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Effectiveness and Safety of Liposomal Amphotericin B for Visceral Leishmaniasis under Routine Program Conditions in Bihar, India

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Abstract. We evaluated, through the prospective monitoring of 251 patients at Sadar Hospital in Bihar, India, the effectiveness and safety of 20 mg/kg body weight of liposomal amphotericin B for the treatment of visceral leishmaniasis. The treatment success rates for the intention-to-treat, per protocol, and intention-to-treat worse-case scenario analyses were 98.8%, 99.6%, and 81.3%, respectively. Nearly one-half of patients experienced mild adverse events, but only 1% developed serious but non–life-threatening lips swelling. The lost to follow-up rate was 17.5%. Our findings indicate that the 20 mg/kg body weight treatment dosage is effective and safe under routine program conditions. Given that the exorbitant cost of liposomal amphotericin B is a barrier to its widespread use, we recommend further study to monitor and evaluate a lowered dosage and a shorter treatment course.

INTRODUCTION

Visceral leishmaniasis (kala-azar) is a vector-borne parasitological disease transmitted to humans by the bite of an infected sandfly. The disease manifests with fever, asthenia, weight loss, anemia, pancytopenia, and splenomegaly, and it is fatal without effective treatment. Approximately one-fifth of the estimated 500,000 annual incident cases worldwide occur among the rural poor of India, Bangladesh, and Nepal.[1] In India, an estimated 165.4 million people are at risk of the disease, and cases have been reported in 52 districts. Disease underrecognition and poor access to medical facilities and treatment contribute to uncertain mortality figures.[2]

Kala-azar transmission in India is anthroponotic; successful treatment of infected humans, therefore, advances disease-control efforts.[2] However, ineffective and incomplete treatments hinder these efforts, with the latter contributing to parasite resistance to the limited drugs currently available.[3] New drugs or compounds are unlikely to become available in the near future because of under-funding and non-prioritization of kala-azar research and development initiatives.[3] Current efforts, therefore, focus on the identification and delivery of an effective, safe, and affordable treatment option from the existing drug regimen.

In Bihar, studies have identified drug resistance and toxicity of existing kala-azar treatments. Parasite resistance to sodium stibogluconate (SSG), a pentavalent antimonial and current first-line treatment of kala-azar in many endemic countries, has been proven, with fewer than 50% of patients achieving cure.[4–8] Moreover, sporadic availability, toxic and fatal side effects, and a 30-day painful intramuscular delivery contribute to poor adherence to the SSG treatment regimen and high healthcare costs.[4,9,10] Miltefosine (Impavido; Paladin Laboratories Inc., Quebec, Canada), an orally administered antileishmanial drug, cannot be used in women of childbearing age unless contraception is used for the duration of therapy and an additional 2 months after because of its teratogenic potential.[3,11,12] Treatment with miltefosine is also associated with gastrointestinal side effects.[11–13] Amphotericin B deoxycholate (Nicholas Piramal India Limited, Mumbai, India), requiring prolonged treatment and hospitalization with clinical and laboratory monitoring, is associated with adverse events, high treatment cost, and inconsistent availability in India.[3,14] Paromomycin (Gland Pharma Ltd., Hyderabad, India), a well-tolerated and effective aminoglycoside antibiotic, is currently being assessed in a phase IV clinical trial. It requires an inexpensive 21-day intramuscular injection course; treatment may be potentially affordable because of lowered costs through local manufacturing.[14]

Liposomal amphotericin B (AmBisome; Gilead Sciences, Foster City, CA) has a relatively short treatment course and causes minor adverse events (e.g., fever and/or rigor).[15] Encouraged by its high efficacy and low toxicity, the World Health Organization (WHO) selected liposomal amphotericin B as having the highest therapeutic index of existing antileishmanial drugs.[16] Previously, liposomal amphotericin B was available at 30 times the cost of conventional formulations[17,18]; the current preferential cost of 20 United States dollars (USD) per 50 mg vial[16] remains unaffordable for the impoverished. In India, efficacy and safety of liposomal amphotericin B have been studied at a range of total dosage.[15,19–23] Prior assessments varied from 3.75 mg/kg body weight to 15 mg/kg, with 89–100% shown efficacy. Thakur[22] reported a single 15-mg/kg dose to be well-tolerated and effective, whereas Sundar and others[20] showed the potential of a 7.5-mg/kg single dose and reported a 90% cure rate. Single-dose liposomal amphotericin B at 10 mg/kg has been shown to be non-inferior to conventional amphotericin B in a phase III clinical trial.[24] To date, however, a common limitation of published studies to show efficacy and safety of 20 mg/kg liposomal amphotericin B has been the small patient cohort.

