

Liposomal Amphotericin B for Visceral Leishmaniasis in Human Immunodeficiency Virus-Coinfected Patients: 2-Year Treatment Outcomes in Bihar, India

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Background. Reports on treatment outcomes of visceral leishmaniasis (VL)–human immunodeficiency virus (HIV) coinfection in India are lacking. To our knowledge, none have studied the efficacy of liposomal amphotericin B in VL-HIV coinfection. We report the 2-year treatment outcomes of VL-HIV-coinfected patients treated with liposomal amphotericin B followed by combination antiretroviral treatment (cART) in Bihar, India.

Methods. The study included all patients with newly diagnosed VL-HIV coinfection and initiating treatment with liposomal amphotericin B (20–25 mg/kg in 4–15 days) between July 2007 and September 2010. Kaplan–Meier estimates of the cumulative incidence of death/treatment failure were calculated.

Results. Fifty-five patients were included (83.6% male; median age, 35 years; 62% migrant laborers; median follow-up, 1 year). The median CD4 cell count at VL diagnosis was 66 cells/ μ L (interquartile range, 38–112). Twenty-seven patients (49.1%) presented with VL relapse of VL. The overall tolerance of liposomal amphotericin B was excellent, with no interrupted treatment. Survival by 1 and 2 years after VL treatment was estimated at 85.5%. No patients had initial treatment failure. The probabilities of VL relapse were 0%, 8.1%, and 26.5% at 0.5, 1, and 2 years after VL treatment, respectively; relapse rates were similar for primary VL and VL relapse. CD4 counts <200 cells/ μ L at 6 months after cART initiation were predictive of subsequent relapse. The mean CD4 cell counts at 6 and 24 months after cART initiation were 187 and 261 cells/ μ L, respectively. The rate for retention in HIV care was 83.6%.

Conclusions. Good long-term survival and retention rates were obtained for VL-HIV-coinfected patients treated with liposomal amphotericin B and cART. Although the initial VL treatment response was excellent, VL relapse within 2 years remained frequent.

More than 60% of the estimated 500 000 annual cases of visceral leishmaniasis (VL), also known as kala-azar, occur in the Indian subcontinent [1, 2]. In this region, as in East Africa, VL is caused by *Leishmania donovani*, and

humans act as the reservoir. In response to this disease burden, a VL elimination program was launched by the governments of India, Nepal, and Bangladesh, relying on outpatient treatment with miltefosine and aiming to eliminate VL as a public health problem by 2015. With close to 40% of all cases worldwide and about 80%–90% of all cases in India occurring in Bihar state, Bihar lies at the heart of the VL problem regionally as well as globally [3]. Bihar is one of the most backward and populous states of India, with poverty, malnutrition, and poorly functioning health care services chronically embedded.

Human immunodeficiency virus (HIV) infection has been identified as one of the emerging challenges for VL control [4]. HIV infection dramatically increases the risk

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of progression from asymptomatic infection to VL disease, and VL accelerates HIV disease progression [5]. In some regions of East Africa, up to 30% of patients with VL are coinfecting with HIV [5]. Although in early reports VL-HIV coinfection seemed initially to be virtually nonexistent in India [6–8], a progressive increase in prevalence has been suggested in more recent studies from Bihar during the last 5–10 years, with coinfection rates above 2%–5% reported in some studies [5, 9–13]. In contrast with the national decline in HIV prevalence (currently estimated at ~0.3%), data suggest an increasing trend in Bihar, with the extensive migration of laborers from Bihar to and from major urban areas put forward as a main driving factor [14].

With the advent of combination antiretroviral treatment (cART), dramatically improved survival has been reported for VL-HIV coinfection in high-income countries, although high relapse rates seemed to persist [5, 15]. Recent studies from East Africa continue to demonstrate high mortality rates [16–18]. Very limited data are available on VL-HIV coinfection from the Indian subcontinent. The few, very small studies on short-term treatment outcomes in India have consistently shown high rates of mortality, treatment failure and drug-related toxicity [11–13]. None provided long-term outcome data, possibly because of the high associated mortality given the relatively limited availability of cART programs in Bihar until recently. Moreover, none reported outcomes with the use of liposomal amphotericin B, which combines high efficacy with low toxicity and is currently the preferential treatment for VL-HIV coinfection according to World Health Organization (WHO) recommendations [19]. Although liposomal amphotericin B was initially prohibitively expensive, recent price reductions have made it an option for low- and middle-income countries [20]. Although the use of liposomal amphotericin B, combined with cART, could offer new treatment options, the effectiveness of this approach has not been documented or explored in the Indian subcontinent.

