# Limited Effectiveness of High-Dose Liposomal Amphotericin B (AmBisome) for Treatment of Visceral Leishmaniasis in an Ethiopian Population With High HIV Prevalence

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***Background.*** Due to unacceptably high mortality with pentavalent antimonials, Médecins Sans Frontières in 2006 began using liposomal amphotericin B (AmBisome) for visceral leishmaniasis (VL) patients in Ethiopia who were severely ill or positive for human immunodeficiency virus (HIV).

***Methods.*** We used clinical data obtained from January 2007 to January 2009 to compare outcomes by HIV status and VL episode (primary vs relapse) and to identify risk factors for treatment failure among patients treated with AmBisome monotherapy at a total dose of 30 mg/kg in 6 doses on alternate days, a higher dose than recommended by the World Health Organization (20 mg/kg).

***Results.*** Among 94 HIV-negative severely ill VL patients, 93% had initial cure and 6% died. Among 195 HIV-positive patients (116 primary, 79 relapse VL), 60% had initial cure, 7% died, and 32% were parasitological failures. AmBisome was less effective in the 79 HIV-positive VL relapse patients (38% initial cure, 5% mortality, 56% parasitological failure) than in the 116 HIV-positive primary VL patients (74% initial cure, 8% mortality, 16% parasitological failure). Sodium stibogluconate (SSG) rescue treatment increased the overall cure rate among all HIV-positive VL patients from 60% to 83%, but 16% (9 of 59) of rescue treatment patients died, mainly due to SSG toxicity.

***Conclusions.*** High-dose AmBisome for VL is safe and effective in severely ill HIV-negative patients, and safe but less effective in HIV-positive patients. Combining AmBisome with another drug may enhance its effectiveness in HIV-positive VL patients. SSG should be avoided for treatment of VL in HIV-positive patients.

Visceral leishmaniasis (VL; "kala-azar") is a systemic parasitic disease caused by the *Leishmania donovani* species complex. Transmission, via sandflies, is anthroponotic. VL is endemic in large areas of East Africa, including the lowlands between Humera and Metema in northern Ethiopia. Médecins Sans Frontières (MSF) has diagnosed and treated up to 2000 VL patients annually in this area since 1997. The VL-affected population comprises mainly male (>90%) migrant farm laborers. Many patients present with advanced severe VL because of difficulties in accessing treatment. Pentavalent antimonials (sodium stibogluconate; SSG), the mainstay of VL treatment in East Africa, remains very efficacious [1–5], but treatment-associated mortality in VL patients with human immunodeficiency virus (HIV) infection [3, 4, 6] and immunocompetent patients with severe VL [7, 8] is unacceptably high.

HIV has expanded into northern Ethiopia via the migrant rural workforce. The HIV coinfection rate in VL patients rose from 19% in 1999 to 34% in 2009 [3, 4, 6, 9]. Most studies of HIV/VL coinfection originated pre-1996 in southern Europe and are not representative of

the situation in Ethiopia where the parasite is more virulent and different comorbidities occur [10–13]. HIV/VL coinfection in Africa is characterized by high parasite load, parasite dissemination to unusual sites, lower initial and final cure rates, greater susceptibility to pentavalent antimonial drug toxicity, increased risk of secondary drug resistance, higher rates of death, and very high rates of relapse [3, 4, 14, 15].

Studies from Europe, India, and Brazil have demonstrated that liposomal amphotericin B (AmBisome) has high efficacy and low toxicity in immunocompetent VL patients [16]. It is also the preferred antileishmanial drug in HIV-positive VL patients, although the data are more limited and originate predominantly from Europe. The World Health Organization (WHO) recommends a total AmBisome dose of 20 mg/kg to treat immunocompetent children and adults [17], but we reported that 16% (10 of 64) of patients in Sudan did not respond to treatment at this dose [18]. We attributed this high failure rate to high initial parasite loads and immunosuppression due to underlying HIV infection and/or tuberculosis, and we speculated that higher doses should be used. Total doses of 30–40 mg/kg were well tolerated in HIV-positive VL patients in Europe [19]. Hence, we introduced AmBisome into MSF programs in Ethiopia in 2006 as first-line treatment for HIV-positive and severely ill immunocompetent VL patients at a total dose of 30 mg/kg. In this retrospective cohort analysis we assess the effectiveness of high-dose AmBisome monotherapy and identify risk factors for treatment failure.