In collaboration with Indian health authorities at the national, state, and district levels, Médecins Sans Frontières (MSF) diagnoses and treats kala-azar patients at the Sadar District Hospital in the municipality of Hajipur, Vaishali District, Bihar State, India, a major epicenter of the disease. Throughout Vaishali District, Indian health authorities have replaced SSG with liposomal amphotericin B as the first-line treatment of kala-azar. As optimum liposomal amphotericin B

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dosage remains inconclusive, the treatment is administered according to the current WHO recommendation: a total dose of 20 mg/kg administered intravenously at 5-mg/kg doses on days 0, 1, 4, and 9.[16,25]

Through the prospective monitoring of patients treated with liposomal amphotericin B, this study aimed to contribute to the knowledge base for kala-azar medical management in Bihar. We hypothesized that a total dosage of 20 mg/kg over 10 days, if determined to be effective and safe under routine program conditions, could be benchmarked for recommended use in India while lowered drug increments are assessed in future studies. Because impoverished patients in high-prevalence regions are currently unable to afford a complete treatment course of liposomal amphotericin B, a lowered dosage and shorter treatment course, in mono or combination therapy, may broaden access to treatment by lowering drug-procurement and hospitalization costs and reducing the number of lost workdays for patients and caregivers.

The National Vector-Borne Disease Control Program and the Health Ministry Screening Committee of the Rajendra Memorial Research Institute of Medical Sciences (RMRIMS) granted approval for the MSF kala-azar treatment program and patient-monitoring initiatives. The objectives of these initiatives were as follows: (1) evaluate the effectiveness of liposomal amphotericin B for successful treatment of visceral leishmaniasis; (2) assess the drug safety; and (3) based on the findings, recommend future study options for furthering the evidence base for optimum dosage of liposomal amphotericin B.

MATERIALS AND METHODS

Study location and participants. We conducted this observational cohort study from July 2007 to May 2008 at Sadar District Hospital, which serves approximately 2.2 million inhabitants of Vaishali District in Bihar, India. The patient sample size was set at 250, which is sufficient, with an expected 10% loss to follow-up, to detect a treatment success rate of 95% with ± 4% precision.

Patient inclusion criteria were defined as follows: (1) clinical symptoms consistent with kala-azar, (2) ≥ 2 years of age, (3) residence within Vaishali District, (4) provision of written informed consent, and (5) a positive rK39 rapid diagnostic test (DiaMed-IT-Leish; in northeast India, rk39 has shown 99% sensitivity and 100% specificity with whole-blood samples) or a parasitological diagnostic test result. Patients excluded from the study were those with (1) post–kala-azar dermal leishmaniasis, (2) prior treatment of current kala-azar infection with ≥ 20 mg/kg of liposomal amphotericin B, (3) known allergic reaction to amphotericin B, or (4) human immunodeficiency virus, tuberculosis, or malaria co-infections.

Consenting patients provided 8–12 μL of whole blood, the amount required for rK39 *Leishmania* antibody detection. The rK39-negative patients were classified as non–kala-azar cases and reexamined for an alternative illness. If kala-azar clinical suspicion remained, patients were transferred to RMRIMS, based in Patna (the Bihar State capital), for parasitological analysis by splenic or bone-marrow aspiration. Parasitologically confirmed patients returned to Sadar District Hospital for study enrollment, whereas negative patients remained at RMRIMS for alternative illness diagnosis.

Study procedures. Enrolled patients received a total dose of 20 mg/kg of liposomal amphotericin B intravenously at 5-mg/kg doses on days 0, 1, 4, and 9. Each 5-mg/kg dose was initially constituted with sterile water and diluted with 5% dextrose to achieve a final concentration of 0.50–2.0 mg/mL, and then, it was administered over 2 hours. Patients were hospitalized throughout the 10-day treatment course and evaluated daily by kala-azar–experienced clinicians. Paromomycin, donated by the Institute for OneWorld Health, was made available as a second-line treatment in the event of treatment failure.

After receiving the final treatment dose on day 9, the patient was discharged and requested to return to the Sadar District Hospital for scheduled 3- and 6-month follow-up visits for clinical assessment. Patients were followed for 6 months, because most kala-azar treatment relapses occur during this period.[26] An MSF medical team conducted active follow-up if a patient failed to return for his or her scheduled visit. The medical team consisted of two kala-azar–experienced health professionals who visited the patient at his or her residence to conduct a clinical evaluation to determine treatment success or failure. If the patient was not located, an alternative time was arranged to return to the household within the following 7 days. If the patient was not located within the arranged time, a lost to follow-up outcome was assigned.

Study variables. Patient demographics and clinical history were recorded at study enrolment (day 0). Demographics included age, gender, caste, and pregnancy status for women of childbearing age between 12 and 44 years. Clinical history included self-reported number of weeks ill with current kala-azar symptoms and any prior antileishmanial treatment received for current kala-azar infection.

Clinical characteristics, assessed at study enrollment, end treatment (day 9), and 3 and 6 months after treatment initiation, included kala-azar symptoms (fever, asthenia, weight loss, anemia, and splenomegaly). Fever was defined as auxiliary body temperature ≥ 37.5°C. Asthenia, a self-reported weakness or loss of strength, was recorded as present or not. Nutritional status was monitored using a mid upper-arm circumference measurement (MUAC; mm) and body mass index (BMI) for adults ≥ 17 years and BMI percentiles for children and adolescents ≤ 16 years. BMI was calculated using weight (kg) versus height (cm) comparisons (kg/cm^2). Plotting the BMI on the 2000 Centers for Disease Control and Prevention (CDC) age-for-growth chart generated BMI percentiles.[27] Anemia was defined as a hemoglobin concentration in grams per deciliter of blood (g/dL) < 11 for all patients, except males ≥ 17 years, who were defined as anemic if hemoglobin was < 13 g/dL. Splenomegaly was defined as a palpable spleen, measured in centimeters. Patients self-reported other medical problems (e.g., cough, abdominal pain, and diarrhea).