For assessments of treatment effectiveness in HIV-negative patients, definitive cure is usually assessed 6 months after treatment. However, this assessment is less clear for coinfecting patients, given their high rate of relapse even after the first year of treatment [21]; VL-HIV coinfection seems to be a chronic condition, requiring long-term monitoring of both conditions. In this study, we report on the long-term treatment outcomes (survival and treatment success) in VL-HIV-coinfecting patients treated with liposomal amphotericin B in a VL treatment program in Bihar, India.

METHODS

Study Setting

In collaboration with the Indian health authorities, Médecins Sans Frontières (MSF) started a VL treatment program in Vaishali district in Bihar, in July 2007, with liposomal amphotericin B as

first-line treatment. Whereas most cases were treated at the district hospital in Hajipur (district capital), complicated cases were referred for further diagnostic work-up to the Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), located in Patna (Bihar State capital). By 1 September 2010, 6626 patients had received VL treatment within the entire program, with excellent treatment outcomes reported [22].

Study Design and Population

We conducted a retrospective cohort study using routine program data. The study included all patients with newly diagnosed VL-HIV coinfection who started treatment with liposomal amphotericin B between July 2007 and September 2010.

Visceral Leishmaniasis Diagnoses and Treatment Protocols

Diagnosis was based on the combination of clinical signs and symptoms consistent with VL (fever for >2 weeks, splenomegaly or lymphadenopathy, and weight loss), and a positive rK39 rapid diagnostic test (DiaMed-IT-Leish) for *Leishmania* antibodies, after exclusion of malaria and bacterial infections. A provider-initiated testing and counseling strategy for HIV infection was implemented at the moment of VL diagnosis. VL-HIV-coinfecting patients were referred to RMRIMS for parasitologic diagnosis by spleen aspiration, or bone marrow aspiration in case of contraindications. Parasitologic diagnosis and grading was done as reported before [22]. For unstable cases, treatment was usually started at the hospital level, with patients referred to RMRIMS after stabilization. Consequently, no tissue aspirate was done in these cases, and diagnosis was based on clinical or serologic criteria. The same applied when HIV infection was diagnosed after initiation of VL treatment. Still, all were referred to RMRIMS for test of cure (TOC) and cART initiation.

Treatment consisted of intravenous liposomal amphotericin B (AmBisome; Gilead Sciences) administered over 2 hours and reconstituted according to the manufacturer's instructions. A total dose of 20 mg/kg was given, divided over 4 doses given on days 1, 2, 5, and 10. For less advanced cases, possibly requiring shorter hospitalization, the same total dose could be given over 4 consecutive days (days 1–4). For patients relapsing after having previously received a full course of liposomal amphotericin B, a total dose of 25 mg/kg was given in 5 doses (days 1, 2, 5, 10, and 15).

Treatment Monitoring and Follow-up

Before and after VL treatment initiation at RMRIMS, full blood counts, liver function tests, and creatinine measurements were performed. Follow-up visits and laboratory testing was scheduled at 1, 3, 6, 12, and 24 months after treatment initiation. During these visits, patients were clinically assessed for signs and symptoms of relapse, with parasitologic evaluation performed for suspected cases. All patients were clearly informed about the risk of relapse and strongly counseled to present for evaluation

with the least sign of relapse. To assess for initial parasitologic cure, TOC was planned for all patients at 1 month after treatment initiation. For some, TOC was not done at the scheduled visit for programmatic reasons (patients presenting late in the day; aspiration material unavailable), and TOC was cancelled if these patients were clinically cured.

