10-13-2023

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Clinical Infectious Diseases

MAJOR ARTICLE

Single High Dose of Liposomal Amphotericin B in Human Immunodeficiency Virus/AIDS-Related Disseminated Histoplasmosis: A Randomized Trial

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Background. Histoplasmosis is a major AIDS-defining illness in Latin America. Liposomal amphotericin B (L-AmB) is the drug of choice for treatment, but access is restricted due to the high drug and hospitalization costs of the conventional long regimens.

Methods. Prospective randomized multicenter open-label trial of 1- or 2-dose induction therapy with L-AmB versus control for disseminated histoplasmosis in AIDS, followed by oral itraconazole therapy. We randomized subjects to: (i) single dose 10 mg/kg of L-AmB; (ii) 10 mg/kg of L-AmB on D1, and 5 mg/kg of L-AmB on D3; (iii) 3 mg/kg of L-AmB daily for 2 weeks (control). The primary outcome was clinical response (resolution of fever and signs/symptoms attributable to histoplasmosis) at day 14.

Results. A total of 118 subjects were randomized, and median $CD4^+$ counts, and clinical presentations were similar between arms. Infusion-related toxicity, kidney toxicity at multiple time-points, and frequency of anemia, hypokalemia, hypomagnesemia, and liver toxicity were similar. Day 14 clinical response was 84% for single-dose L-AmB, 69% 2-dose L-AmB, and 74% for control arm ($P = .69$). Overall survival on D14 was 89.0% (34/38) for single-dose L-AmB, 78.0% (29/37) for 2-dose L-AmB, and 92.1% (35/38) for control arm ($P = .82$).

Conclusions. One day induction therapy with 10 mg/kg of L-AmB in AIDS-related histoplasmosis was safe. Although clinical response may be non-inferior to standard L-AmB therapy, a confirmatory phase III clinical trial is needed. A single induction dose would markedly reduce drug-acquisition costs (>4-fold) and markedly shorten and simplify treatment, which are key points in terms of increased access.

Keywords. disseminated histoplasmosis; liposomal amphotericin B; AIDS; HIV; treatment.

Disseminated histoplasmosis (DH) is a major killer of people with human immunodeficiency virus (HIV)/AIDS (PWHA), resulting in more deaths than tuberculosis in the Americas [1]. The real burden of DH is not completely known, given the low recognition of disease and limited access to rapid *Histoplasma* antigen detection [2]. In different parts of the world, lack of access to rapid diagnostics leads to late diagnoses and high mortality [3]. Advanced HIV disease (AHD), despite increasing access to antiretroviral treatment, is still a major problem in most countries of the region [4]. Disruptions of HIV care driven by the coronavirus disease 2019 (COVID-19) pandemic has led to more AHD [5] and may also contribute to more HIV-associated histoplasmosis.

The recommended treatment for DH is liposomal amphotericin (L-AmB) for 2 weeks [6]; however, access to this drug is limited in most countries where histoplasmosis is endemic

Received 17 February 2023; editorial decision 15 May 2023; published online 26 May 2023

This work has been partially presented as a Late Breaking Clinical Trial during ID Week 2022, Washington D.C., USA.

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Clinical Infectious Diseases® 2023;77(8):1126–32

https://doi.org/10.1093/cid/ciad313

[7]. Patients are usually treated with high-dose regimens of amphotericin B deoxycholate (d-AmB) for at least 2 weeks, requiring hospitalization and costly laboratory monitoring of adverse effects. Nephrotoxicity is the main adverse event and is associated to increased mortality [8]. Other adverse reactions such as anemia and electrolyte abnormalities contribute to poor outcomes and intensive use of health resources [9]. Alternative modalities of treatment are ultimately needed.