The occurrence of adverse events was used to assess drug safety. Adverse events were monitored during the infusion of the medication and daily over the 10-day treatment, and they were graded using Common Toxicity Criteria.[28] Adverse events were defined as clinically apparent symptoms, reported by a manufacturer-sponsored clinical trial and judged by a kala-azar–experienced clinician, to be associated with liposomal amphotericin B treatment.[29] Biochemical laboratory tests and electrocardiograms were not performed for adverse-event monitoring.

Treatment outcome, the measure of drug effectiveness, was evaluated at end treatment and 3 and 6 months after treatment initiation. Treatment success was defined as the absence of kala-azar symptoms and improvement in clinical characteristics,

as judged by a kala-azar–experienced clinician. Likewise, treatment failure was defined as the presence of kala-azar symptoms or non-improvement in clinical characteristics confirmed by a positive bone-marrow or splenic aspirate at end treatment or anytime during the 6 months after treatment initiation. Relapse was a type of treatment failure occurring after an initial success was achieved at end treatment. Default was defined as not completing, for any reason, the 10-day treatment course. A patient was considered lost to follow-up when unavailable for clinical assessment at day 9 or 3 or 6 months after treatment initiation.

Treatment success rates were calculated according to intention-to-treat (ITT), per protocol, and worst-case ITT scenario analyses; these approaches were used to estimate treatment efficacy in human African trypanosomiasis trials.[30–32] An ITT analysis considered relapses and all deaths as failures; successes included lost to follow-up patients considered cured at least one time without a relapse. A per protocol analysis considered only relapses and deaths clinically attributed to kala-azar as failures. A worst-case ITT scenario analysis considered all relapses, deaths, and lost to follow-up patients as failures.

Data analysis. Data were entered in Microsoft Excel and analyzed on SAS software (version 9.2; SAS Institute, Cary, NC). McNemar χ^2 tests detected differences between paired proportions. Repeated measures *t* tests were used to compare the mean difference in the measure of continuous variables before and after exposure to the treatment variable. It tests the null hypothesis that the differences between a series of the paired observations comes from a population whose mean is zero. For example, the difference between hemoglobin concentration measured at study enrollment and end of treatment was calculated for each patient. The repeated measures *t* test was used to determine if the mean difference was significantly greater than zero. P values < 0.05 were considered statistically significant.

RESULTS

A total of 251 kala-azar–confirmed patients were enrolled in the observational cohort (Table 1). All reported Vaishali District residence. Patients' ages ranged from 2 to 65 years; nearly one-half (45.8%) were adults (≥ 17 years), and 10.8% were children ≤ 5 years. Males constituted the majority of the cohort (58%); the male:female ratio was 1.4:1. Pregnancy was noted for 3 of 46 (6.5%) women of childbearing age.

The mean number of weeks before study enrolment that patients self-reported illness with kala-azar–compatible symptoms was 6.2 [standard deviation (SD) ± 4]. Nearly 20% of patients had been ill for 9 weeks or longer. At enrollment, in congruence with the used clinical-case definition, nearly all patients presented with an enlarged spleen and fever (98.8% and 96.8%, respectively). Of 230 patients with a hemoglobin measurement at study enrollment, 97.4% were anemic. Other medical problems self-reported at enrollment were: cough (11.6%), abdominal pain (4.8%), weight loss (4.0%), asthenia (2.0%), and diarrhea (1.6%). Despite having received prior antileishmanial treatment of current symptoms, 31 patients (12.4%) were enrolled with symptomatic and diagnostically confirmed visceral leishmaniasis. Of these 31 patients, 16 received less than the recommended 20 mg/kg of liposomal amphotericin B before study enrolment.

TABLE 1

Method of diagnosis, patient demographics, and clinical history and characteristics at study enrollment among patients presenting to Sadar District Hospital, Vaishali District, India (July 2007 to May 2008; $N = 251$)

