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Five-Year Retrospective Italian Multicenter Study of Visceral Leishmaniasis Treatment

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The treatment of visceral leishmaniasis (VL) is poorly standardized in Italy in spite of the existing evidence. All consecutive patients with VL admitted at 15 Italian centers as inpatients or outpatients between January 2004 and December 2008 were retrospectively considered; outcome data at 1 year after treatment were obtained for all but 1 patient. Demographic characteristics, underlying diseases, diagnostic procedures, treatment regimens and outcomes, as well as side effects were recorded. A confirmed diagnosis of VL was reported for 166 patients: 120 (72.3%) immunocompetent, 21 (12.6%) patients with immune deficiencies other than HIV infection, and 25 (15.1%) coinfected with HIV. Liposomal amphotericin B (L-AmB) was the drug almost universally used for treatment, administered to 153 (92.2%) patients. Thirty-seven different regimens, including L-AmB were used. The mean doses were 29.4 ± 7.9 mg/kg in immunocompetent patients, 32.9 ± 8.6 mg/kg in patients with non-HIV-related immunodeficiencies, and 40.8 ± 6.7 mg/kg in HIV-infected patients ($P < 0.001$). The mean numbers of infusion days were 7.8 ± 3.1 in immunocompetent patients, 9.6 ± 3.9 in non-HIV-immunodeficient patients, and 12.0 ± 3.4 in HIV-infected patients ($P < 0.001$). Mild and reversible adverse events were observed in 12.2% of cases. Responsive patients were 154 (93.3%). Successes were 98.4% among immunocompetent patients, 90.5% among non-HIV-immunodeficient patients, and 72.0% among HIV-infected patients. Among predictors of primary response to treatment, HIV infection and age held independent associations in the final multivariate models, whereas the doses and duration of L-AmB treatment were not significantly associated. Longer treatments and higher doses of L-AmB were not able to significantly modify treatment outcomes either in the immunocompetent or in the immunocompromised population.

Visceral Leishmaniasis (VL) in the Mediterranean basin is a zoonotic disease caused by *Leishmania infantum* (1). It is the only vector-borne disease present in all Mediterranean countries, with an incidence of symptomatic cases ranging from 5 to 200 for each country, with Italy being close to the upper limit in recent years (2). Overt clinical disease with typical features (high fever, anemia, splenomegaly, hypergammaglobulinemia, and wasting syndrome) is common in immunocompetent adults and children (3–5), whereas immunocompromised patients often present lower rates of response to treatment and atypical disease features. These include organ transplant recipients, patients with advanced HIV disease or CD4 T-cell idiopathic deficiency, hematological malignancies, and patients on long-term steroids or monoclonal antibody treatments (6–9).

In spite of several treatment options (pentavalent antimony salts, paramomycin, pentamidine, amphotericin B formulations, and miltefosine), treatment of VL is still a complex and debated issue, since the use of each of drug poses different problems either related to limited efficacy or to potential serious adverse effects or to costs (10–23). Consensus recommendations from the World Health Organization (WHO) recognized in 2010 the prevalent use of liposomal amphotericin B (L-AmB) as the first choice for VL caused by *Leishmania infantum*, although they did not shed light on the great variety of administration schedules used (24). Indeed, several clinical trials demonstrated 90 to 98% efficacy of L-AmB in immunocompetent patients, using a total dose of 18 to 21 mg/kg (14, 25). As a consequence, U.S. regulatory agencies recommend 3 mg/kg on days 1 to 5, 14. and 21 and a total dose of 21 mg/kg (26).

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For pediatric patients, current recommendations indicate L-AmB at a dose of 10 mg/kg/day for 2 consecutive days, with the yield of similarly high response rates (27). Finally, in HIV-infected patients with VL, the WHO and the U.S. Food and Drug Administration recommend a total dose of 40 mg of L-AmB/kg.

In spite of such evidence and recommendations, L-AmB for VL is not yet reimbursable in Italy, and it is widely prescribed "off label." This retrospective investigation was endorsed by the National Society for Infectious and Tropical Diseases of Italy (SIMIT) in 2009 to gather further information on the epidemiology, clinical features, and therapeutic options in use for patients with VLat several Italian centers.