Human Immunodeficiency Virus Diagnosis and Antiretroviral Treatment

HIV diagnosis was based on parallel testing with 2 rapid diagnostic tests (SD Bioline-HIV 1/2 and Determine-HIV 1/2) with confirmation by Western blot analysis (SRL Ranbaxy). All patients with diagnoses of VL-HIV coinfection were eligible for cART, as recommended by WHO and national guidelines [19]. The preferential first-line regimen consisted of a generic fixed dose combination containing stavudine, lamivudine, and nevirapine. Treatment was initiated after the patient's general condition had improved, usually shortly after the end of VL treatment, with monthly follow-up visits after cART initiation. A CD4 cell count test was performed before cART initiation (shortly after VL treatment initiation) and at 6-month intervals once treatment started. Patients could choose to initiate cART within the VL-HIV project (the majority) or be transferred to another antiretroviral treatment (ART) center.

Visceral Leishmaniasis and Human Immunodeficiency Virus Treatment Outcomes

For VL treatment, initial treatment response was determined 1 month after treatment initiation. Initial cure was defined as parasite clearance—as demonstrated by TOC—combined with clinical improvement or clinical cure alone if no TOC was done. Relapse was defined as recurrence of clinical signs or symptoms of VL with parasitologic confirmation, after initial or clinical cure. Patients achieving initial or clinical cure and remaining relapse free during follow-up were defined as cured. Additional treatment outcomes included death or being unavailable for follow-up, defined as patients not presenting for planned visits and unable to be contacted by phone or through home visits. Those alive and receiving cART or transferred out were considered retained in HIV care.

Data Collection and Statistical Analysis

Clinical and laboratory data were systematically collected on standardized forms. The primary outcome was time to death or treatment failure (initial or relapse) up to 2 years after VL treatment. Death and treatment failure were analyzed separately in secondary analysis. For each patient, person-time at risk was calculated, starting from the date of VL treatment initiation up to either the date of death, date of treatment failure, or date of last visit for those unavailable for follow-up or transferred out, and 1 September 2010 for the remainder. The cumulative incidence of the outcome was estimated using Kaplan–Meier

methods. For the individual outcome estimates, adjustments were made for competing risks [23, 24]. Comparisons between groups were based on the log-rank test. For the main outcome, independent risk factors were determined in multivariate Cox regression analysis with backward selection, including those factors with P values $< .05$ in univariate analysis. For the separate outcomes, no multivariate analysis was performed, given the smaller number of events and the occurrence of groups without event. To visualize the association of independent continuous variables and the outcome, a nonparametric method called *LOWESS smoothing* was used. Pre- and posttreatment comparisons were based on the Wilcoxon signed rank test.

Ethical Considerations

The data included in this retrospective analysis constituted part of routine programmatic data collected for monitoring and evaluation purposes. The VL-HIV clinical treatment guideline has been reviewed and approved by the RMRIMS ethics committee.

RESULTS

Baseline Characteristics

A total of 55 cART-naive VL-HIV infected patients started treatment with liposomal amphotericin B within the VL program (Figure 1). The vast majority (83.6%) were male (Table 1). The median CD4 cell count at VL diagnosis was 66 cells/ μ L. Sixty-two percent of patients reported that they were migrant laborers within India, most commonly in Delhi and Kolkata.

Clinical presentation is summarized in Table 2. For 43 patients (78.2%), diagnosis was parasitologically confirmed. In the remainder of patients, in whom VL treatment was initiated before referral to RMRIMS, diagnosis was based on the combination of clinical and serologic data. All but one of these were primary VL cases with a typical clinical response to VL treatment. In terms of treatment history, 27 (49.1%) patients presented with VL relapse.

Visceral Leishmaniasis and Human Immunodeficiency Virus Treatment Outcomes

Overall, tolerance of liposomal amphotericin B was excellent, with no interruptions of treatment due to intolerance. Treatment was associated with significant increases in body weight, hemoglobin levels, and platelet counts and decreases in spleen size (Table 3). No significant changes in the results of kidney or liver function tests were observed.