Variables	*n* (%)	Mean, SD, and range*
Positive rK39 rapid diagnostic test (DiaMed-IT-Leish) only	245 (97.6)	–
Positive rK39 rapid diagnostic test (DiaMed-IT-Leish) plus a positive parasitological diagnostic test result	6 (2.4)	–
Age group (years)		
≤ 5	27 (10.8)	20.5 ± 15.5 (2–65)
6–16	109 (43.4)	20.5 ± 15.5 (2–65)
≥ 17	115 (45.8)	20.5 ± 15.5 (2–65)
Gender ($N = 250$)		
Male	145 (58.0)	–
Female	105 (42.0)	–
Cast ($N = 246$)		
Musahar	28 (11.4)	–
Other	218 (88.6)	–
Pregnant† ($N = 46$)		
No	43 (93.5)	–
Yes	3 (6.5)	–
Number of weeks ill before enrollment		
≤ 2	40 (15.9)	6.2 ± 4.0 (2–24)
3–8	163 (64.9)	6.2 ± 4.0 (2–24)
≥ 9	48 (19.1)	6.2 ± 4.0 (2–24)
Symptoms‡		
Splenomegaly	248 (98.8)	–
Fever	243 (96.8)	–
Anemia§	224 (97.4)	–
Cough	29 (11.6)	–
Abdominal pain	12 (4.8)	–
Weight loss	10 (4.0)	–
Asthenia	5 (2.0)	–
Diarrhea	4 (1.6)	–
Previous treatment		
No	220 (87.6)	–
Yes	31 (12.4)	–
Previous visceral leishmaniasis treatment type ($N = 31$)		
Liposomal amphotericin B¶	16 (51.6)	–
Sodium stibogluconate	12 (38.7)	–
Miltefosine	2 (6.5)	–
Unspecified	1 (3.2)	–

SD = standard deviation; – = not measured.
*Values are means ± SD. The range is in parenthesis.
† Includes females of childbearing age between 12 and 44 years.
‡ One or more symptoms recorded for each patient.
§ Number of patients with hemoglobin concentration measured at enrollment ($N = 230$).
¶ Incomplete treatment (i.e., < 20 mg/kg total dose).

Of 251 enrolled patients, 5 (2.0%) defaulted, because they received fewer than four doses (20 mg/kg) of the liposomal amphotericin B during the 10-day treatment course (Table 2). An anemic (5.1 g/dL) 6-year-old male, who experienced nausea and vomiting during treatment, received only two doses (10 mg/kg) of the drug. Treatment was halted after three doses (15 mg/kg) for an additional three children (≤ 8 years) because of severe, non–life-threatening lip swelling during infusion of the medication. Similarly, a 30-year-old male received only three doses, but the reason for this was not recorded. Despite not completing the full recommended course of treatment, four of the five defaulters were cured at day 9.

During the 10-day treatment course, concomitant upper-respiratory infection, occurring among 39 patients (15.5%), was the most frequent of apparent adverse events experienced. More serious infections of pneumonia were experienced by

TABLE 2
Dosage of liposomal amphotericin B received and apparent adverse events experienced during the 10-day treatment course among study cohort patients at Sadar District Hospital, Vaishali District, India (July 2007 to May 2008; $N = 251$)

Variables	N (%)
Number of doses received of liposomal amphotericin B	
Two doses (10 mg/kg)	1 (0.4)
Three doses (15 mg/kg)	4 (1.6)
Four doses (20 mg/kg)	246 (98.0)
Apparent adverse event(s) experienced	
No	129 (51.4)
Yes	122 (48.6)
Apparent adverse event experienced*	
Muscle-skeletal system	
Back pain	2 (0.8)
Digestive system	
Nausea and vomiting	25 (10.0)
Diarrhea	20 (8.0)
Dyspepsia	12 (4.8)
Jaundice	2 (0.8)
Respiratory system	
Upper respiratory infection†	39 (15.5)
Pneumonia	16 (6.4)
Coagulation system	
Epistaxis	3 (1.2)
Skin and appendages	
Rash	4 (1.6)
Urinary tract infection	4 (1.6)
Unspecified infections	6 (2.4)
Apparent adverse event during infusion	
Chills/rigor	8 (3.2)
Severe lip swelling	3 (1.2)

* One or more symptoms recorded for each patient.
† Includes cough and sore throat only.

16 patients (6.4%). Ten percent of patients experienced nausea and vomiting, 20 (8%) patients experienced diarrhea, and 12 patients (4.8%) suffered from dyspepsia. Three patients experienced epistaxis related to splenomegaly-induced coagulopathy; however, no laboratory tests were conducted. No other bleeding disorders were identified. Incidents of chills/rigor that did not require treatment termination were noted for 8 (3.2%) patients. Fewer than 5% of patients experienced other apparent adverse events: unspecified infections (6; 2.4%), urinary tract infections (4; 1.6%), non–infusion-related rashes (4; 1.6%), hepatomegaly (2; 0.8%), or back pain (2; 0.8%) (Table 2).

Clinical characteristic measurements that quantified treatment effectiveness endpoints are presented in Table 3. Nearly all patients (96.8%) were febrile at enrollment, but those with fever at 3 and 6 months dropped significantly ($P < 0.0001$). Patient nutritional status, as measured by MUAC, improved significantly from study enrolment to follow-up periods. No patient had a MUAC measurement ≤ 135 mm at 6 months follow-up. The mean BMI at enrollment among adults aged ≥ 17 years was 18.3 (SD ± 2.5). By 6 months, their mean BMI was approximately 20.4, an increase from enrolment of 2.1 (SD ± 1.8; $P < 0.0001$). Children and adolescents ≤ 16 years at enrollment were, on average, in the 19th BMI percentile. Their BMI increased by 19 BMI percentile points at 6 month follow-up ($P < 0.0001$).