Treatment strategies based on high single doses of L-AmB have been successful for visceral leishmaniasis and cryptococcosis [10, 11]. In visceral leishmaniasis, doses of 10 mg/kg of L-AmB are routinely used. Recently, based on the results of a randomized clinical trial, the World Health Organization has endorsed a single high-dose regimen of L-AmB for the treatment of cryptococcal meningitis [12]. Drug characteristics like the safer profile, long tissue half-life, and a higher concentration in the reticuloendothelial system (one of the sites in which *Histoplasma capsulatum* concentrates) make this strategy particularly attractive for the treatment of DH [13–15]. However, such treatment strategy has never been studied in histoplasmosis. Here we conducted an open-label, phase 2, multicenter, randomized trial, to investigate safety, clinical and mycological response of high-dose regimens of L-AmB for the treatment of histoplasmosis. We evaluated two experimental regimens (single dose of 10 mg per kilogram of L-AmB and one dose of 10 mg/kg on day 1 followed by a second dose of 5 mg per kilogram on day 3) along with a control arm using the World Health Organization (WHO)/ Pan American Health Organization (PAHO) recommended regimen (3 mg/kg per day for 14 days).

METHODS

Study Design and Oversight

This was a prospective multicenter randomized phase II non-inferiority open label trial, conducted from February 2020 to April 2022 in 6 Brazilian tertiary medical centers (Supplementary Appendix). The trial was registered at ClinicalTrials.gov: NCT04059770. The trial was conducted in accordance with the International Council for Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. The study was approved by the Brazilian National Committee of Ethics in Research and by the ethical committees of all participating centers. Participation in the study was voluntary and signed consent was obtained for all research individuals.

Trial Participants

The trial enrolled adult PWHA hospitalized patients diagnosed with DH using antigen detection, polymerase chain reaction, or culture/histopathology. Inclusion and exclusion criteria are detailed in the Supplementary Appendix.

Interventions and Procedures

We randomized study participants for induction therapy for DH for one of the three regimens: Single-dose L-AmB, 10 mg/kg single intravenous dose of L-AmB; 2-dose L-AmB, 10 mg/kg L-AmB (day 1), followed by 5 mg/kg (day 3); and control arm, L-AmB at 3 mg/kg/days intravenously for 2 weeks. After induction therapy, all patients received itraconazole capsules 400 mg daily (no recommendation regarding loading dose), which was started within 24 hours after induction therapy, and continued for 1 year. Physicians were recommended to initiate antiretroviral therapy as soon as possible, according to international guidelines [6]. Clinical and laboratory data collection and the preparation of the study drug are described in the Supplementary Appendix. Electrolyte supplements and prehydration were given to all individuals participating in the trial.

Randomization

Participants were subjected to a simple centralized randomization method 1:1:1, to one of the three study arms [16].

Primary Endpoint

Clinical resolution on day 14 was the primary endpoint, defined as resolution of fever and signs/symptoms attributable to histoplasmosis, except for manifestations that are expected to last for more than 14 days after treatment initiation: skin lesions, hepatosplenomegaly, jaundice, and pancytopenia.

Secondary Endpoints

Secondary endpoints were also determined on day 14 and included overall survival; renal function abnormalities; anemia; and liver function abnormalities. Kidney toxicity was classified according to the KDIGO system [17], and liver toxicity was determined using DAIDS Adverse Event Grading Tables [18]. Patients were followed up for one year after entering the study, and survival was evaluated at weeks 12, 24, and 48.

Statistical Analysis

Using a non-inferiority design, assuming an expected proportion of favorable response equals to 88% [15], a sample size of 29 subjects per arm would provide the trial with 80% of power to show non-inferiority of the alternative arms to the PAHO/ WHO recommended regimen, given a specified non-inferiority margin of 20% and 1-side $\alpha = 0.05$. Considering a dropout rate of 10%, the sample size per arm was calculated to 33 patients, and the total sample size for the study was 99 patients.

The primary analysis was performed in an intention-to-treat fashion, which included all the participants who had undergone randomization. Statistical tests were used in an exploratory way according to a pre-specified analysis plan. The primary outcome was evaluated using the proportions in each arm and respective confidence intervals. Patients who did not complete the initial 14 days of treatment were analyzed for the safety purposes only. Fishers exact test was used

for the comparisons of proportions. A Kaplan-Meier curve was constructed for the analysis of survival of the three trial arms. For the comparison of continuous variables of secondary outcomes, the Mann-Whitney test was utilized. Analyses were conducted using SPSS software, version 18.0 (IBM Corporation, New York, USA).