Seven patients (12.7%) died, 3 shortly after initiation of treatment for suspected opportunistic infections. Four were reported dead after discharge or during active case finding. Five never started cART (see Figure 1). Whereas none of the 55 patients had initial treatment failure, 8 (14.5%) experienced

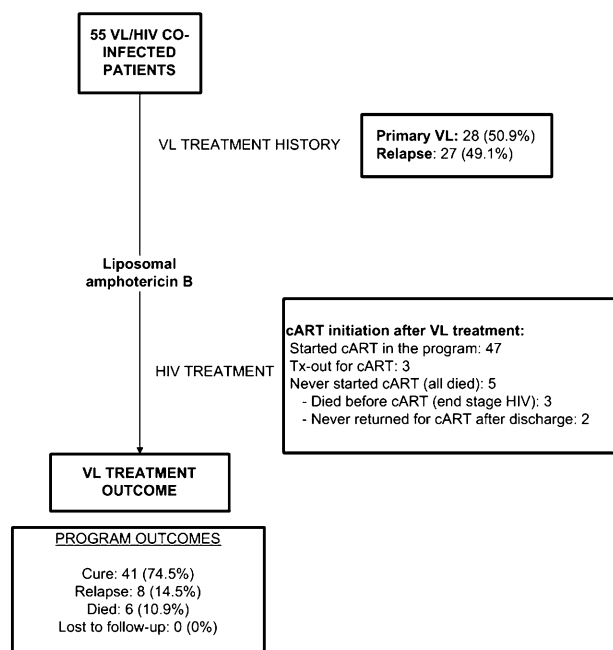


Figure 1. Flow chart for patients in this study coinfecting with visceral leishmaniasis (VL) and human immunodeficiency virus (HIV). In addition to the 6 deaths shown here, 1 patient with relapse was subsequently reported to have died. cART, combination antiretroviral treatment.

relapse during follow-up at a median of 1.3 years (interquartile range, 0.7–1.4) after VL treatment. None of these relapses occurred within the first 6 months after treatment. As shown in Figure 2, CD4 cell counts of >250–300 cells/ μ L at 6 months after VL treatment seemed to be associated with a very low risk of subsequent relapse. Four of the eight patients developing VL relapse were patients with previous VL relapse and had received VL treatment (conventional amphotericin B) before the initiation of liposomal amphotericin B within the program. Despite good initial treatment response for all relapse episodes, 4 of the 8 patients with relapse experienced another relapse within the next 3–8 months. All 4 patients had CD4 cell counts <100 cells/ μ L at 6 months after the first VL treatment. The mean CD4 cell counts at 6, 12, and 24 months after cART initiation were 187 (95% CI, 153–221), 234 (95% CI, 164–303) and 261 (95% CI, 37–486) cells/ μ L, respectively.

In terms of overall VL treatment response, the estimated probabilities of death or treatment failure by 6, 12, and 24 months were 11.7%, 19.8% and 38.3%, respectively. Mortality by 2 years after VL treatment was estimated at 14.5% (Figure 3). The probabilities of relapse were 0%, 8.1%, and 26.5% at 6, 12 and 24 months, respectively. The overall rate of retention in HIV care was 83.6%.

Risk Factor Analysis for Death and Treatment Failure

In univariate analysis, a body mass index (BMI) <16 kg/m² and diagnosis of tuberculosis were identified as risk factors for the

Table 1. Baseline Characteristics of Patients With Visceral Leishmaniasis (VL) and Human Immunodeficiency Virus (HIV) Coinfection Treated With Liposomal Amphotericin B, July 2007–July 2010;

Patient characteristics	Patients, no. (%) (n = 55)
Sex	
Female	9 (16.4)
Male	46 (83.6)
Age, median (IQR), years	35 (30–40)
Pediatric patients (<15 years old)	2 (3.6)
Caste^a	
Forward	6 (10.9)
Backward	35 (63.6)
Lowest	12 (21.8)
Other	2 (3.6)
Risk factor for HIV infection	
Migrant laborer	34 (61.8)
High-mobility profession (driver, transport)	3 (5.4)
Husband with high-mobility profession	4 (7.3)
Other ^b	5 (9.1)
None reported	9 (16.4)
CD4 cell count at VL diagnosis, median (IQR), cells/ μ L (n = 53)	66 (38–112)
CD4 cell count <200 cells/ μ L	48 (90.6)
Baseline body mass index, median (IQR), kg/m ²	17.1 (15.6–18.5)
Tuberculosis treatment during current VL episode	9 (16.4)
Time from VL diagnosis to cART initiation, median (IQR), days (n = 47)	19 (11–39)

Data represent no. (%) of patients unless otherwise indicated (n = 55 unless otherwise stated).