Patients' mean hemoglobin at enrolment was 7.8 g/dL (SD ± 1.9). Mean hemoglobin concentration significantly increased at each subsequent measurement period (8.7 to 11.8 to 12.1). The percentage of females with anemia dropped from 98.9% at enrollment to 34.3% at 3 months ($P < 0.0001$) and 30.4% at 6 months ($P < 0.0001$). Likewise, approximately 96% of adult males (≥ 17 years) were anemic at enrollment, and the percentage with anemia decreased, respectively, to 38.8% and 35.7% at 3 and 6 months ($P < 0.0001$). The percentage of males ≤ 16 years with anemia declined from 96.7% at enrollment to 31.3% at 3 months ($P < 0.0001$) and 25.9% at 6 months ($P < 0.0001$).

Compared with enrollment measurements, patients' spleen size reduced significantly by end of treatment and at 3 and 6 months follow-up. At 6 months, the mean spleen size had decreased significantly by 6 cm (SD ± 3.8; $P < 0.0001$), and spleens were non-palpable for all patients. During the entire follow-up period, negative spleen aspirates indicated parasite clearance for eight patients for whom clinicians sought confirmation for their clinical diagnosis of treatment success (data not shown).

Tables 4 and 5 provide treatment effectiveness endpoint data. No treatment relapses were detected during study follow-up. The ITT analysis included as failure all three deaths during the study time frame, which occurred among children ≤ 8 years, yielding a treatment effectiveness of 248/251 or 98.8% [95% confidence interval (CI) = 96.6–99.8]. The anemic 6-year-old male (hemoglobin value = 5.1 gm/dL) who received only two doses of medication died during the treatment phase. Another child died before his 3-month follow-up visit because of measles. A third child died between the 3- and 6-month follow-up visits, but the reason is unknown. The per protocol analysis 1 considered only relapses and kala-azar–related deaths as failures, yielding a treatment effectiveness of 250/251 or 99.6% (95% CI = 97.8–99.9). Per protocol analysis 1 considered the unknown death as related to kala-azar. Per protocol analysis 2 considered the unknown death as unrelated to kala-azar and found treatment effectiveness to be 251/251 or 100% (95% CI = 98.8–100.0, one sided CI). The worst-case ITT scenario considers all deaths and lost to follow-ups as failures. At 6 months after their treatment initiation, 204 of 251 study participants were clinically determined to be free of kala-azar (81.3%; 95% CI = 75.9–85.6). χ^2 and Student paired t test analyses indicated that patient demographics, clinical history, and characteristics of lost to follow-up patients ($N = 44$) and those who remained in the study did not differ significantly with regard to age, gender, caste, nutrition status, previous antileishmanial treatment received, number of weeks ill, or study-monitored dosage of liposomal amphotericin B received (data not shown).

DISCUSSION

This observational cohort study used patient clinical data to evaluate the effectiveness and safety of liposomal amphotericin B for successful treatment of kala-azar in Bihar state, India. High cure rates (99.6% per protocol analysis treatment success rate) indicate that a total dose of 20 mg/kg body weight of liposomal amphotericin B, administered intravenously at 5 mg/kg doses on days 0, 1, 4, and 9, is a highly effective treatment of kala-azar under routine program conditions in Bihar. Per protocol analysis of 251/251, indicating 100% treatment effectiveness, cannot be confirmed because of insufficient data on one child who did not present for 6 month follow-up. The MSF team attempted to trace the child; however, the family had relocated, and neighbors reported that the child had died. There is no confirmation of this death or reason to suspect the death was related to kala-azar or the treatment.

TABLE 3

Number and frequency or mean and standard deviation of patient clinical characteristics at enrollment, end treatment, and 3- and 6-month follow-ups at Sadar District Hospital, Vaishali District, India (July 2007 to May 2008; $N = 251$)