RESULTS

Trial Population

During the period of study, we screened 882 patients for the trial, the majority of whom did not have histoplasmosis (Figure 1). Of those screened, we excluded 764, resulting in 118 patients for the intention-to-treat population. Patients were randomized to a single high dose of L-AmB (10 mg/kg of L-AmB; n = 40); 2-dose L-AmB (10 mg/kg of L-AmB on day 1, followed by 5 mg/kg on day 3; n = 39); and control arm (3 mg/kg of L-AmB for 2 weeks; n = 39).

Most patients in the trial were male (97/118; 81.9%), and median age was 38 years old (range, 18–74 years old). Patients in the study had advanced HIV disease with median CD4 count of 25 cells/μL (range, 1–361 cells/μL) and median HIV viral load of 5.56 $\log_{10}$ copies/mL (range, 1.6–7.1 $\log_{10}$ copies/mL). Histoplasmosis was the AIDS-defining illness for 51% (60/118) patients in the study.

Clinical manifestations of DH included, weight loss 70% (83/118) of patients, respiratory complaints 67% (79/118), fever 66% (77/117), abdominal findings 59% (70/118), skin lesions 38% (45/118), lymphadenopathy 38% (45/118), and oral lesions 36% (42/118). Pancytopenia was present in 53% (59/112) of patients: hemoglobin <10 g/dL in 73%, lymphocytes < 500 cells/μL in 59%, platelets <100 000 cells/μL in 49%, and leukocytes <2500 cells/μL in 39%. In addition, ferritin was >3000 ng/mL in 75.0% of patients, serum aspartate aminotransferase (AST) was >3 times the upper limit of normal in 46%, lactate dehydrogenase was >1000 U/L in 38% (normal range, 100–190 U/l), and serum creatinine was >1.5 times the upper limit in 7% (protocol violations). Baseline characteristics of the study participants were similar across all study arms (Table 1).

Histoplasmosis was mainly diagnosed by the detection of *Histoplasma* antigen in the urine (114/118; 97%). Median antigen titers were 31.2 enzyme immunoassay (EIA) units (range, <0.01 to >500 EIA units). Alternative diagnostic methods included fungal culture (48/118; 41%), microscopy (37/118; 31%), analysis of peripheral blood smears (8/118; 7%), biopsy (4/118; 3%), and detection of antibodies (3/118; 2%). No patient was diagnosed using molecular methods or lysis centrifugation (Isolator™).

Primary Endpoint

Clinical response on day 14 was 84.0% for the single-dose L-AmB arm—(32/38—1 patient was excluded due to concomitant tuberculosis, and another was lost to follow-up). In contrast, clinical response occurred for 69.0% (25/36) for the 2-dose L-AmB arm, with 3 patients being excluded from efficacy analysis (1 individual with central nervous system histoplasmosis, 1 patient with concomitant tuberculosis, and another patient lost to follow-up); in the control group, response rate was 74.0% (28/38), with 1 patient being lost to follow-up. Reasons for failure included death (n = 15), persistent fever (n = 10), hypotension (n = 1), and respiratory failure (n = 1). Regarding clinical response, the absolute risk reductions (ARR) and 95% confidence interval (CI) for the single-dose L-AmB arm in comparison with control group was +10.5% (95% CI, −7.7% to 28.7%). For the 2-dose L-AmB arm, the ARR was −4.2% (95% CI, −24.8% to 16.3%) versus controls.

Secondary Endpoints

Overall survival on day 14 were, respectively: 89.0% (34/38) (single-dose L-AmB—with 2 patients being excluded from efficacy analysis); 78.0% (29/37) (2-dose L-AmB arm—2 patients excluded from efficacy analysis); and 89.7% (35/38) (control group—1 patient excluded from efficacy analysis). Survival did no differ at 1 year among study groups ($P = .440$) (Figure 2). Survival ARR for the single-dose L-AmB arm, in comparison to controls, was −2.6% (95% CI, −15.6% to 10.4%). Survival ARR for the 2-dose L-AmB arm was −13.7% (95%CI, −29.5% to 2.1%), in comparison to control group.