## METHODS

### Study Design and Population
Our study population comprised severely ill and HIV-positive VL patients diagnosed and treated within an integrated HIV/VL program at MSF clinics in Humera (western Tigray) and Abdurafi (northern Amhara) in northern Ethiopia from January 2007 to January 2009. All investigations and treatments for all patients were free of charge. Our analysis included all patients treated with AmBisome monotherapy. Patients given AmBisome in combination with another antileishmanial drug, or who were switched from SSG to AmBisome due to SSG intolerance, were excluded.

### Visceral Leishmaniasis Diagnosis
Diagnosis was according to standard MSF practice [20]. The WHO case definition of VL was used as a starting point: history of fever for >2 weeks, malaria excluded, in combination with wasting, and either splenomegaly or lymphadenopathy [21]. Patients whose illness met this case definition, and who had no previous VL treatment, were diagnosed serologically either by positive rK39 rapid diagnostic test (DiaMed-IT-Leish, DiaMed AG) [22] or by high-titer (≥1:6400) leishmania direct

agglutination test (DAT) (Royal Tropical Institute) [23]. Patients with an intermediate DAT titer (1:800–1:3200) underwent splenic or lymph node aspiration, and VL was confirmed parasitologically. Patients with suspected VL but a negative rK39 test and low DAT titer (<1:400) were evaluated for alternative illnesses and retested if their illness persisted. Severely ill patients with a negative rK39 test were aspirated without delay. Patients fulfilling the case definition but with previous VL treatment (suspected VL relapse patients) were treated only if they had a positive aspirate. A clinical diagnosis of relapse was made if splenic aspiration was contraindicated due to a barely palpable spleen, pregnancy, bleeding tendency, severe anemia, jaundice, or state of collapse.

### HIV Testing and Treatment
Patients were offered counseling and testing for HIV immediately after confirmation of VL diagnosis. Two rapid diagnostic HIV tests were used in parallel (HIV-Determine, Abbott Diagnostics and Unigold, Trinity Biotech) using venous blood [24]. All positive HIV results were confirmed by repeat tests on a second blood sample. Patients with discordant results were asked to retest after 6 weeks and were considered HIV negative if test results were again discordant. VL patients coinfected with HIV were offered follow-up care in our clinic for HIV/AIDS, including diagnosis and treatment of opportunistic infections, *Pneumocystis jiroveci* pneumonia prophylaxis, tuberculosis treatment, psychosocial support, shelter for homeless patients, and therapeutic feeding. HIV-positive VL patients were offered antiretroviral treatment (ART) if they were well motivated for lifelong ART and had not defaulted from the follow-up clinic.

### Assessment of Visceral Leishmaniasis Severity
Severity of VL disease was assessed by a scoring system combining scores for different factors associated with >20% increased risk of death, including general weakness, old age, low body mass index, and/or low hemoglobin levels [7].

### Visceral Leishmaniasis Treatment Regimens
Primary nonsevere VL was treated with SSG (Albert David, Calcutta and International Dispensary Association) 20 mg/kg/day by intramuscular injection for 30 days. Severely ill VL patients, patients with known intolerance to SSG, patients with VL relapse, and HIV-positive VL patients were treated with AmBisome (Gilead Sciences) at a total dose of 30 mg/kg, divided into 6 infusions of 5 mg/kg on alternate days. Primary and relapse VL patients failing AmBisome treatment received rescue treatment with SSG (20 mg/kg/day) for 30 days and 40 days, respectively.

### Visceral Leishmaniasis Treatment Outcomes
In HIV-negative primary VL, initial cure was established clinically by observing fever resolution, spleen regression, hemoglobin increase, and weight gain. A parasitological test of cure by splenic

aspirate was performed on day 28 in HIV-positive and relapsed patients or if clinical response to primary VL treatment was uncertain. If a test-of-cure aspirate could not be done because of absence of palpable spleen or lymph nodes after treatment, initial cure was established clinically. Patients were considered to have failed treatment if they showed parasitological failure after AmBisome monotherapy irrespective of clinical response, died during treatment, defaulted from treatment, or were transferred to another hospital because of treatment complications.