Variable	Study enrollment ($N = 251$)	Change at end treatment	P value	Change at 3-month follow-up ($N = 227$)*	P value	Change at 6-month follow-up ($N = 204$)*	P value
Categorical variables†‡							
Fever	$N = 251$	–	–	$N = 227$	< 0.0001	$N = 204$	< 0.0001
No	8 (3.2)	–	–	210 (92.5)		200 (98.1)	
Yes	243 (96.8)	–	–	17 (7.7)		4 (2.0)	
MUAC categorization 1	$N = 247$	–	–	$N = 220$	na	$N = 203$	na
< 110 mm	3 (1.2)	–	–	2 (0.9)	na	0 (0.0)	na
110–124 mm	9 (3.6)	–	–	0 (0.0)	na	0 (0.0)	na
125–135 mm	35 (14.2)	–	–	10 (4.6)	na	0 (0.0)	na
> 135 mm	200 (81.0)	–	–	208 (94.6)	na	203 (100.0)	na
MUAC categorization 2	$N = 247$	–	–	$N = 220$	< 0.0001	$N = 203$	na
≤ 135 mm	47 (19.0)	–	–	12 (5.5)		0 (0.0)	na
> 135 mm	200 (81.0)	–	–	208 (94.6)		203 (100.0)	na
< 5th BMI percentile (2–16 years)	$N = 135$; 40 (44.4)	–	–	$N = 121$; 16 (13.2)	< 0.0001	$N = 114$; 15 (13.2)	< 0.0001
BMI < 18.5 (≥ 17 years)	$N = 115$; 65 (56.5)	–	–	$N = 99$; 24 (24.2)	< 0.0001	$N = 89$; 17 (19.1)	< 0.0001
Female anemia (< 11 g/dL)	$N = 94$; 93 (98.9)	–	–	$N = 67$; 23 (34.3)	< 0.0001	$N = 46$; 14 (30.4)	< 0.0001
Male anemia categorization 1 (≤ 16 years, < 11 g/dL)	$N = 61$; 59 (96.7)	–	–	$N = 32$; 10 (31.3)	< 0.0001	$N = 27$; 7 (25.9)	< 0.0001
Male anemia categorization 2 (≥ 17 years, < 13 g/dL)	$N = 75$; 72 (96.0)	–	–	$N = 49$; 19 (38.8)	< 0.0001	$N = 28$; 10 (35.7)	< 0.0001
Continuous variables§¶							
BMI (≤ 17 years)	$N = 115$; 18.3 ± 2.5	–	–	$N = 99$; +1.7 ± 1.2	< 0.0001	$N = 89$; +2.1 ± 1.8	> 0.0001
Child BMI (≤ 16 years)	$N = 135$; 19.9 ± 26.8	–	–	$N = 121$; +16.3 ± 31.1	< 0.0001	$N = 114$; +19.0 ± 33.6	< 0.0001
Hemoglobin (g/dL)	$N = 231$; 7.8 ± 1.9	$N = 229$; +0.9 ± 1.4	< 0.0001	$N = 135$; +4.0 ± 2.2	< 0.0001	$N = 92$; +4.3 ± 2.0	< 0.0001
Spleen size (cm)	$N = 251$; 6.1 ± 3.8	$N = 249$; −4.4 ± 2.9	< 0.0001	$N = 222$; −5.9 ± 3.6	< 0.0001	$N = 204$; −6.0 ± 3.8	< 0.0001

– = not measured; na = not applicable.
* Number excludes patients who died or were lost to follow-up.
† Number and percentage reported.
‡ P values calculated using McNemar χ^2 test.
§ Values are means ± SD. Change columns are in relation to the mean value of the study enrollment measurement.
¶ P values calculated using repeated measures t test.

TABLE 4
Treatment endpoints among study cohort patients at Sadar District Hospital, Vaishali District, India (July 2007 to May 2008; $N = 251$)

Endpoints	*N* (%)
Relapse	0 (0.0)
Deaths during 10-day treatment course	1 (0.4)
Deaths during follow-up period	2 (0.8)
Lost to follow-up	44 (17.5)

To evaluate treatment safety, we assessed apparent clinical adverse events experienced during the 10-day treatment course. Nearly one-half of patients experienced mild adverse events; 1% of patients experienced severe, non–life-threatening lip swelling. Minimal adverse events indicate that liposomal amphotericin B is a safe treatment. We also found that liposomal amphotericin B is safe and effective for pregnant and lactating women and children; we successfully treated 3 pregnant and lactating women and 27 children.

A common limitation of previous studies seeking to show the efficacy and safety of liposomal amphotericin B was the low number of enrolled patients. Additionally, efficacy and safety have not been assessed through a phase III trial. Our 251 patient cohort study was unique to Bihar, India in that 20 mg/kg of liposomal amphotericin B was used as first-line treatment of primary kala-azar on a large scale and outside the framework of a clinical trial. Our study had sufficient power to detect true liposomal amphotericin B treatment success rates, which corroborated previous reports of liposomal amphotericin B efficacy, effectiveness, and safety at varying dosage.[15,19–23]

Our study has potential limitations. Our prospective cohort was managed and followed-up under field conditions; hence, rigorous biochemical monitoring of adverse events was not undertaken, and patient follow-up was limited to two visits during a 6-month period. However, previous studies have shown the biochemical safety of AmBisome both *in vitro* and *in vivo*.[20,33,34] Follow-up for the 251 patient cohort was attempted, and study conditions accurately reflect routine program circumstances at the Sadar Hospital, making it a reasonable measure of feasibility in a field-hospital setting. This is particularly relevant in Bihar where kala-azar is endemic in health resource-poor areas and biochemical testing facilities are not always available. A total of 44 (17.5%) patients were lost to follow-up. There is no reason to believe that patients with poor outcomes were selectively lost to follow-up. We found no systematic differences in risk factors with regard to age, gender, caste, nutrition status, previous antileishmanial treatment, number of weeks ill, or study-monitored dosage of liposomal amphotericin B received among the group whose outcome was determined in the follow-up period and the 44 patients who were lost-to-follow-up.

TABLE 5
Effectiveness estimates among study cohort patients at Sadar District Hospital, Vaishali District, India (July 2007 to May 2008; $N = 251$)

Effectiveness estimates	*N* (%) [95% CI]
Intention-to-treat analysis	
Cured	248 (98.8) [96.6–99.8]
Patients in analysis	251
Per protocol analysis 1*	
Cured	250 (99.6) [97.8–99.9]
Patients in analysis	251
Per protocol analysis 2*	
Cured	251 (100.0) [98.8–100.0]†
Patients in analysis	251
Intention-to-treat worst-case scenario analysis	
Cured	204 (81.3) [75.9–85.6]
Patients in analysis	251

*Per protocol analysis 1 considered only relapses and kala-azar–related deaths as failures. Per protocol analysis 2 considered a single death for an unknown reason to be unrelated to kala-azar.
†One-sided confidence interval.