Kidney and liver toxicities were similar between the interventional groups and the control group (Figure 3). Serum potassium levels of <3.5 mg/dL on day 14 occurred in 9% (3/34) of patients in single-dose L-AmB arm; 19% (6/31) in 2 doses of L-AmB; and 33% (12/36) in control group ($P = .040$). Severe hypokalemia (<2.5 mg/dl) was not seen on day 14 in single-dose of L-AmB and 2 doses of L-AmB arms, as opposed to 6% (2/36) in control group ($P = .094$). Serum magnesium levels of <1.8 mg/dL were present in 57% (17/30) of patients in single-dose of L-AmB arm, 41% (12/29) in 2 doses of L-AmB arm, 42% (13/31) in control group ($P = .405$). Severe hypomagnesemia (<1.25 mg/dl) was seen in no patient in single-dose of L-AmB, in comparison to 7% (2/29) (2 doses of L-AmB), 3% (1/31) (control group) ($P = .493$).

Infusion-related toxicity occurred in 18.9% (7/37) of patients on day 1 in the single high-dose arm, in comparison to 14.3% (5/35) (2 doses arm), and 10.3% (4/39) (control arm) ($P = .561$). On day 3, infusion-related toxicity was reported in 0% (single high-dose arm), 2.9% (1/35) (2 doses arm), and 5.1% (2/39) (control arm). No patient discontinued the drug due to infusion-related toxicity in the study. Hemoglobin dropped >2 g/dL by day 14 in 21.2% (7/33), 13.3% (4/30), and 22.2% (8/36) of single high dose of AmB, 2 doses of L-AmB, and control group, respectively ($P = .618$).

DISCUSSION

In this phase II trial, we demonstrated that a regimen using a single high dose of L-AmB can be safely used as induction therapy

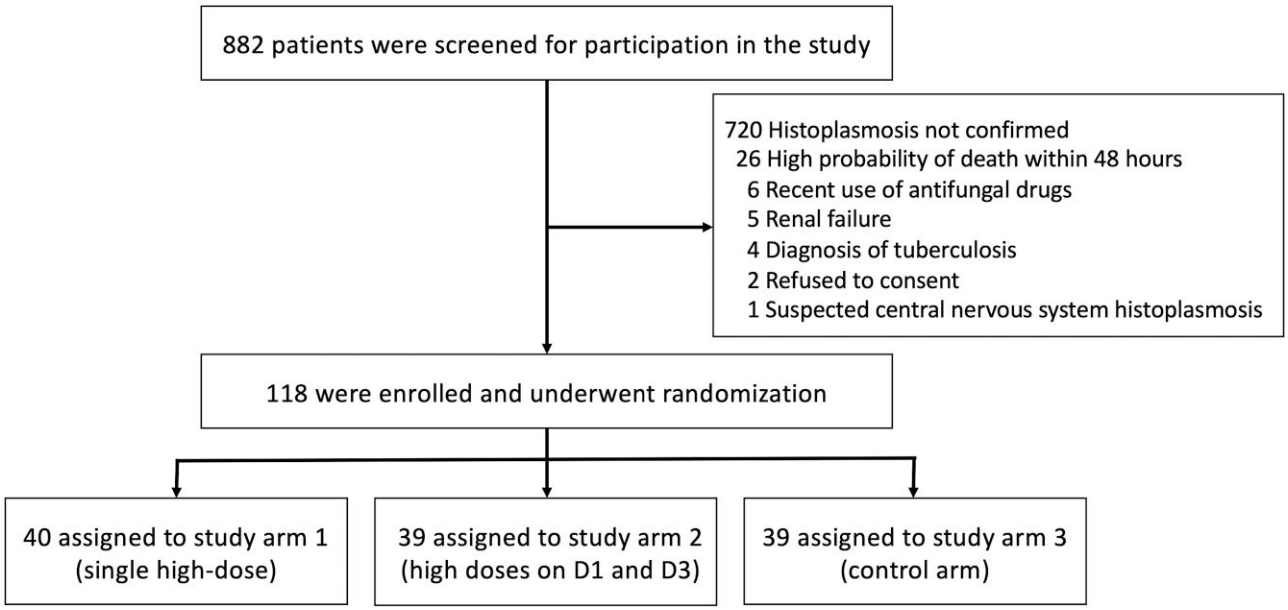

Figure 1. Randomization process.

