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Adjunctive sertraline for asymptomatic cryptococcal antigenemia: A randomized clinical trial

David R Boulware[1,*,†], Elizabeth Nalintya[2,†], Radha Rajasingham[1], Paul Kirumira[2], Rose Naluyima[2], Fred Turya[2], Sylvia Namanda[2], Morris K Rutakingirwa[2], Caleb P Skipper[1,2], Yofesi Nikweri[3], Kathy Huppler Hullsiek[1], Ananta S Bangdiwala[1] and David B Meya[1,2,4]

[1]University of Minnesota, Minneapolis, MN, USA, [2]Infectious Diseases Institute, Makerere University, Uganda, [3]Medical Research Council/Uganda Virus Research Institute & London School of Hygiene and Tropical Medicine (MRC/UVRI & LSHTM), Uganda Research Unit, Masaka Station and [4]Department of Medicine, School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda

*To whom correspondence should be addressed. David R Boulware. MD, MPH, University of Minnesota, 689 23rd Avenue SE, Minneapolis, MN 55455-1507, USA. E-mail: boulw001@umn.edu.

†Equal contribution

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Abstract

Cryptococcal antigen (CrAg) screening in HIV-infected persons with CD4 < 100 cells/μl can reduce meningitis and death, yet preemptive fluconazole therapy fails in ~25%. Sertraline has *in vitro* and *in vivo* activity against *Cryptococcus* and is synergistic with fluconazole in mice. We evaluated the efficacy and safety of sertraline in asymptomatic cryptococcal antigenemia. We conducted a randomized trial of asymptomatic CrAg-positive Ugandans from November 2017 to February 2018. All subjects received WHO standard therapy of fluconazole 800 mg for 2 weeks, then 400 mg for 10 weeks, then 200 mg through 24 weeks. Participants were randomized to receive adjunctive sertraline or placebo, given in once-weekly escalating 100 mg/day doses up to 400 mg/day, which was then given for 8 weeks, then tapered. The primary endpoint was meningitis-free 6-month survival. The data and safety monitoring board halted the trial after 21 subjects were enrolled due to safety concerns. Meningitis-free 6-month survival occurred in 9 of 11 of placebo participants and 10 of 10 of sertraline participants. However, seven serious adverse events (SAEs) occurred ($n = 4$ sertraline group; $n = 3$ placebo group). Three SAEs in the sertraline group presented with psychosis and aggressive behavioral changes with one meeting Hunter's criteria for serotonin syndrome while receiving 200 mg/day sertraline. Two transient psychoses were associated with antecedent fluconazole and sertraline interruption. The serotonin syndrome resolved within 1 day, but psychosis persisted for 4 months after sertraline discontinuation. Sertraline was associated with excess SAEs of psychosis. Due to early stopping, we were unable to determine any efficacy for cryptococcal antigenemia.

Key words: cryptococcosis, cryptococcal meningitis, preemptive therapy, sertraline, clinical trial.

Introduction

Cryptococcosis causes 15% of AIDS-related mortality;[1] however, there is a prolonged duration of early disseminated asymptomatic infection during which the cryptococcal antigen (CrAg) is detectable in peripheral blood.[2,3] This asymptomatic cryptococcal antigenemia lasts for weeks to months prior to onset on meningitis symptoms.[4] Preemptive fluconazole therapy given to asymptomatic CrAg-positive persons has a survival benefit over human immunodeficiency virus (HIV) antiretroviral therapy (ART) alone;[3,5–7] however, 25–30% of preemptively treated asymptomatic CrAg-positive patients die or unmask meningitis

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within 6 months after starting ART.[8] Enhanced antifungal therapy is likely needed in persons with higher cryptococcal burden. However, limited antifungal options exist in low and middle income countries where flucytosine is not available and amphotericin is scarce and impractical to administer.[9]

Sertraline, the selective serotonin reuptake inhibitor (SSRI) antidepressant, has *in vitro* and *in vivo* activity against *Cryptococcus neoformans* in murine models.[10,11] Sertraline's putative mechanism of action is interference with *Cryptococcus* protein synthesis via eukaryotic translational initiation factor 3 (Tif3).[11] Four studies have demonstrated a median minimum inhibitory concentration (MIC_{50}) of 4 μg/ml among 503 clinical isolates.[11–14] Mouse model studies demonstrated the synergy between sertraline and fluconazole with reductions in *Cryptococcus* in brain and a survival benefit in mice.