Abbreviations: cART, combination antiretroviral treatment; IQR, interquartile range.

^a The Indian caste system is a system of social organization in which communities are defined by thousands of hereditary groups. Our patient population is almost uniformly from relatively “lower” castes. Three commonly used broader categories were defined, from higher to lower on the social ladder: forward, backward, and scheduled castes (also called the “untouchables”).

^b The “other” category included multiple blood transfusions and vertical transmission (HIV-positive parent).

combined outcome of death and treatment failure ($P < .01$ for both). Only the association with BMI (hazard ratio, 3.6; 95% confidence interval [CI], 1.0–12.9) remained significant in multivariate analysis.

With regard to mortality, CD4 cell counts of <50 cells/ μ L at VL diagnosis, diagnosis of tuberculosis, and BMI <16 kg/m² were identified as risk factors in univariate analysis. Relating to the risk of relapse, a significant association was observed with CD4 cell counts <200 cells/ μ L at 6 months after VL treatment. No significant associations were seen between mortality or relapse and cART use at time of VL diagnosis, history of relapse, or any other baseline characteristic.

Table 2. Clinical Presentation, Diagnosis, and Treatment Details in Patients With Visceral Leishmaniasis (VL) and Human Immunodeficiency Virus (HIV) Coinfection Treated With Liposomal Amphotericin B, July 2007–July 2010

Variable	Patients, no. (%) (n = 55)
Main symptoms at presentation (n = 54)	
Fever	54 (100)
Cough	15 (27.8)
Weakness/asthenia	8 (14.8)
Weight loss	7 (13.0)
Abdominal pain/distension	2 (3.7)
Duration of illness, median (IQR), weeks	6 (4–12)
VL diagnosis	
Clinical case definition and positive rK39 rapid diagnostic test	12 (21.8)
Clinical case definition, positive rK39 rapid diagnostic test, and parasitologic confirmation ^a	43 (78.2)
Grading of parasite density, median (IQR) (n = 42)	4 (3–5)
VL treatment history	
First episode (primary VL)	28 (50.9)
Relapse	27 (49.1)
≥2 previous VL episodes	8 (14.5)
Time since most recent VL treatment, median (IQR), months	11.2 (8.2–15.7)
Treatment at most recent VL episode	
Amphotericin B	12 (21.8)
Antimonials	6 (10.9)
Liposomal amphotericin B	5 (9.1)
Miltefosine	4 (7.3)
Liposomal amphotericin B treatment regimen	
20 mg/kg in 4 doses	50 (90.9)
25 mg/kg in 5 doses	5 (9.1)
Follow-up time after VL treatment, median (IQR), months	11.4 (3.4–20.3)
Test of cure performed	43 (78.2)

Data represent no. (%) of patients, unless otherwise indicated.

Abbreviations: cART, combination antiretroviral treatment; IQR, interquartile range.

^a Confirmation based on spleen (n = 40) or bone marrow (n = 3) aspiration.

DISCUSSION

To our knowledge, this study reports on the largest cohort of VL-HIV-coinfected patients in Asia. Moreover, it is the first to provide long-term outcomes with the use of liposomal amphotericin B and cART for VL-HIV coinfection from a resource-constrained setting. Overall survival was relatively good, initial treatment failure appeared very rare. Although the 2-year relapse rate was substantial, all relapse cases responded well to liposomal amphotericin B retreatment. It is also important to note that all relapse cases would have gone unreported with routine efficacy

Table 3. Clinical and Laboratory Parameters Before and After Treatment With Liposomal Amphotericin B, July 2007–July 2010