Table 1. Baseline Demographic, Clinical, and Laboratorial Findings of AIDS Patients With Disseminated Histoplasmosis Included in Each of the Three Study Arms of Induction Therapy With Liposomal Amphotericin B in Brazil (2020–2022)

	Arm 1	Arm 2	Arm 3
Age in years (median)	40	39	39
Male sex	88%	72%	87%
On antiretroviral therapy	32%	38%	26%
CD4 count cells/μL (median)	27	27	22
Viral load, log_{10} (median)	5.3	5.2	5.6
Histoplasma antigen, EIV (median)	47.6	30.7	31.0
Karnofsky score ≤70	52%	49%	51%
Fever	70%	58%	69%
Weight loss	80%	56%	74%
Pulmonary symptoms	75%	69%	56%
Oral lesions	42%	33%	31%
Skin lesions	42%	31%	41%
Hemoglobin (median)	8.80	9.10	9.35
Leukocytes (median)	2930	3030	2850
Neutrophils (median)	2025	2363	2285
Lymphocytes (median)	443	313	431
Platelets (median)	90 000	135 000	93 000
Serum creatinine (median)	0.80	0.78	0.77
Lactate dehydrogenase (median)	496	618	691
Ferritin (median)	5193	8250	8250
Aspartate aminotransferase (median)	43	102	88
Alanine aminotransferase (median)	43	58	59

Arms 1 received single (10 mg/kg) high dose of lipossomal amphotericin B (n = 40).
Arm 2 received 10 mg/kg on day 1, followed by 5 mg/kg on day 3 (n = 39).
Arm 3 is the control group (3 mg/kg of liposomal amphotericin B for 2 weeks) (n = 39).
Abbreviation: EIV, enzyme immunoassay index value.

of AIDS patients with DH. Infusion-related toxicity in the single high-dose arm was observed at an acceptable rate (19%) and did not result in drug withdrawal. Nephrotoxicity in the single high-dose arm was low: 14% and 12% on days 7 and 14 of treatment, respectively, with no significant difference between arms. Electrolyte abnormalities were infrequent in the single high-dose arm. Hepatic dysfunction was not observed and reductions in hemoglobin were comparable to the control arm. Considering that the efficacy in the single high-dose arm was non-inferior to the standard of care, our results indicate that a shorter L-AmB regimen is feasible, may be better tolerated, and should be further evaluated for non-inferiority in a larger phase III trial.

We did not observe any severe infusion reactions and the documented episodes did not drive any treatment interruption. In the AMBITION-cm trial, where patients with HIV-related cryptococcal meningitis were evaluated, data on infusion reactions were not systematically collected, but 2 severe (grade 3) infusion reactions (which did not lead to drug withdrawal) were observed among 407 patients who received a single high-dose regimen of L-AmB [10]. Our rate of infusion reactions was 19%, still far lower than the 40% (of 304 patients) reported by Sundar and collaborators in a single high-dose amphotericin trial for the treatment of visceral leishmaniasis [11].

Our trial showed a non-significant reduction in renal toxicity in the single high dose of liposomal amphotericin as compared to the standard of care with 14 days of treatment. Data from studies of d-AmB have demonstrated association between