### Data Entry and Statistical Analysis

Data were extracted from medical records and single-entered into an Excel (Microsoft) spreadsheet. Characteristics of groups (HIV positive vs HIV negative; primary vs relapse VL) were compared using Fisher exact test for categorical variables and Student $t$ test for continuous variables (Epi Info 2002 software, revision 2, Centers for Disease Control and Prevention). Multivariable logistic regression models were used to identify independent risk factors for parasitological failure (Stata software version 10, StataCorp).

### Ethical Approval

Data were collected as part of routine patient care. No additional investigations were performed other than those indicated for medical management. The study underwent ethical review according to MSF procedures for routine programmatic data.

## RESULTS

### Patients

Between January 2007 and January 2009, 362 patients (277 in Humera, 85 in Abdurafi) were treated with AmBisome (Figure 1). Forty-nine patients who had started SSG treatment but who were switched to AmBisome due to SSG toxicity were excluded from our analysis, as were 24 patients with unknown HIV status. Of the 289 patients included in our analysis, 94 (32.5%) tested HIV negative, 195 (67.5%) HIV positive. Of the 94 HIV-negative patients, 84 (89.4%) were severely ill primary VL patients and 10 were VL relapse patients. Of the 195 HIV-positive patients, 116 (59.5%) had primary VL and 79 (40.5%) had VL relapse.

In total, 173 (86.5%) primary VL patients were diagnostically confirmed by serological test (rK39 and/or DAT) and 17 (8.5%) by splenic aspirate, and 10 (5.0%) patients were diagnosed clinically (negative serological test but strong clinical suspicion of VL and aspiration contraindicated). Eighty-nine patients were diagnosed with VL relapse (67 first relapse, 22 second or further episode), of whom 76 (85.4%) were confirmed parasitologically by splenic aspiration, 13 (14.6%) were diagnosed clinically because aspiration was contraindicated, and 79 (88.8%) were HIV-positive.

Patients were predominantly (91%) male, with no difference in this proportion by HIV status (Table 1) or VL episode (Table 2). Two patients were pregnant (1 HIV-negative primary VL, 1 HIV-positive VL relapse); both were discharged without test of cure (contraindicated because of pregnancy) but with good response to treatment. HIV-positive patients were older, more malnourished, and less anemic than HIV-negative patients (Table 1). The prevalence of tuberculosis coinfection was higher in HIV-positive (29.7%) than in HIV-negative (7.4%) patients. Jaundice was more common among HIV-negative (12.8%) than among HIV-positive (0.5%) patients. VL relapse was more common among HIV-positive patients (40.5%) than among HIV-negative patients (10.6%). Among HIV-positive patients, relapse patients had larger spleens and were more malnourished compared with primary VL patients (Table 2).

### Treatment Outcomes

The total AmBisome dose received by patients who completed treatment ranged from 25.0 mg/kg to 40.5 mg/kg (median 30.0 mg/kg). Two patients received 7 instead of 6 doses. A test-of-cure aspirate was performed in 129 of 179 (72.1%) HIV-positive primary VL patients and 75 of 84 (89.3%) VL relapse patients. Reasons for not performing a test of cure included no palpable spleen (n = 13), nonattendance at test-of-cure appointment (n = 13), and splenic aspiration contraindicated (n = 7). Nineteen patients (6.6%) died during AmBisome treatment. The most common causes of death were sepsis (31%), congestive heart failure (19%), and bleeding (19%).

Initial cure was 92.6% in HIV-negative patients compared with 59.5% in HIV-positive patients ($P$ < .001), mainly due to parasitological failures (0% in HIV-negative vs 32.3% in HIV-positive patients; $P$ < .001; Table 3). There was no difference in mortality by HIV status (6.4% in HIV-negative vs 6.7% in HIV-positive patients; $P$ = .87).

AmBisome was much less effective in HIV-positive VL relapse patients than in HIV-positive primary VL patients, with initial cure rates of 38.0% versus 74.1%, respectively ($P$ < .001) (Table 4). Parasitological failures were 55.7% versus 16.4% ($P$ < .001), but there was no difference in mortality (7.8% vs 5.1%; $P$ = .65). Parasitological failures in patients with multiple relapses were 68.2% (15 of 22) versus 50.9% (29 of 57) in patients admitted for first relapse ($P$ = .17).