Nevertheless, in a phase III trial of adjunctive sertraline given with amphotericin B and fluconazole for cryptococcal meningitis, there was no survival benefit or improved fungal cerebrospinal fluid (CSF) clearance.[15] In that trial, sertraline was given at 400 mg/day for 2 weeks before being reduced to 200 mg/day. The higher 400 mg sertraline dose was used to compensate for the induction of hepatic P450 metabolism by efavirenz.[12] However, in the meningitis trial, only 2 weeks of high dose sertraline was given due to an unknown long-term safety profile. Pharmacokinetic measurements revealed that it took 1 week to reach steady state, therapeutic levels that were achieved for 1 week before the dose was lowered. The induction of metabolism was much greater than anticipated. The authors hypothesized that the lack of observed benefit was due to the short duration of therapeutic sertraline levels, lack of further benefit in the setting of concomitant amphotericin B, or the overall critically ill condition of meningitis patients where further antimicrobial-directed therapy may have minimal survival benefit.[15]

In this trial, we hypothesized that adjunctive sertraline when added to standard of care preemptive fluconazole therapy for asymptomatic CrAg-positive persons would have a survival benefit. By augmenting fluconazole activity, we hypothesized this would improve antifungal activity and also cover for emerging azole resistance[14] or selection of fluconazole hetero-resistance.[16]

Methods

Study design

We conducted a randomized, double blind, placebo-controlled clinical trial testing adjunctive sertraline among HIV-infected persons with asymptomatic cryptococcal antigenemia (ClinicalTrials.gov Identifier: NCT03002012). Three sites in Kampala, Masaka, and Mbarara, Uganda, were planned to participate. Two sites opened for enrollment in Kampala and Masaka. We enrolled participants from 15 November 2017 to 27 February 2018. All CrAg-positive subjects received standard therapy of fluconazole 800 mg for 2 weeks, then 400 mg through 12 weeks, then 200 mg through 24 weeks.[17,18]

Participants were randomized 1:1 in permutated-blocks to adjunctive sertraline or placebo in blinded 100 mg tablets. A computer-generated randomization list, accessible only to the central study pharmacist in Kampala, was used to prepare blinded medications for the three study sites. All study medicines had a unique code recorded separately by the pharmacist and study medical officer, which was verified by the biostatistician. Sertraline was initiated at 100 mg/day for 1 week, thereafter escalating by 100 mg each week up to 400 mg/day by week 4 and thereafter continued at 400 mg/day for 8 weeks (through week 12), and then tapered over 3 weeks for 15 weeks in total. For both the escalation phase and tapering phase, participants were dispensed blister packs with the requisite number of tablets present. A US FDA-approved generic Sertraline (NorthStarRx) was used, and a matched placebo was manufactured by KPI, Kampala, Uganda.

ART was initiated after receiving 2 weeks of antifungal therapy in accordance with international guidelines for CrAg-positive persons.[18,19] For those who presented while on ART with suspected virologic failure, HIV viral load testing was done at study entry, and participants were re-counseled regarding ART adherence at next visit (week 2). A repeat viral load was obtained four weeks later, and participants with virologic failure (>1000 copies/ml) were switched to second-line ART at the next visit (~8 weeks), in accordance with World Health Organization (WHO) recommendations and Ugandan national guidelines.[20]