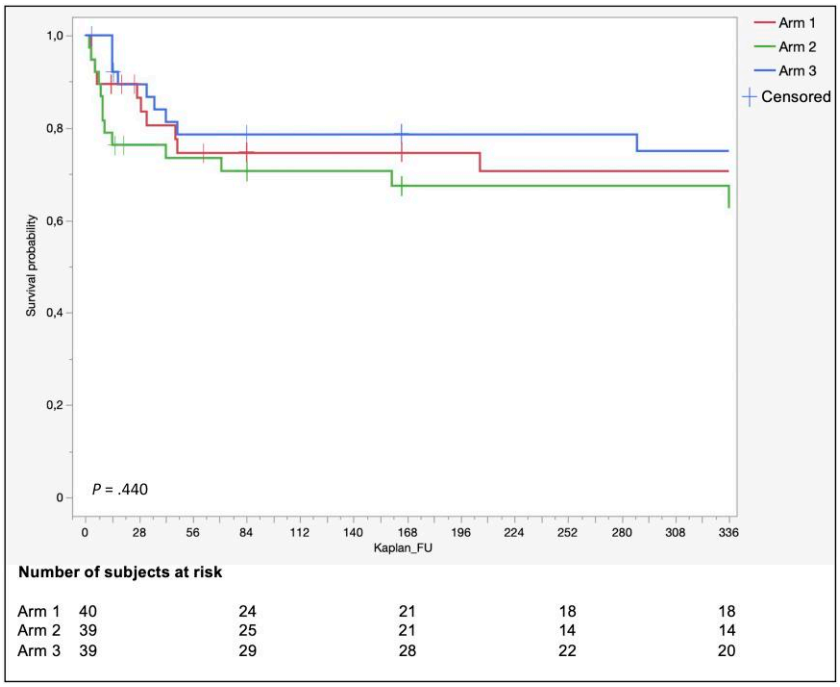

Figure 2. Cumulative survival per study arm. Arm 1 received single (10 mg/kg) high dose of lipossomal amphotericin B (n = 40). Arm 2 received 10 mg/kg on day 1, followed by 5 mg/kg on day 3 (n = 39). Arm 3 is the control group (3 mg/kg of liposomal amphotericin B for 2 weeks) (n = 39).

longer courses of treatment and a higher risk of nephrotoxicity [19, 20]. In fact, Luber and collaborators found that a greater cumulative dose of d-AmB and the use of concomitant nephrotoxic drugs were associated to an increased risk of renal toxicity [20]. However, extrapolation of these data must be interpreted with caution, because L-AmB and d-AmB are suggested to have distinct mechanisms of nephrotoxicity [21].

In line with our observations, single high-dose regimens have not been associated with increased kidney toxicity [10, 11]. The use of a 10 mg/kg dose of L-AmB for the treatment of cryptococcal meningitis was associated with only 4% Grade 3 (1.5 to < 2.0×participants' baseline) and 1.2% Grade 4 (of ≥2.0×participants' baseline) glomerular toxicity [10]. Our study, using the acute kidney injury definition by KDIGO [17] detected acute kidney injury in 12% of patients using the single high-dose L-AmB treatment. Even though not statistically different (P = .10), the rate was half of the observed in the control arm.

Sundar and collaborators also reported low rates (2%) of nephrotoxicity, defined by a doubled serum creatinine level from baseline and that exceeded 2.0 mg/dL, or that was more than 2.5 mg/dL, for the treatment of visceral leishmaniasis [11], reporting no severe electrolyte abnormalities. Rates of hypokalemia and hypomagnesemia observed in the single high-dose arm of our study were low and tend to be lower than the control arm.

Another practical concern regarding the use of a high dose of L-AmB are increases in serum bilirubin, alkaline phosphatase, and serum transaminases, which have been infrequently observed before [14]. Indeed, rises in serum transaminases appeared to occur with prolonged treatments [14]. We did not observe any signs of liver toxicity or increases of serum transaminases aside from what is expected in DH, a disease primarily affecting the reticuloendothelial system and consequently involving the liver [22, 23].

Considering the occurrence of this disease in AHD patients, anemia is a potentially life-threatening adverse event and must be addressed when using amphotericin B therapy. In a small retrospective study, L-AmB has been associated with anemia and thrombocytopenia in a dose-dependent manner [24]. However, the strategy of a single high dose of L-AmB was not associated to increased risk of anemia in the treatment of cryptococcosis and visceral leishmaniasis, performing better than the comparators in both studies [10, 11]. In our study, no differences between the high and standard dosing groups were observed regarding to hematological toxicity.