The drug used for previous treatment was known in 83.5% (66 of 79) of HIV-positive VL relapse patients. Of these, 25.8% (17 of 66 ) had previous exposure to AmBisome or amphotericin B. Previous AmBisome or amphotericin B exposure was reported by 14 of 44 (31.8%) HIV-positive VL relapse patients who experienced parasitological failure versus 2 of 30 (6.7%) patients who were cured ($P$ = .01). The median interval between VL episodes in patients who received previous AmBisome treatment (n = 13) was 4 months (range, 2–8 months), significantly
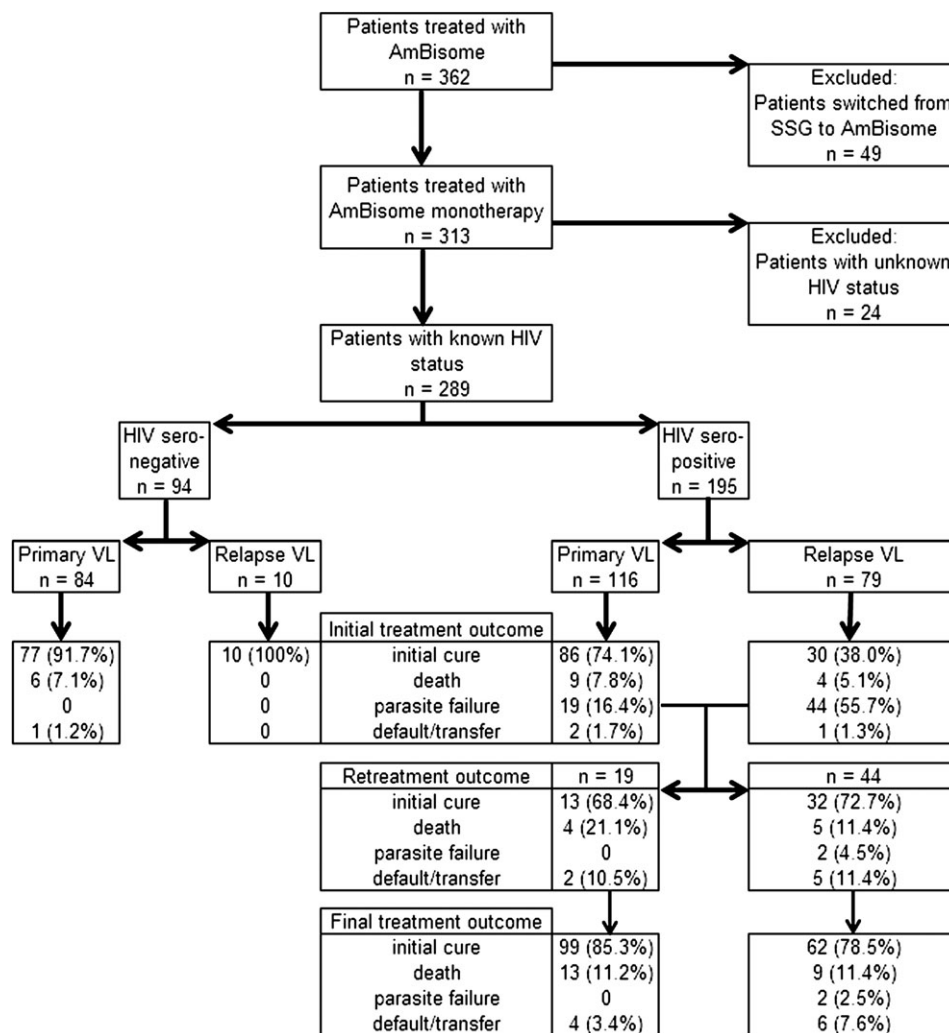
**Figure 1.** Patient flow diagram.

shorter than the median 18 months (range, 4–120 months) in patients who had received SSG (n = 44; $P < .001$).