Participants

Participants were identified by lab-based reflex CrAg screening, per national and WHO guidelines, which recommend CrAg screening in CD4 < 100 cells/μl.[18,19] We processed leftover plasma remnants with a CrAg LFA (Immy Inc., Norman, OK, USA) at central CD4 testing facilities. Participants were identified at their outlying clinics and referred to research clinics at the Infectious Diseases Institute or Masaka Medical Research Council Field Station. Additional outreach occurred for referrals of CrAg-positive individuals from nearby community HIV clinics. Any outside laboratory CrAg result was verified by research staff on plasma. Inclusion criteria were: HIV-infected adults aged from ≥18 years to <65 years with CrAg positivity in blood who provided written informed consent. All women of childbearing potential had to consent to use one reliable method of contraception while receiving fluconazole ≥400 mg/day, due to the teratogenicity of high-dose fluconazole. Exclusion criteria included: prior history of cryptococcal meningitis, suspected meningitis or mania, suspected or known cirrhosis, jaundice, or ALT > 5× upper limit of normal, receiving an antidepressant, receiving antifungal therapy for >1 week, breastfeeding or pregnant, contraindication to sertraline or fluconazole, current rifampin

use, or electrocardiogram with QTc interval of >450 ms. Any person with a positive blood CrAg whose symptoms were suggestive of possible meningitis had a lumbar puncture performed to exclude CSF CrAg positivity. Persons with a negative CSF CrAg remained eligible. Persons without central nervous system (CNS) symptoms (i.e., asymptomatic CrAg-positive) did not routinely have lumbar punctures performed.

Endpoints and monitoring

The primary endpoint was 6-month meningitis-free survival. Secondary endpoints included: 6-month survival time, incidence of symptomatic cryptococcal meningitis, incidence of grade 3–5 clinical adverse events or serious adverse events, incidence of grade 3–5 laboratory adverse events, incidence of premature study drug/placebo discontinuation, and prevalence of depression by PHQ-9 score over 4, 8, and 12 weeks. The protocol specified an expected mortality in the standard of care control group of 25%,[7,21,22] and a 30% incidence of SAEs due to hospitalizations (with an overall expected 40% SAE incidence).

Prior to entry, ALT was measured at a screening visit. Thereafter participants returned, typically the next day, for enrollment. At baseline, 4, 8, and 12 weeks, monitoring included: complete blood count with platelets; serum Na^+, K^+, creatinine, ALT, total-bilirubin; and EKG. There was no QT prolongation with fluconazole 800 mg/day and sertraline 400 mg/day in HIV-infected Ugandans;[12] however, electrocardiograms were performed for an abundance of caution. As suicidal ideation is listed as a black box warning for sertraline, participants were contacted weekly over the first 6 weeks to monitor for thoughts of self-harm.

The National Institute of Allergy and Infectious Diseases (NIAID) HIV Complications and Coinfections data and safety monitoring board (DSMB) provided safety oversight with reviews of the trial prior to initiation and at 3.5 months after initiation.

Statistical methods

The trial was powered with 300 subjects per group ($n = 600$ in total) to achieve 80% power at a 0.05 significance level to detect a hazard ratio of 0.60 via a two-sided log-rank test when the proportion with meningitis-free survival with retention-in-care in the control group was 75%, equating to an approximate survival of 84% in the intervention group.[23] There was 90% power to detect a hazard ratio of 0.55 equating to 85% meningitis-free survival. The original analysis plan is provided in the clinical trial protocol (Supplemental Appendix 1).

Results

We screened 48 CrAg-positive persons with CD4 < 100 cell/μl and enrolled 22 participants beginning 15 November 2017. Reasons for ineligibility (not mutually exclusive) included: symptomatic CNS cryptococcosis ($n = 14$), repeat CrAg-negative on plasma ($n = 6$), active TB ($n = 3$), pregnancy ($n = 1$), prior fluconazole for >1 week ($n = 2$), and elevated ALT ($n = 1$). Of the 22 randomized participants, one person was administratively withdrawn within 30 minutes after randomization and prior to receipt of any therapy, following review of a head computed tomography (CT) scan, which prompted a lumbar puncture, revealing positive CSF CrAg.