L-AmB has a dose-related, non-linear, saturation-like pharmacokinetics. The maximum area under the curve (AUC) and maximum concentrations (C_{max}) values are reached following the administration of 10 mg/kg/day, declining at 12.5 and 15 mg/kg/day [14]. In the phase I/II studies, the drug was well tolerated, without dose-limiting adverse effects, across this dose range [14]. When taken into effect measured in animal models, the best correlation with outcome is the ratio of C_{max} to minimal inhibitory concentration (C_{max}/MIC) [13].

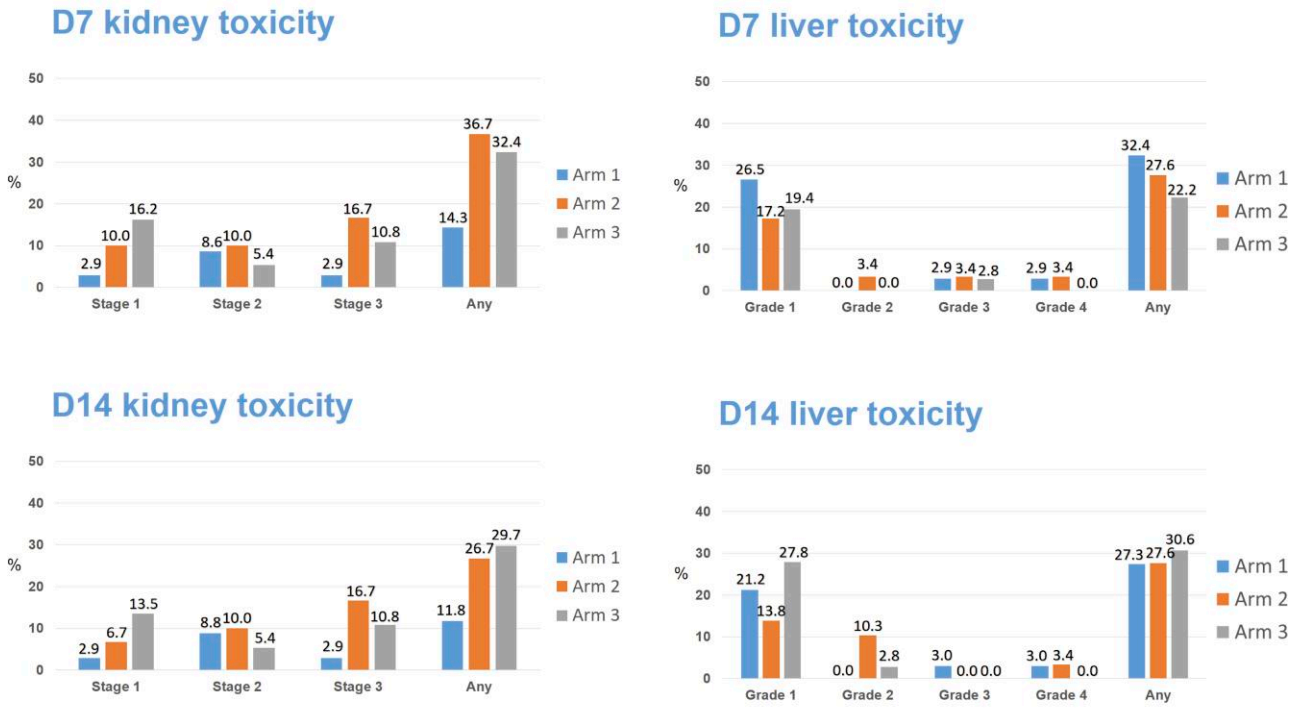

Figure 3. Kidney and liver toxicities per study arm on days 7 and 14 of study. Arm 1 received single (10 mg/kg) high dose of liposomal amphotericin B (n = 40). Arm 2 received 10 mg/kg on day 1, followed by 5 mg/kg on day 3 (n = 39). Arm 3 is the control group (3 mg/kg of liposomal amphotericin B for 2 weeks) (n = 39).