MATERIALS AND METHODS

The steering committee of the present study was instituted by the SIMIT central board early in 2009, based on the proposal of the Abruzzo regional board. Each infectious disease or pediatric unit adhering to SIMIT (146 units) was contacted individually and asked to participate. Local ethical committees were notified whenever a unit joined the study, in accordance with Italian rules for observational investigations. Centers adhering in the retrospection were requested to collect data relative to all consecutive patients diagnosed with VL since 2004 through 2008, with the aid of an electronic datasheet, according with a questionnaire prepared by the steering committee and approved by all of the participating centers. For all included patients, follow-up data up to 1 year after treatment completion were requested. The diagnosis of VL was based on the presence of amastigotes in bone marrow (BM) specimens or other tissue specimens at microscopic examination in the majority of cases; in a few cases, it was based on positive serological tests with indirect immunofluorescence and/or enzyme-linked immunosorbent assays for leishmanial antibodies, together with positive results of leishmania PCR assays on bone marrow aspirates or peripheral blood. The following variables were requested: demographics (age, sex, ethnicity, and region of origin); year of VL diagnosis, HIV status, CD4 T-cell counts, both at nadir and enrollment, antiretrovirals used for HIV-infected patients, other immunodeficiencies, and/or comorbidities; hemoglobin levels, white blood cell (WBC) counts, platelet counts, serum transaminases, serum albumin; type and schedule of drug(s) used for treatment of VL; treatment of relapse(s); support therapies (RBC transfusions and albumin infusions); adverse events recorded at any stage during treatment or follow-up; and treatment outcomes (healing, relapse, and death). The primary outcome of treatment was defined as the clinical cure of VL, that is, the absence of relapses 1 year after treatment completion; this included both successes obtained after first line treatment and those gained after retreatment of relapsers. Multivariable logistic regression analyses were used to evaluate potential independent predictors of failure to first line treatment, which was the dependent variable. Age and gender were included into regression models *a priori*, along with any other possible significant variable, selecting in a stepwise forward process. For continuous covariates for which a defined threshold has been indicated in the literature (for example, platelets [PLT] < 50 for severe thrombocytopenia), we tested the inclusion of both the continuous and the categorical form and selected the one that was included in the model with highest pseudo or adjusted R^2 values. All variables not included into the final models were not significant. Overall, only two subjects with one missing data in one of the variables were excluded from multivariate models. Thus, no missing data imputation technique was adopted. The results of the logistic analysis are presented as odds ratios (OR) and 95% confidence intervals (CI). Statistical significance was defined as a two-sided P value of <0.05 for all analyses, which were performed using STATA 10.1 (Stata Corp., College Station, TX).

RESULTS

Only 113 of the 146 infectious disease or pediatric units adhering to SIMIT answered to our request after the second notification.

TABLE 1 Demographic and selected clinical characteristics of 166 patients with VL[a]

Characteristic	No. of patients (%)
Male	108 (65)
Immunocompetent	120 (72.3)
HIV positive	25 (15)
Hematological malignancy	9 (5.4)
Transplant recipients	5 (3.0)
Long-term steroid treatment	4 (2.4)
Methotrexate treatment	2 (1.2)
Splenectomy	1 (0.6)
With comorbidities	
None	116 (69.9)
Multiple	12 (7.2)
Hepatitis	11 (6.6)
Cardiovascular diseases	6 (3.6)
Diabetes	5 (3.0)
Rheumatologic diseases	6 (3.6)
Chronic renal failure	3 (1.8)
COPD	3 (1.8)
Sepsis	2 (1.2)
Pneumonia/TB	2 (1.2)
Thyroid diseases	1 (0.6)

[a] The mean age of the patients ± the standard deviation was 34.9 ± 24.3 years (range, 1 to 85 years). COPD, chronic obstructive pulmonary disease; TB, tuberculosis.

Thirty-two centers replied they had no cases to contribute over the requested time span. Fifty-five additional centers replied they were unwilling (28) or unable (23) to participate. Twelve centers did not provide data after adhering to the survey. As a consequence, 15 Italian centers from 14 Italian towns contributed all of their retrospective data on patients diagnosed with VL between 2004 and 2008, stating that they were consecutively collected. A confirmed diagnosis of VL was reported for 166 patients (145 of European descent). Follow-up data at 1 year after treatment were provided for all but one patient. The final sample included 120 (72.3%) immunocompetent patients, 21 (12.6%) patients with immunodeficiencies other than HIV infection, and 25 (15.1) HIV-coinfected patients (Table 1).