The results of our study show that liposomal amphotericin B at 20 mg/kg is a safe and effective treatment of kala-azar in Bihar, where other existing treatment regimens are complicated by drug resistance, toxicity, and teratogenicity. On the basis of these findings, we recommend adoption of liposomal amphotericin B as a first-line treatment of kala-azar across endemic areas of India, where possible. However, the exorbitant cost of treatment is a formidable barrier to widespread use. The current preferential cost of a 50-mg vial of AmBisome is 20 USD.[16] Complete treatment requires 16 vials of medication for a 40-kg adult and costs 320 USD. Impoverished patients in high-prevalence regions are currently unable to afford a complete treatment course of liposomal amphotericin B.

A lowered dosage and shorter treatment course could broaden access to treatment by lowering drug-procurement and hospitalization costs and reducing the number of lost workdays for patients and caregivers. Lowering dosage to 15 mg/kg or 10 mg/kg is a possibility, and hospital stays could be halved if dosage is reduced by just 5–15 mg/kg. (It may be noteworthy that five patients enrolled in our study did not complete the full recommended treatment course and instead, received either 10 mg/kg or 15 mg/kg only. Four of these patients were cured). Alternatively, if the current dosage of 20 mg/kg is maintained, hospital stays could be shortened by changing the treatment regimen from days 0, 1, 3, and 9 to 1, 2, 3, and 4. However, some patients may continue to benefit from a longer hospital stay (e.g., those requiring parenteral antibiotics for concurrent bacterial infections or nutritional therapy for severe malnutrition).

Treatment using combination therapies could also reduce costs. A reduced dosage of liposomal amphotericin B, combined with a partner drug, might be effective for successful treatment. It would also protect the drugs from parasite resistance. Moreover, patients with primary kala-azar and manageable clinical manifestations could benefit from an ambulatory delivery of the combination treatment, which could be administered through outpatient consultations, thereby reducing hospitalization time for some patients. For example, on day 0, patients would receive a thorough clinical examination followed by on-site treatment and a day 9 post-treatment clinical assessment. A combination treatment trial is currently being evaluated by the Drugs for Neglected Diseases initiative.

In the interim, a recent phase III trial evaluating 10 mg/kg of liposomal amphotericin B as a single dose showed noninferiority compared with conventional amphotericin B.[24] Single-dose treatment would likely broaden the access to treatment of impoverished people. The consensus among experts in Bihar is that 10 mg/kg single-dose first-line treatment should be prioritized for implementation in all endemic areas in India. Implementing AmBisome at 10 mg/kg as a single-dose treatment and/or as a component of combination therapy under routine program conditions, in conjunction with a monitoring and evaluation initiative, would advance knowledge regarding

optimal dosage and case-management strategy. The monitoring and evaluation initiative is crucial, particularly in light of our finding that 16 kala-azar–confirmed patients received < 20 mg/kg of liposomal amphotericin B for their kala-azar infection before study enrolment. Of equal importance, the pending results of combination trials should guide future treatment recommendations in Bihar. Finally, non-inferiority trials of generic versions of liposomal amphotericin B, ideally at lower cost, locally available, and validated by reliable institutions, are recommended.