For the 21 persons randomized and in the final data analysis cohort, the median age was 32 years (interquartile range [IQR], 30 to 35), and 12 (57%) were women. The median CD4 T cell count was 40 (IQR, 21 to 83) cells/μl. Somewhat unexpectedly, 67% ($n = 14$) were receiving ART at time of CrAg screening, with 7 having virologic failure and 7 who had recently started ART in the weeks prior. Baseline demographics are presented in Table 1 by randomized group.

Enrollment was halted on 27 February 2018 by the DSMB after two subjects had a SAE requiring hospitalization for mania and psychosis (sertraline group), and one additional subject had died at home of progressive symptoms consistent with cryptococcal meningitis (placebo group). By the time of the DSMB meeting on 13 March 2018, six SAEs had occurred (five hospitalizations and one death) among the 21 subjects (29% SAE incidence). A third subject also had SAE for mania (sertraline group), and one subject was hospitalized for pulmonary embolus (placebo group). At time of stopping, four SAEs had occurred in the sertraline arm and two in the placebo arm, including one death (Table 2). One SAE in the placebo arm of meningitis occurred after trial discontinuation. DSMB reports are provided as Supplemental Appendix 2. The DSMB was 'extremely concerned about the three SAEs and believes the events are likely related to' sertraline. 'The DSMB felt that this <SAE> rate was above the acceptable rate to continue the study.'

Overall, meningitis-free 6-month survival occurred in 10 of 10 of sertraline participants and 9 of 11 of control participants. Among the control participants receiving placebo, one presented with symptomatic meningitis, and a second subject died at home from suspected meningitis coupled with seizures. In the control arm, the three subjects who experienced SAEs ($n = 2$ hospitalizations, $n = 1$ death) included SAEs of death, symptomatic meningitis, and bilateral pulmonary embolism. In the sertraline arm, the four subjects who experienced SAEs were hospitalized.

Three SAEs in the sertraline group were classified as psychosis and aggressive behavioral change with one of these meeting criteria for possible serotonin syndrome based on agitation and inducible clonus. CSF examination at time of the SAEs did not reveal meningeal cryptococcosis, tuberculosis, or other viral pathogens. Transient psychosis among two of the participants was associated with fluconazole and sertraline interruption with documented low plasma levels of both of these medicines.

The first participant had stopped their medications at 6.5 weeks for 9 days while traveling for work. They restarted

Table 1. Baseline characteristics by treatment group.

	Sertraline		Placebo		Overall	
	No. with data	*N* (%) or median (IQR)	No. with data	*N* (%) or median (IQR)	No. with data	*N* (%) or median (IQR)
Participants, n		10		11		21
Age, years	10	32 [26, 32]	11	35 [32, 38]	21	32 [30, 35]
Men	10	5 (50.0%)	11	4 (36.4%)	21	9 (42.9%)
On antiretroviral therapy (ART)	10	6 (60.0%)	11	8 (72.7%)	21	14 (66.7%)
Months on ART	6	24.2 [0.7, 58.8]	8	14.1 [0.6, 31.2]	14	14.1 [0.7, 47.5]
PHQ depression score	10	7 [3, 8]	11	5 [4, 8]	21	5 [4, 8]
Heart rate, beats/minute	10	83 [68, 96]	11	78 [69, 92]	21	80 [69, 92]
QTc interval, msec	10	396 [384, 409]	11	404 [393, 425]	21	398 [388, 410]
$CD4^+$ T cells/μl	10	39 [24, 86]	7	50 [7, 83]	17	40 [21, 83]
White blood cells $\times 10^9$/l	10	3.6 [2.7, 4.4]	11	3.4 [3.3, 4.8]	21	3.4 [2.9, 4.4]
Hemoglobin, g/dl	10	11.3 [9.7, 12.0]	11	12.9 [11.2, 13.9]	21	11.6 [11.1, 13.4]
Platelets $\times 10^9$/l	10	195 [175, 261]	11	217 [170, 250]	21	206 [175, 260]
Sodium, mEq/l	10	136 [135, 137]	11	135 [132, 137]	21	135 [134, 137]
Potassium, mEq/l	10	4.3 [4.0, 4.4]	11	4.4 [4.1, 4.7]	21	4.3 [4.1, 4.5]
Creatinine, mg/dl	10	0.8 [0.7, 0.9]	10	0.8 [0.7, 0.8]	20	0.8 [0.7, 0.9]
ALT, U/l	10	16 [14, 45]	11	20 [13, 36]	21	17 [14, 36]
Total bilirubin, mg/dl	9	0.3 [0.3, 0.4]	11	0.3 [0.2, 0.4]	20	0.3 [0.3, 0.4]
Plasma CrAg Titer ≤1:40	10	8 (80%)	10	9 (82%)	20	17 (81%)
1:160		0		1 (9%)		1 (4.5%)
1:320		2 (20%)		0 (0%)		2 (10%)
Baseline LPs performed to exclude meningitis	10	2 (20%)	11	1 (9%)	21	3 (14%)