Time-kill studies proved the concentration-dependent fungicidal activity and prolonged post-antifungal effects for a duration of up to 12 hours [14]. These data support greater efficacy of higher doses of L-AmB, in comparison to standard regimens.

Furthermore, because DH is a disease that severely affects the liver, spleen, and reticuloendothelial system, the significant L-AmB penetration in these tissues, as a whole, supports the use of high dose for the treatment of DH evaluated in our trial [14, 22].

Our data indicate a similar clinical response and survival between the single high-dose regimen and the control arm. These observations provide a framework for the development of a clinical phase III trial comparing the single high-dose regimen to the WHO recommended regimen.

The study arm that used two doses of L-AmB (10 mg/kg on day 1 and 5 mg/kg on day 3) had a lower rate of clinical response, although not statistically significant. It seems likely that this was not due to a true biological effect but possibly likely statistical artefact due to the small sample size. Moreover, the absence of any practical or economical advantage of a regimen involving two doses, eliminates the need to further evaluate this regimen. It is worth noting that the clinical response and survival reported in our study is consonant with the only published trial using L-AmB for the treatment of DH to date [15].

Unfortunately, the use of L-AmB is not easily accessible in regions where it is most needed, for the treatment of diseases like cryptococcosis and histoplasmosis in persons with AHD [7, 25]. The single high-dose strategy evaluated here is feasible and its further study is urgently needed: if incorporated into clinical practice, it could be translated to a 4-fold cost-reduction in drug acquisition. This might be a critical step to significantly improve access.

Our study has limitations. The small number of patients included, which is a characteristic of phase II studies, precluded definitive conclusions about efficacy. Nonetheless, one of most important clinical trials including patients with DH and AIDS involved only 81 participants [15]. The modification of the protocol including previous liver abnormalities is aligned to the disease presentation and in fact reflects clinical practice. Even though the study was open-label, blindness would be not feasible in such a study design. Also, nearly all patients in the trial had "probable" histoplasmosis based on positive *Histoplasma* antigen rather than "proven" histoplasmosis based on positive culture and/or histopathology (therefore, earlier diagnoses were obtained, in comparison to those performed by classical mycological methods).

In conclusion, a single high dose of L-AmB followed by itraconazole proved to be safe and efficacious as induction therapy of DH in PWHA. There seems to be no advantage for a second

L-AmB dose on day 3. Toxicity was not an issue as the high dose was as safe as standard therapy. A phase III trial is currently being planned (NCT05814432).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. This was a collaborative study between the principal investigator (A. C. Pasqualotto) and Gilead Sciences. Gilead Sciences has provided researchers with the study drug, and has also given financial support to run the study. All *Histoplasma* antigen detection kits used in the study were kindly donated by IMMY.

Potential conflicts of interest. A. C. P. has received research grants from Gilead, Pfizer, and IMMY (who also provided diagnostic tests for the study); he reports travel support from Pfizer, United Medical, and Merck; participation on a Data Safety Monitoring or Advisory Board for Gilead; and payment or honoraria for talks on behalf of Gilead, United Medical, Pfizer, Merck, Sharp & Dohme (MSD), IMMY, Astra-Zeneca, and Astellas Pharma. D. R. F. has received research grants and consulting fees from Pfizer, MSD, and Gilead Sciences. D. R. F. also reports travel support from Pfizer, United Medical, Janssen, and Merck; participation on Data Safety Monitoring or Advisory Boards for Gilead Sciences, Merck, and GlaxoSmithKline (GSK); has given paid lectures on behalf of United Medical, Pfizer, Janssen, GSK, Merck, Gilead Sciences, Knight Pharmaceuticals, and MSD, and received non-financial research support from IMMY. N. C. B. reports grants from National Institutes of Health (NIH) (grant numbers NINDS K23 NS110470 and NIAID R01 AI170158) and Karyopharm therapeutics (Site PI for 2020 clinical trial; funds paid to institution); and participation as chair of the Data and Safety Monitoring Board (DSMB) for NCT04335123. A. S. reports grants from Astellas and Mayne, and consulting fees from GSK and F2G. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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