Variable	Baseline (n = 55)	End of treatment (n = 53)	Within-patient difference [P]	1 month after treatment (n = 45)	Within-patient difference [P]
Weight, kg	45 (40–48)	45 (41–49)	1 (0–2) [.010]	48 (45–52)	3 (0–5) [<.001]
Spleen size, cm	5 (4–8)	2 (0–3)	–3 (–5 to –2) [<.001]	0 (0–1)	–5 (–7 to –3) [<.001]
Hemoglobin, g/dL ^a	7.9 (6.9–9.3)	8.5 (7.4–9.4)	0.1 (–0.5 to 0.7) [.30]	8.9 (8.3–10.0)	1.0 (0.0–1.5) [<.001]
Creatinine, mg/dL (n = 49)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	0.0 (–0. to 0.2) [.39]	ND	...
SGPT, U/L (n = 49)	25 (19–38)	25 (20–36)	0 (–9 to 9) [.55]	ND	...
Platelet count, cells/μL (n = 50) ^b	124 000 (100 000–166 000)	168 000 (124 500–215 500)	25 000 (0–89 000) [<.01]	ND	...

Data represent medians (interquartile ranges).

Abbreviations: ND, not done; SGPT, serum glutamic pyruvic transaminase (liver function test).

^a For hemoglobin levels, n = 52 at the end of treatment and n = 46 at 1 month after treatment.

^b For platelet counts, n = 48 at the end of treatment.

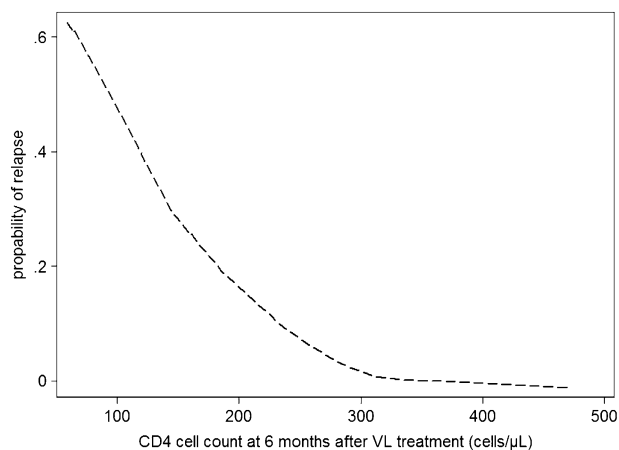


Figure 2. Association between CD4 cell counts at 6 months after initiation of treatment for visceral leishmaniasis (VL) and the estimated risk of subsequent VL relapse (LOWESS graph).

monitoring, using 6-month outcomes to define the final treatment response.

Mindful of the reported high early mortality (up to 20%–30%) for patients beginning cART with advanced HIV infection throughout the world [25, 26], irrespective of VL, the 2-year survival rates reported for this VL-HIV-coinfected population are rather encouraging. Moreover, these survival rates compare favorably with those in a recent study from Ethiopia, which found a case-fatality rate for coinfecting patients of 17.4% by 1 month after VL treatment initiation [18]. Still, given the <1% mortality reported in our program for HIV-negative patients with VL treated with liposomal amphotericin B [22], it remains true that VL-HIV coinfection is still a deadly combination, probably mainly because of the advanced HIV disease [27].

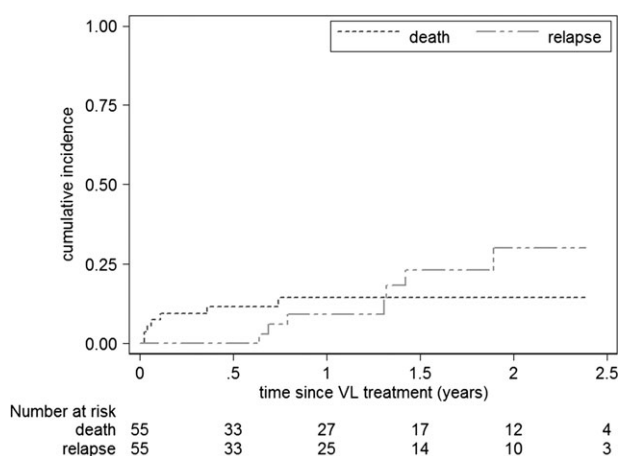


Figure 3. Kaplan–Meier estimates of cumulative incidence of death or relapse at different time points after initiation of visceral leishmaniasis (VL) treatment.

