/hivaids/consolidated-guidelines-prevention-and-treatment-hiv-uganda

19. Tsai S-T, Lin F-Y, Chen P-S, Chiang H-Y, Kuo C-C. Three-year mortality in cryptococcal meningitis: Hyperglycemia predict unfavorable outcome. PLoS ONE. 2021; 16: e0251749. https://doi.org/10.1371/journal.pone.0251749 PMID: 34048463

20. Boulware DR, Meya DB, Bergemann TL, Wiesner DL, Rhein J, Musubire A, et al. Clinical Features and Serum Biomarkers in HIV Immune Reconstitution Inflammatory Syndrome after Cryptococcal Meningi-tis: A Prospective Cohort Study. PLoS Med. 2010; 7: e1000384. https://doi.org/10.1371/journal.pmed.1000384 PMID: 21253011

21. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. Timing of Antire-troviral Therapy after Diagnosis of Cryptococcal Meningitis. N Engl J Med. 2014; 370: 2487–2498. https://doi.org/10.1056/NEJMoa1312884 PMID: 24963568

22. Pastick KA, Bangdiwala AS, Abassi M, Flynn AG, Morawski BM, Musubire AK, et al. Seizures in Human Immunodeficiency Virus-Associated Cryptococcal Meningitis: Predictors and Outcomes. Open Forum Infect Dis. 2019; 6: ofz478. https://doi.org/10.1093/ofid/ofz478 PMID: 32042847

23. Link A, Okwir M, Iribarren S, Meya D, Bohjanen PR, Kasprzyk D. Lack of education, knowledge, and supplies are barriers to cryptococcal meningitis care among nurses and other healthcare providers in rural Uganda: A mixed methods study. Nurse Educ Today. 2023; 128: 105873. https://doi.org/10.1016/j.nedt.2023.105873 PMID: 37390523

24. Long-Term Cryptococcal Meningitis Survival, Uganda (DATA). figshare; 2024. https://doi.org/10.6084/m9.figshare.25447570.v1

25. Lira Hospital.pdf. https://budget.finance.go.ug/sites/default/files/Sector%20Spending%20Agency%20Budgets%20and%20Performance/Lira%20Hospital.pdf

26. WHO 2022: World Health Organization 2022. Guidelines for diagnosing, preventing, and managing cryptococcal disease among adults, adolescents and children living with HIV. [cited 17 May 2023]. https://www.who.int/publications-detail-redirect/9789240052178

27. Link A, Okwir M, Nabongo B, Meya DB, Iribarren S, Bohjanen P, et al. Delays in Cryptococcal Meningitis Diagnosis and Care: A Mixed Methods Study in Rural Uganda. Ann Glob Health. 2022; 88. https://doi.org/10.5334/aogh.3524 PMID: 35415076

28. Stadelman AM, Ssebambulidde K, Tugume L, Pastick KA, Hullsiek KH, Lofgren S, et al. Impact of biological sex on cryptococcal meningitis mortality in Uganda and South Africa. Med Mycol. 2021; 59: 712–719. https://doi.org/10.1093/mmy/myaa108 PMID: 33399865

29. Kitonsa J, Nsubuga R, Mayanja Y, Kiwanuka J, Nikweri Y, Onyango M, et al. Determinants of two-year mortality among HIV positive patients with Cryptococcal meningitis initiating standard antifungal treatment with or without adjunctive dexamethasone in Uganda. PLoS Negl Trop Dis. 2020; 14: e0008823. https://doi.org/10.1371/journal.pntd.0008823 PMID: 33253210

30. Kitonsa J, Mayanja Y, Aling E, Kiwanuka J, Namutundu J, Anywaine Z, et al. Factors affecting mortality among HIV positive patients two years after completing recommended therapy for Cryptococcal meningitis in Uganda. PLOS ONE. 2019; 14: e0210287. https://doi.org/10.1371/journal.pone.0210287 PMID: 30699151

31. Lessells RJ, Mutevedzi PC, Heller T, Newell M-L. Poor long-term outcomes for cryptococcal meningitis in rural South Africa. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 2011; 101: 251–252. https://doi.org/10.7196/samj.4378 PMID: 21786729

32. Jarvis JN, Meintjes G, Williams Z, Rebe K, Harrison TS. Symptomatic relapse of HIV-associated cryptococcal meningitis in South Africa: the role of inadequate secondary prophylaxis. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 2010; 100: 378–382. https://doi.org/10.7196/samj.3515 PMID: 20526411

33. Bongomin F, Oladele RO, Gago S, Moore CB, Richardson MD. A systematic review of fluconazole resistance in clinical isolates of Cryptococcus species. Mycoses. 2018; 61: 290–297. https://doi.org/10.1111/myc.12747 PMID: 29377368

34. Newman H, Hardie D. HIV-1 viral load testing in resource-limited settings: Challenges and solutions for specimen integrity. Rev Med Virol. 2021; 31: e2165. https://doi.org/10.1002/rmv.2165 PMID: 32978882

35. Meya DB, Kiragga AN, Nalintya E, Morawski BM, Rajasingham R, Park BJ, et al. Reflexive Laboratory-Based Cryptococcal Antigen Screening and Preemptive Fluconazole Therapy for Cryptococcal Antigenemia in HIV-Infected Individuals with CD4 <100 Cells/µL: A Stepped-Wedge, Cluster-Randomized Trial. JAIDS J Acquir Immune Defic Syndr. 2019; 80: 182. https://doi.org/10.1097/QAI.0000000000001894 PMID: 30399034

36. Rajasingham R, Meya DB, Greene GS, Jordan A, Nakawuka M, Chiller TM, 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: e0210105. https://doi.org/10.1371/journal.pone.0210105 PMID: 30629619

37. Drain PK, Galagan SR, Govere S, Krows M, Thulare H, Wallis CL, et al. Laboratory Reflex and Clinic-based Point-of-care Cryptococcal Antigen Screening for Preventing Meningitis and Mortality among People Living with HIV. J Acquir Immune Defic Syndr 1999. 2021; 87: 1205–1213. https://doi.org/10.1097/QAI.0000000000002717 PMID: 33990495

38. de Oliveira L, Melhem de MSC, Buccheri R, Chagas OJ, Vidal JE, Diaz-Quijano FA. Early clinical and microbiological predictors of outcome in hospitalized patients with cryptococcal meningitis. BMC Infect Dis. 2022; 22: 138. https://doi.org/10.1186/s12879-022-07118-7 PMID: 35139801

39. Mora DJ, Ferreira-Paim K, Andrade-Silva LE, Bragine T, Rocha IH, Ribeiro B de M, et al. Cytokine patterns in a prospective cohort of HIV-infected patients with cryptococcal meningitis following initiation of antifungal and antiretroviral therapy. PLOS ONE. 2017; 12: e0176304. https://doi.org/10.1371/journal.pone.0176304 PMID: 28486489

40. Qu J, Jiang J, Lv X. The utility of cerebrospinal fluid white cell counts during the prognostic assessment for cryptococcal meningitis patients: a retrospective study. BMC Infect Dis. 2020; 20: 571. https://doi.org/10.1186/s12879-020-05287-x PMID: 32758162

41. Vlasova-St Louis I, Musubire AK, Meya DB, Nabeta HW, Mohei H, Boulware DR, et al. Transcriptomic biomarker pathways associated with death in HIV-infected patients with cryptococcal meningitis. BMC Med Genomics. 2021; 14: 108. https://doi.org/10.1186/s12920-021-00914-1 PMID: 33863324