Clinical characteristics and diagnosis. Mean hemoglobin values at diagnosis were 9.2 ± 2.0 g/dl (range, 4.8 to 16 g/dl), the mean WBC counts were 3,385 ± 1,978/mm^3 (range, 400 to 12,700/mm^3), the mean platelet counts were 116,027 ± 58,466/mm^3 (range, 4,500 to 301,000/mm^3), and the mean serum albumin lavel was 3.2 ± 0.8 g/dl, with 13% of the patients presenting values of ≤2.5 g/dl. All 166 patients had a definite diagnosis of VL; males predominated (65.1%), the mean age was 34.9 ± 24.3 years, with 49 (29.5%) patients aged <18 years. The mean age significantly differed in the three groups considered: 29.8 ± 25.3 years in the 120 immunocompetent patients, 40.8 ± 9.0 years in the 25 HIV-infected patients, and 57.4 ± 15.8 years in the 21 patients with immunodeficiencies other than HIV ($P < 0.001$, Table 1). Among the HIV-infected patients included, the mean nadir CD4 T-cell counts were 28.9 ± 18.9 cells/mm^3 (range, 1 to 60/mm^3) and the current CD4 T-cell counts at the time of diagnosis of VL were 125.4 ± 180.7 cells/mm^3 (range, 7 to 855/mm^3). Eight HIV patients (32%) were naive to antiretrovirals at the time of VL diagnosis. Among the patients diagnosed with other immunodeficiencies before VL, nine had hematological malignancies, four

TABLE 2 Tests performed for VL diagnosis, with the relative proportions of positive tests

Diagnostic method	No. (%) of tests	
	Total	Positive
Bone marrow	160 (96.4)	136 (85.0)
Serological tests	153 (92.2)	127 (83.0)
Qualitative PCR	65 (39.1)	58 (89.2)
Quantitative PCR	2 (1.2)	2 (100)

had received bone marrow transplants, one had a renal transplant, one had undergone a splenectomy, six had a diagnosis of rheumatoid arthritis. Among these latter individuals, four had been treated with long-term steroids (>2 months). and two had been treated with methotrexate. Patients without comorbidities were 116 (69.9%, Table 1).

Direct examination of BM smears and serological tests were performed in nearly all of the 166 enrolled patients, whereas qualitative PCR amplifications from BM cells or blood were performed in approximately half of the cases (Table 2).

The distribution of patients as to the Italian region of origin was in good agreement with that observed in other series, in spite of underrepresentation of sites from regions usually reporting cases. Sicily had the highest number of cases throughout the study period. Cases of VL decreased steadily in immunocompetent hosts during the 5 years of observation, from 41 cases in 2004 to 13 in 2008, whereas they remained constant in immunocompromised hosts.

Treatment regimens and outcomes. Twenty-seven (16.3%) patients needed blood transfusions and/or albumin infusions early after diagnosis. L-AmB was the most widely used treatment drug (153 cases, 92.2%), followed by colloidal dispersion of amphotericin B (7 cases, 4.2%) and antimonial derivatives (6 cases, 3.6%). The mean cumulative doses of L-AmB were 29.4 ± 7.9 mg/kg in immunocompetent patients, 32.9 ± 8.6 mg/kg in patients with non-HIV-related immunodeficiencies, and 40.8 ± 6.7 mg/kg in HIV-infected patients ($P < 0.001$). The mean numbers of infusion days were 7.8 ± 3.1 in immunocompetent patients, 9.6 ± 3.9 in non-HIV-immunodeficient patients, and 12.0 ± 3.4 in HIV-infected patients ($P < 0.001$). Thirty-seven different regimens, including L-AmB were used, three of which accounted for 67 (43.8%) of the treatments: (i) 3 mg/kg/day for 15 days, repeated in a single dose after 5 and 30 days (22 patients), (ii) 3 to 4 mg/kg/day for 6 to 7 days (22 patients), and (iii) 3 mg/kg/day for 5 days, repeated in a single dose after 5 days (23 patients). The longest treatment prescribed included 10 consecutive 3-mg/kg daily doses of L-AmB, followed by 10 additional doses, used in a single patient with HIV infection. Patients responsive to treatment were 154 (93.3%) among the 165 evaluable patients, for which a follow-up of at least 1 year after treatment completion was available. Successes included 117 (98.3%) among the 119 immunocompetent patients, 19 (90.5%) among the 21 non-HIV-immunodeficient patients, and 18 (72.0%) among the 25 HIV-infected patients (Table 3). Primary nonresponses were rare overall: two in immunocompetent patients (1.6%), two (9.5%) among the non-HIV-immunodeficient patients, and three (12%) among the HIV patients (Table 3). Among the 11 relapsers, all five immunocompetent patients were rescued after retreatment, versus two (33.3%) of the six retreated HIV patients (Table 3). Nonresponders and relapsers were exposed to higher cumulative doses than patients cured (37.4 ± 9.4 mg versus 30.9 ± 8.5 mg, $P = 0.004$). No severe adverse events were reported. Side effects were recorded in 12.2% of patients, without significant differences among the three groups of patients. None of the side effects affected the duration of treatment. Most were mild, including nausea (2.4%), rash (1.8%), diarrhea (1.8%), headache (1.8%), mild hypokaliemia (0.6%), and mild hypertransaminasemia (0.6%). Mild and reversible increases in serum creatinine were reported in four patients (2.4%), three among immunocompetent patients, and one in a non-HIV-immunodeficient patient. Side effects were more frequent in HIV-negative patients receiving higher doses of L-AmB (21.6% versus 8.8%, $P = 0.04$); the frequency of side effects did not differ, however, among patients undergoing retreatment of relapses (8.3% versus 12.5%, $P = 0.6$).