Values are *n* (%), median (IQR). PHQ depression score: 0–4 none; 5–9 mild, 10–14 moderate, 15–19 moderate-severe, or 20–27 severe depression.

Table 2. Serious adverse events (SAEs) observed.

ID number	Group	Days to event	AE grade	Certainty of diagnosis	Expected AE?	Related to study drug?[a]	Related to HIV?	Final SAE diagnosis
11015	Placebo	13	5	Definite	Yes	Not related	Definite	Death due to meningitis syndrome (declined LP)
11044	Placebo	38	3	Definite	No	Possible	Probable	Bilateral pulmonary embolism
11054	Placebo	88	3	Probable	Yes	Not related	Possible	Meningitis, complicated by CSF leak
11017	Sertraline	98	3	Probable	Yes	Possible	Definite	Acute gastrointestinal bleed, anemia
11019	Sertraline	58	3	Definite	No	Possible	Possible	Brief psychotic episode with gastroenteritis[b]
11028	Sertraline	40	3	Definite	No	Possible	Possible	Mania[a]
11055	Sertraline	17	3	Definite	No	Probable	Possible	Psychosis, serotonin syndrome (w/clonus)[b]
	Sertraline	32	3	Definite	No	Not related	Probable	Mania, psychosis (ongoing without sertraline)

[a] Assessment of relationship to the sertraline study drug was blinded.

[b] *Cryptococcus* RNA was detected in the CSF by metagenomic next-generation sequencing.

fluconazole and sertraline on day 55 having undetectable sertraline levels < 1 ng/ml and trace fluconazole levels of 0.2 μg/ml. On day 56, they developed an acute gastroenteritis illness with vomiting and diarrhea, being treated with metronidazole by an outside clinic. On day 58, the participant presented with aggressive behavior, increased talkativeness, transient athetoid movements, visual and auditory hallucinations. CSF exam was negative. Head CT scan revealed an old skull fracture, consistent with the participant's medical history. Ceftriaxone and haloperidol were given with resolution of the gastrointestinal illness and clouded sensorium.

In the second participant with an SAE, they presented with excessive talking, euphoric mood, increased energy, reduced need for sleep, and hypersexuality on day 40. They had stopped all their medicines prior to the SAE. CSF exam was negative. Brain magnetic resonance imaging (MRI) was unremarkable. All medications were restarted. Sertraline plasma levels were 175 ng/ml (<10th percentile) and fluconazole levels 1.2 μg/ml (<5th percentile) two weeks prior to the adverse event, and 386 ng/ml (60th percentile) and 6.4 μg/ml (10th percentile), respectively, after the SAE had resolved.

In the first two SAEs, prompt psychiatric consultation occurred. Haloperidol was given, and the altered mental status resolved. Fluconazole 400 mg and blinded-study medicine (sertraline 400 mg) were restarted during hospitalization at the direction of the psychiatrist. The haloperidol was then tapered off over several weeks, and the psychosis did not recur. Thus, with rechallenge of the potential offending medication of sertraline, the psychosis did not recur.