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REFERENCES

1. WHO, 2009. *Communicable Diseases, Kala-Azar status in SEA Region*. Available at: http://www.searo.who.int/en/section10/section2163_11668.htm. Accessed December 16, 2009.
2. Desjeux P, 2004. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis 27:* 305–318.
3. Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S, 2005. Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980–2004. *Lancet Infect Dis 5:* 763–774.
4. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, Kumar PC, Murray HW, 2000. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis 31:* 1104–1107.
5. Lira R, Sundar S, Makharia A, Kenney R, Gam A, Saraiva E, Sacks D, 1999. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *J Infect Dis 180:* 564–567.
6. Sundar S, Singh VP, Sharma S, Makharia MK, Murry HW, 1997. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis 176:* 1117–1119.
7. Thakur CP, Narayan S, Ranjan A, 2004. Epidemiological, clinical and pharmacological study of antimony-resistant visceral leishmaniasis in Bihar, India. *Indian J Med Res 120:* 166–172.
8. Das VN, Ranjan A, Bimal S, Siddique NA, Pandey K, Kumar N, Verma N, Singh VP, Sinha PK, Bhattacharya SK, 2005. Magnitude of unresponsiveness to sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar. *Natl Med J India 18:* 131–133.
9. Thakur CP, Sinha GP, Pandey AK, Kumar N, Kumar P, Hassan SM, Narain S, Roy RK, 1998. Do the diminishing efficacy and increasing toxicity of sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar, India justify its continued use as a first-line drug? An observational study of 80 cases. *Ann Trop Med Parasitol 92:* 561–569.
10. Ahasan HA, Chowdhury MA, Azhar MA, Rafiqueuddin AK, Azad KA, 1996. Deaths in visceral leishmaniasis (kala-azar) during treatment. *Med J Malaysia 51:* 29–32.
11. Sindermann H, Engel J, 2006. Development of miltefosine as an oral treatment for leishmaniasis. *Trans R Soc Trop Med Hyg 100 (Suppl 1):* S17–S20.
12. Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, Junge K, Bryceson A, Berman J, 2002. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med 347:* 1739–1746.
13. Sundar S, Murray HW, 2005. Availability of miltefosine for the treatment of kala-azar in India. *Bull World Health Organ 83:* 394–395.
14. Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK, 2007. Injectable paromomycin for visceral leishmaniasis in India. *N Engl J Med 356:* 2571–2581.
15. Sundar S, Mehta H, Suresh AV, Singh SP, Rai M, Murray HW, 2004. Amphotericin B treatment for Indian visceral leishmaniasis: conventional versus lipid formulations. *Clin Infect Dis 38:* 377–383.
16. WHO, 2005. *Report of a WHO Informal Consultation on Liposomal Amphotericin B in the Treatment of Visceral Leishmaniasis*. Available at: http://www.who.int/neglected_diseases/resources/AmBisomeReport.pdf. Accessed December 17, 2009.
17. Sundar S, Rai M, 2002. Advances in the treatment of leishmaniasis. *Curr Opin Infect Dis 15:* 593–598.
18. Rosenthal E, Marty P, 2003. Recent understanding in the treatment of visceral leishmaniasis. *J Postgrad Med 49:* 61–68.
19. Sundar S, Agrawal G, Rai M, Makharia MK, Murray HW, 2001. Treatment of Indian visceral leishmaniasis with single or daily infusions of low dose liposomal amphotericin B: randomised trial. *BMJ 323:* 419–422.
20. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R, 2003. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis 37:* 800–804.
21. Thakur CP, Pandey AK, Sinha GP, Roy S, Behbehani K, Olliaro P, 1996. Comparison of three treatment regimens with liposomal amphotericin B (AmBisome) for visceral leishmaniasis in India: a randomised dose finding study. *Trans R Soc Trop Med Hyg 90:* 319–322.
22. Thakur CP, 2001. A single dose treatment of kala-azar with AmBisome (amphotericin B lipid complex): a pilot study. *Int J Antimicrob Agents 17:* 67–70.
23. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VR, Buffels R, 2002. Low dose liposomal amphotericin B in refractory Indian visceral leishmaniasis: a multicenter study. *Am J Trop Med Hyg 66:* 143–146.
24. Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW, 2010. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N Engl J Med 362*: 504–512.
25. Bern C, Adler-Moore J, Berenguer J, Boelaert M, den Boer M, Davidson RN, Figueras C, Gradoni L, Kafetzis DA, Ritmeijer K, Rosenthal E, Royce C, Russo R, Sundar S, Alvar J, 2006. Liposomal amphotericin B for the treatment visceral leishmaniasis. *Clin Infect Dis 43:* 917–924.
26. Collin S, Davidson R, Ritmeijer K, Keus K, Melaku Y, Kipngetich S, Davies C, 2004. Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. *Clin Infect Dis 38:* 612–619.
27. Centres for Disease Control and Prevention, 2009. *The SAS Program for the CDC Growth Charts*. Available at: http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/sas.htm. Accessed April 14, 2009.
28. National Cancer Institute Cancer Therapy Evaluation Program, 2003. *Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE) DCTD, NCI, NIH, DHHS*. Available at: http://

ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf. Accessed December 21, 2009.
29. Astellas Pharma US, Inc., 2007. *AmBisome (Amphotericin B) Liposome for Injection*. Available at: http://www.ambisome.com/index2.php?section=about&page=trials. Accessed March 19, 2009.
30. Schmid C, Nkunku S, Merolle A, Vounatsou P, Burri C, 2004. Efficacy of 10-day melarsoprol schedule 2 years after treatment for late-stage gambiense sleeping sickness. *Lancet 364:* 789–790.
31. Schmid C, Richer M, Bilenge CM, Josenando T, Chappuis F, Manthelot CR, Nangouma A, Doua F, Asumu PN, Simarro PP, Burri C, 2005. Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis: confirmation from a multinational study (impamel II). *J Infect Dis 191:* 1922–1931.
32. Checchi F, Piola P, Ayikoru H, Thomas F, Legros D, Priotto G, 2007. Nifurtimox plus eflornithine for late-stage sleeping sickness in Uganda: a case series. *PLoS Negl Trop Dis 1:* e64.
33. Berman JD, Badaro R, Thakur CP, Wasunna KM, Behbehani K, Davidson R, Kuzoe F, Pang L, Weerasuriya K, Bryceson AD, 1998. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. *Bull World Health Organ 76:* 25–32.
34. Adler-Moore J, Proffitt RT, 2002. Ambisome: liposomal formulation, structure, mechanism of action and pre-clinical experience. *J Antimicrob Chemother 49 (Suppl 1)*: 21–30.