Independent risk factors for parasitological failure in HIV-positive VL patients were a diagnosis of relapse VL versus primary VL (odds ratio [OR] = 4.43; 95% confidence interval [CI], 1.57–12.53; $P = .005$) and larger spleen size on admission (OR = 1.18; 95% CI, 1.01–1.39; $P = .04$; Table 5). Age, body mass index, hemoglobin, and tuberculosis infection were not associated with parasitological failure in HIV-positive VL patients.

### Rescue Treatment

Of 63 HIV-positive patients with parasitological failure after AmBisome monotherapy, 58 (92.1%) received SSG rescue treatment and 5 were retreated with AmBisome due to SSG intolerance. Of the 58 patients retreated with SSG, 41 (70.7%) were cured parasitologically, 9 (15.5%) died, 7 (12.1%) defaulted or were transferred, and 1 (relapse after previous SSG treatment) failed treatment parasitologically. Of the 5 patients

retreated with AmBisome, 3 had a negative test of cure, 1 was transferred, and 1 failed parasitologically. Rescue treatment increased the overall cure rate in HIV-positive patients to 82.1% (160 of 195), with no difference between primary and relapse VL patients (85.3% vs 78.5%; $P = .25$).

### HIV Treatment

Of the 195 HIV-positive patients, ART status was known for 87 patients: 42 were previously on ART, 28 were initiated on ART after VL admission, and 17 were not on ART before or after admission. CD4$^+$ cell counts on admission for VL treatment were available for 43 patients, of whom 21 (49%) had a CD4$^+$ count of <100 cells/uL, and 29 (67%) <200 cells/uL. The mean (SD) CD4$^+$ count was 155 (123) cells/uL. There was no difference in mean CD4 counts between patients on ART versus those not on ART (189 vs 134 cells/uL; $P = .18$), between patients with primary versus relapse VL (150 vs 159 cells/uL; $P = .83$), or between patients with initial cure versus parasitological failure

**Table 1. Patient Characteristics According to HIV Status**

| | HIV negative (n = 94) | | HIV positive (n = 195) | | |
|---|---|---|---|---|---|
| | Median | Range | Median | Range | P value[a] |
| Age (y) | 24 | 1.5–67 | 30 | 10–56 | <.001 |
| Spleen size (cm) | 8 | 0–20 | 8 | 0–20 | .94 |
| Hb (g/dL) | 5.7 | 2.1–14.5 | 7.6 | 2.5–13.1 | .001 |
| BMI (kg/m²) | 16.2 | 11.0–22.2 | 15.9 | 10.8–25.5 | .01 |
| | No. | % | No. | % | P value[b] |
| Sex (male) | 83 | 88.3 | 179 | 91.8 | .46 |
| TB coinfection | 7 | 7.4 | 58 | 29.7 | <.001 |
| Jaundice | 12 | 12.8 | 1 | <1 | <.001 |
| VL relapse | 10 | 10.6 | 79 | 40.5 | <.001 |

Abbreviations: BMI, body mass index; Hb, hemoglobin; HIV, human immunodeficiency virus; TB, tuberculosis; VL, visceral leishmaniasis.

[a] Student *t* test comparing mean values.

[b] Fisher exact test.

(119 vs 183 cells/uL; $P = .07$). In total, 55% (12 of 22) of patients who were on ART before admission for VL had a $CD4^+$ cell count of <200 cells/uL.

## DISCUSSION

High-dose AmBisome monotherapy (30 mg/kg) was safe and effective in severely ill HIV-negative VL patients. Treatment outcomes (92.6% initial cure, 6.4% mortality) were similar to those observed in 2010 in nonsevere VL treated with SSG/paromomycin in southern Sudan (94.3% and 4.0%, respectively; N = 2,618) and with SSG monotherapy in eastern Sudan (92.9% and 3.3%, respectively; N = 878) [MSF, unpublished data].