In a third subject, agitation occurred coupled with transient fever and hyperreflexia on day 16. Psychiatric symptoms included: abnormal behavior characterized by over talking, agitation, restlessness, delusions of grandeur, disorientation in time and day, and divine ideations. Inducible clonus and tachycardia were present on day 17. This met Hunter's criteria for possible serotonin syndrome. Sertraline was being taken at 200 mg/day and was discontinued on day 12 by the participant. Sertraline was not restarted. The clonus, fever and agitation rapidly resolved within 24 hours but psychosis requiring antipsychotic medications persisted for 4 months. Brain MRI was unremarkable. Further case details for the three SAEs are provided in Supplemental Appendix 3.

Four participants received diagnostic lumbar punctures to evaluate for CNS disease. One subject (placebo) had clinical meningitis despite a negative CSF CrAg. Three subjects with SAEs of psychosis all had negative diagnostic lumbar punctures. Diagnostic work up included CSF cell count, CSF protein, CSF CrAg, quantitative fungal culture with limit of detection of 10 cfu/ml, Biofire FilmArray meningitis-encephalitis multiplex polymerase chain reaction (PCR) (Biofire, Salt Lake City, UT, USA), Gram's stain, bacterial culture, and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) for tuberculosis meningitis diagnostics. This diagnostic evaluation was negative in all three SAE subjects. Cryopreserved CSF was then tested in the laboratory of Dr. Michael Wilson at the University of California at San Francisco for metagenomic next-generation sequencing of RNA. *Cryptococcus* RNA was found in the CSF of each of the three sertraline participants with SAEs, but no other pathogens.

Discussion

We conducted a randomized controlled trial to determine whether adjunctive sertraline was synergistic with fluconazole to improve upon the 75% meningitis-free survival, which occurs with standard of care fluconazole preemptive therapy in HIV-infected persons with asymptomatic cryptococcal antigenemia. Due to drug-drug interaction induction of sertraline metabolism with efavirenz and other antiretrovirals,[12,24] the sertraline dose was higher in this study than typically used for depression. The trial was stopped early due to an unacceptably high adverse event rate of neuropsychiatric side effects in the sertraline arm.

The DSMB stated the three psychosis events were 'likely related' to sertraline. Initially, the DSMB was concerned these events were due to toxicity. When the sertraline plasma drug levels were revealed as undetectable or low in two of the participants, the DSMB stated the psychoses may have been due to stopping or restarting sertraline. There were four nonfatal SAEs in the sertraline arm, and three SAEs (one fatal) in the placebo arm. The DSMB felt that this SAE rate was above the acceptable rate to continue the study. The overall 33% SAE incidence was lower than the *a priori* 40% expected SAE incidence cited in the protocol.

We are unable to make an assessment regarding the possible efficacy of adjunctive sertraline to treat cryptococcal antigenemia. Despite the meningitis-free survival among participants who received sertraline, four subjects had serious adverse events in the sertraline arm versus three subjects in placebo arm. This was an unacceptable excess risk, due to the nature of the adverse events. In the 460 subject phase III randomized trial of sertraline for cryptococcal meningitis, there was not an excess of adverse events in the sertraline arm who received directly observed therapy with 400 mg/day of sertraline for 2 weeks, then 200 mg/day.[15]

Sertraline does not typically cause psychosis. Four case reports have described sertraline being associated with paranoid reactions, hallucinations, and psychosis,[25,26] as observed in the participants. Making the situation more complicated, mania occurs with CNS cryptococcosis, which is why mania was an exclusion criterion for the trial. The exact prevalence of mania in cryptococcosis is unclear as this has mostly been described in case reports.[27–32] Our prospective, unpublished experience has psychiatric and behavioral changes as common presenting features of CNS cryptococcosis. During the Cryptococcal Optimal

ART Timing trial and thereafter in 2010–2013,[33] 6% (19/301) of cryptococcal meningitis research participants presented with behavioral abnormalities (e.g., mania, aggressive behavior). Similarly, overt psychosis or mania requiring haloperidol administration at meningitis presentation occurred in 4% (22/536) of cryptococcal meningitis research participants enrolled during 2013–2017 in Uganda.[12,15] Indeed, 1% (5/536) were first admitted to a psychiatric unit for psychosis or mania before eventually being transferred to a medicine ward for meningitis care. Other cryptococcal cohorts have not described mania. Thus, perhaps mania/psychosis is a Ugandan specific event due to *Cryptococcus* or host genetics. Alternatively, mania may be underreported. We suggest that new mania or neuropsychiatric conditions presenting in an HIV-infected persons with advanced HIV disease (CD4 < 200 cells/μl) should always prompt CrAg testing.