This highlights the importance of early detection and treatment of concurrent opportunistic infections, particularly tuberculosis, in VL-HIV-coinfected patients, along with early cART initiation.

The apparent low rates of initial treatment failure in this study are in contrast with those in another MSF program with a similar set-up in Ethiopia; in that program, initial failure occurred in 33% of HIV-coinfected patients and in even more patients with relapse, despite the use of high doses of liposomal amphotericin B (25–30 mg/kg) [28]. However, it needs to be acknowledged that there are also pronounced regional differences in the treatment efficacy of liposomal amphotericin B in the general population. In India, high treatment success rates have consistently been documented in HIV-negative patients at doses of liposomal amphotericin B that were clearly ineffective in other regions, including Europe, South America, and East Africa [29, 30].

In line with findings from Europe, the relapse rate was substantial. Longitudinal studies integrating parasite genotyping would be needed to quantify the contribution of reactivation—given ongoing immune suppression—and reinfection. As reported by others, our data suggest that with progressive immune recovery—with CD4 cell counts reaching 250–300 cells/μL—the risk of relapse seems to be low [5]. This underscores the need for timely cART initiation. More systematic HIV testing should be considered, especially for patients with relapse or HIV risk factors. On the other hand, there seems to be a population with poor immune recovery after cART initiation, with a high risk of (subsequent) relapse. Close monitoring for VL and VL relapse should be integrated in ART programs for patients living in or coming from VL-endemic areas. With relapse predominantly occurring relatively late after VL treatment, follow-up for several years after treatment is required, particularly for those with poor increases in CD4 cell counts after ART initiation.

A number of limitations have to be mentioned. This is a retrospective analysis of a relatively small patient population, using data from operational settings. In this respect, the incomplete data on parasitologic diagnosis or TOC are concerning. The former could have led to erroneous diagnosis of VL, possibly leading to both under- or overestimation of treatment outcomes. Still, high specificity (>95%) of rK39 testing has been reported [31], also in HIV-infected patients [32]. Incomplete TOC data could have resulted in underestimation of initial treatment failure rates, especially in patients who died. However, All patients without TOC data manifested a typical response to antileishmanial treatment, and treatment failure was suspected for none of these. In any event, initial treatment failure appeared to be very rare. Viral load data and information on cART and cotrimoxazole adherence would have strengthened the study findings. Finally, detailed analysis of the causes of death might have been informative.

More scientific and programmatic attention should be placed on VL-HIV coinfection. The expansion of HIV in this VL-hyperendemic and highly populous state could fuel VL-HIV epidemics and could contribute to the spread of VL to non-endemic regions. Although the VL elimination program in the Indian subcontinent continues to rely on the use of miltefosine, recently revised WHO guidelines currently recommend liposomal amphotericin B (at a total dose of 10–15 mg/kg) as first-line treatment in this region [19]. Our findings suggest that liposomal amphotericin B is effective in VL-HIV-coinfected Indian patients at a total dose of 20 mg/kg. Especially given the risk of multiple relapses, the risk of inducing drug resistance should be carefully considered; the use of liposomal amphotericin B at an increased dose (25 mg/kg) may need to be reviewed as a possibility for patients who experience relapse after treatment at 20 mg/kg. Now that miltefosine and paromomycin are increasingly available, these drugs could also be considered for use in second-line regimens. The place of combination therapy to improve and preserve drug efficacy for coinfecting patients remains to be determined [33].

In conclusion, our findings support the need for increased availability of liposomal amphotericin B for VL treatment in resource-constrained settings, along with highly accessible cART and treatment for opportunistic infections. Further price reductions for liposomal amphotericin B are much needed to make this drug available within the public health system in poor, disease-endemic countries.

Notes

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Potential conflicts of interest. All authors: No reported 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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