AmBisome was equally safe but much less effective in HIV-positive VL patients (59.5% initial cure, 6.7% mortality) due to

**Table 2. Characteristics of HIV-Positive Patients According to Visceral Leishmaniasis Episode**

| | Primary VL (n = 116) | | Relapse VL (n = 79) | | |
|---|---|---|---|---|---|
| | Median | Range | Median | Range | P value[a] |
| Age (y) | 30 | 18–56 | 30 | 10–56 | .85 |
| Spleen size (cm) | 6 | 0–20 | 8 | 3–20 | .006 |
| Hb (g/dL) | 7.6 | 2.5–13.1 | 7.5 | 2.7–11.7 | .48 |
| BMI (kg/m²) | 16.2 | 10.8–25.5 | 15.4 | 11.0–18.9 | .04 |
| | No. | % | No. | % | P value[b] |
| Sex (male) | 105 | 90.5 | 74 | 93.7 | .60 |
| TB coinfection | 31 | 26.7 | 27 | 34.2 | .34 |
| Jaundice | 0 | 0 | 1 | 1.3 | 1.00 |

Abbreviations: BMI, body mass index; Hb, hemoglobin; HIV, human immunodeficiency virus; TB, tuberculosis; VL, visceral leishmaniasis.

[a] Student *t* test (continuous variables).

[b] Fisher exact test (binary variables).

**Table 3. Initial Treatment Outcome by HIV Status**

| | HIV negative (n = 94) | | HIV positive (n = 195) | | |
|---|---|---|---|---|---|
| | No. | % | No. | % | P value[a] |
| Initial cure | 87 | 92.6 | 116 | 59.5 | <.001 |
| Death | 6 | 6.4 | 13 | 6.7 | .87 |
| Parasitological failure | 0 | 0 | 63 | 32.3 | .001 |
| Default/transfer | 1 | 1.1 | 3 | 1.5 | 1.00 |

Abbreviation: HIV, human immunodeficiency virus.

[a] Fisher exact test.

the higher proportion of parasitological failures (32.3% vs 0% in HIV-negative patients). AmBisome was even less effective in HIV-positive VL relapses (38.0% initial cure, 55.7% parasitological failure). Initial cure rates in HIV-positive VL patients treated in Europe with similar total doses of AmBisome are 80%–100% [25–27], even though $CD4^+$ counts in our patients were similar (or higher) [28, 29]. Although these 2 groups are not directly comparable, the suggestion that *L. donovani* in East Africa might be intrinsically less sensitive to amphotericin B is supported by observations from the Sudan region [18, 30].

Our data confirm that SSG is unacceptably toxic in HIV-positive VL patients. In a previous clinical trial, we showed that mortality during SSG treatment was 15.6% in HIV-positive versus 2.9% in HIV-negative VL patients (and 1.6% in HIV-positive VL patients treated with miltefosine [4]). In our study, rescue treatment of HIV-positive patients with AmBisome treatment failure increased the overall cure rate from 59.5% to 82.6%, but mortality in patients retreated with SSG was high (15.5%), often due to SSG toxicity. Retreatment with AmBisome led to parasitological cure in 3 of 5 patients. Hence, patients with a significant reduction in parasite burden (eg, ≥2 log grades in splenic aspirate) could be considered slow responders who may respond to higher doses of AmBisome. Patients who continue to show inadequate parasitological response should be retreated with miltefosine or paromomycin (with SSG as a last resort),

**Table 4. Initial Treatment Outcome in HIV-Positive Patients by Visceral Leishmaniasis Episode**

| | Primary VL (n = 116) | | Relapse VL (n = 79) | | |
|---|---|---|---|---|---|
| | No. | % | No. | % | P value[a] |
| Initial cure | 86 | 74.1 | 30 | 38.0 | <.001 |
| Death | 9 | 7.8 | 4 | 5.1 | .65 |
| Parasitological failure | 19 | 16.4 | 44 | 55.7 | <.001 |
| Default/transfer | 2 | 1.7 | 1 | 1.3 | 1.00 |

Abbreviations: HIV, human immunodeficiency virus; VL, visceral leishmaniasis.

[a] Fisher exact test.