The investigators believed that the psychosis occurred due to progression of cryptococcosis in the brain, caused by medication interruption. Sertraline may have indeed been contributory with its mild side effects predisposing to medication nonadherence. As this was a double blind trial, the additional pill burden was equal between arms, but this could have also been contributory in those specific individuals. As described, *Cryptococcus* can have brain involvement with symptomatic antigenemia without CSF involvement.[34] *Cryptococcus* readily metabolizes L-dopa into melanin,[35] and *Cryptococcus* can also metabolize inositol as an energy source.[36] In humans without cryptococcosis, decreased L-dopa or decreased inositol have been associated with psychosis and schizophrenia.[37,38] The metabolic derangements observed in cryptococcal meningitis and associated reversible neuropsychiatric manifestations may be an interesting area of future study of CSF metabolomics. Ultimately, mania/psychosis occurred only in the sertraline arm, even in the absence of detectable sertraline. Subsequently, metagenomic next-generation sequencing identified *Cryptococcus* RNA in the CSF of the three sertraline SAE subjects;[39] however, whether detection of DNA/RNA is common in asymptomatic antigenemia is unknown.

One important question which was raised during the trial was the optimal time to initiate ART or switch to second-line ART in the context of cryptococcal antigenemia. When to start or switch ART in asymptomatic CrAg-positive persons remains an area with uncertainty.

The limitations of this study are inherent in the 21 participant sample size. The planned primary and secondary endpoints were unable to be assessed with any statistical power. Ultimately, the safety concern of sertraline was viewed as a danger; and the trial was halted. Yet we hope that this is not the last clinical trial on asymptomatic cryptococcal antigenemia. Improving the outcomes of this CrAg-positive population is important, as cryptococcal antigenemia averages 6% prevalence in CD4 < 100 cells/μl in outpatient settings in low and middle-income countries. CrAg prevalence in hospital settings is 2.5-fold higher than outpatient settings.[7,21,40] One should be able to improve upon a 75% meningitis-free survival rate,[22] and likely ideal care would risk stratify those with a blood CrAg titer $\geq$ 1:160 for enhanced antifungal therapy,[8] which would be cost effective as preemptive therapy as costs of treating overt cryptococcal meningitis would be averted.

The delayed timing of mortality in CrAg-positive persons with higher CrAg titers suggests that more intensive antifungal therapy, prior to immune reconstitution, may have a positive impact on survival. Immune reconstitution alone is insufficient to prevent meningitis and often deadly once CrAg-positive.[3,5] Potential therapeutic options could include: higher/longer dose fluconazole, adjunctive flucytosine, or amphotericin B therapy.[41,42] Flucytosine may soon be available in sub Saharan Africa via the efforts of UNITAID. Amphotericin is generally unavailable in low-income countries but available in middle-income countries. For an entity causing 15% of AIDS-related mortality,[1] more human clinical research is needed to prevent deaths from this neglected disease.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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Authorship Contributions

D.R.B. and D.B.M. conceived of the trial. D.R.B. wrote the clinical protocol with statistical assistance from K.H.H. and input from E.N., R.R., A.S.B., D.B.M., and NIAID personnel. K.H.H. and A.S.B. generated the randomization sequence. K.H.H. and A.S.B. conducted statistical analysis, with the analysis being guaranteed by K.H.H. Site project managers were: E.N. in Kampala and Y.N. in Masaka. Clinical data were collected in Kampala by E.N., P.K., N.R., F.T., S.N.; and among hospitalized participants by M.K.R. and C.P.S. D.R.B. wrote the first draft of the manuscript and is overall study guarantor. All authors reviewed, revised, and approved the final version of the manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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