**Table 5.** Multivariable Logistic Regression Analysis of Risk Factors for Parasitological Failure in HIV-Positive Visceral Leishmaniasis Patients (n = 80)

| Risk factor[a] | No parasites (n = 37) | Parasites (n = 43) | OR (95% CI)[b] | P value |
|---|---|---|---|---|
| Age (y) | 31.3 (7.3) | 33.5 (7.4) | 1.05 (.98–1.13) | .2 |
| BMI (kg/m$^2$) | 16.1 (2.8) | 15.5 (1.7) | 0.90 (.70–1.17) | .4 |
| Hb (g/dL) | 8.3 (2.0) | 7.6 (1.7) | 0.81 (.61–1.10) | .2 |
| Spleen size (range, 0–20) | 7.5 (3.6) | 9.8 (3.8) | 1.18 (1.01–1.39) | .04 |
| TB (%) | 35.1 | 37.2 | 0.70 (.22–2.18) | .5 |
| VL relapse (vs primary VL, %) | 29.7 | 69.8 | 4.43 (1.57–12.53) | .005 |

Abbreviations: BMI, body mass index; CI, confidence interval; Hb, hemoglobin; HIV, human immunodeficiency virus; OR, odds ratio; TB, tuberculosis; VL, visceral leishmaniasis.

[a] Mean (SD) unless otherwise indicated.

[b] Adjusted for all variables in table.

preferably in combination, assuming that a combination of 2 drugs with different modes of action may be more effective than single-drug treatment.

Parasitological clearance at the end of treatment is not a certain predictor of no future relapse [11, 26, 29]. Moreover, clinical response does not necessarily indicate parasitological clearance [28]. Trials of SSG in Sudan [31] and paromomycin in India [32] showed that scanty parasites at the end of treatment in HIV-negative patients usually clear spontaneously before the spleen is reaspirated 1 month later. In contrast, we reported previously that initial parasitological failure in HIV-positive VL patients was invariably followed by further relapses and high mortality [15]. The migrant farm laborers in our study may have difficulty returning for repeat treatment, so initial parasitological cure is crucial.

Our finding that spleen size on admission is a risk factor for parasitological failure is consistent with results from our study in southern Sudan which showed that splenomegaly is a risk factor for VL relapse [33]. Splenomegaly on admission may indicate severity of illness, parasite burden, and degree of immunosuppression. The prevalence of triple coinfection (VL, HIV, and tuberculosis) among HIV-positive VL patients in our study was 29.7%, similar to that observed in another Ethiopian study [14]. However, our regression model indicated that tuberculosis was not an independent risk factor for parasitological failure in HIV-positive VL patients.

Even though VL is considered an AIDS-defining condition and a valid entry point to commence ART irrespective of CD4$^+$ cell count, a significant proportion of HIV-positive patients in our study were not yet on ART. This was because many patients in this predominantly male migrant patient population did not meet the criteria for commencing ART (motivation to start and adhere to lifelong ART, and/or attendance at follow-up clinics). In Europe, ART has reduced the incidence of VL [34], prolonged intervals between relapses, reduced relapse rates [12, 34–36], and improved survival [29]. A study in Ethiopia demonstrated that ART reduced the risk of VL relapse by 50%, although an impact on survival could not be demonstrated [15]. ART does not guarantee adequate CD4$^+$ cell count reconstitution in *L. donovani*–infected patients, because 55% (12 of 22) of patients who had started ART before admission for VL had a CD4$^+$ cell count <200 cells/μL. It is likely that persistent *Leishmania* parasites hold the patient in a state of immunosuppression, and the inability to regain cellular immunity leads to recrudescence of parasites and relapse.

We may have underestimated parasitological failure rates, despite good clinical response to treatment, because 27.9% of HIV-positive patients and 10.7% of relapsed patients did not have a parasitological test of cure. Our observation that the onset of VL relapse in HIV-positive patients occurred much sooner after AmBisome treatment than after SSG treatment may have been biased because patients with previous SSG treatment would have been survivors from the period before the introduction of AmBisome.

High rates of treatment failure indicate that combination therapy, instead of AmBisome monotherapy, should be used to treat VL in HIV-positive patients. Combinations of AmBisome with safer drugs (eg, paromomycin, miltefosine) should be evaluated to circumvent the toxicity of antimonials. Although AmBisome-resistant parasites have not yet been demonstrated in patients coinfected with HIV and *Leishmania infantum* after repetitive treatment or prophylaxis [37], we are concerned that the already suboptimal effectiveness of AmBisome could be eroded by the emergence of drug-resistant *Leishmania* strains.

## Notes

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