TABLE 3 Proportions of responders to first-line treatment and retreatment, as stratified by immune status

Response type[a]	No. (%) of patients		
	Immunocompetent ($n = 119$)	Non-HIV immunodeficient ($n = 21$)	HIV coinfected ($n = 25$)
Primary response	117 (98.3)	19 (90.5)	22 (88)
Nonresponders	2 (1.6)	2 (9.5)	3 (12)
Relapsers	5/117 (4.3)		6/22 (27.2)
Responders to retreatment	5/5 (100)		2/6 (33.3)
Overall responders	117 (98.3)	19 (90.5)	18 (72)

[a] Primary response refers to patients who responded to their first treatment cycle. Nonresponder patients did not respond to their first treatment cycle. Relapsers refers to patients who had a recurrence of VL after a favorable response to their first-line treatment. Responders to retreatment refers to patients who responded to the second line of therapy after relapsing. Overall responders represents the total number of patients who responded to either first- or second-line therapy.

We searched for factors associated with failure to respond to first-line treatment. In univariate analyses, HIV infection, types of other immune deficiency, age, gender, hemoglobin, platelet counts, serum albumin, transaminases, the presence of comorbidities, the length of treatment, the total L-AmB dose, and adverse events were investigated. The final logistic regression model indicated that the only independent predictors of treatment failure were HIV infection (OR = 12.14, 95% CI = 2.75 to 53.65, $P = 0.001$) and age (OR = 1.06 for each 1-year increase, 95% CI = 1.01 to 1.10, $P = 0.008$) (Table 4).

DISCUSSION

We set up the present investigation to shed light on the critical issue of treatment of VL in Italy. Our dataset was therefore mainly

TABLE 4 Final logistic regression model for predictors of primary treatment failure

Variable	Failure		
	OR	95% CI	P[a]
HIV status	12.14	2.7–53.6	0.001
Sex	1.77	0.4–7.2	0.4
Length of treatment	1.11	0.28–4.3	0.9
Age	1.06	1.014–1.1	0.008
PLT	0.99	0.98–1.0	0.5
Side effects	0.32	0.3–3.0	0.3

[a] Logistic model with 164 observations; P value for the goodness of fit = 0.17; area under the ROC curve = 0.8523.

oriented to monitor how patients were treated around Italy. In the final sample collected, males predominated, adults were more numerous than children (who nevertheless represented approximately one-third of the study population), and only a small proportion of the examined patients were coinfected with HIV. These findinsg are is in line with previous data showing that the incidence of VL decreased in HIV-infected patients after the introduction of combination antiretroviral therapy (HAART) in southern Europe (6, 29–31). Nearly one-third of the 25 HIV-infected patients were naive to HAART when VL was diagnosed, VL being the index opportunistic infection in late AIDS presentation (data not shown). Failures were more frequent in HIV patients with lower nadir CD4 counts (55.5 ± 40.3 versus 172 ± 221, $P = 0.1$), whereas HIV suppression by HAART was not protective, a finding in line with similar data in the literature (2, 7).

In the search for factors associated with the failure of first-line treatment, the final logistic-regression model indicated that the only independent predictors were HIV infection and older age, the first reflecting, as discussed above, the profound immune imbalance determined by $CD4^+$ T-cell depletion (32). The pinpointed significance of age was in agreement with another report showing a 5-fold-greater risk of relapse in African patients older than 45 years and treated with antimonial derivatives (28). Our study documented that L-AmB, the first choice for the treatment of VL in high-income countries, was by far the most frequently drug used at all sites participating in the present retrospection, in spite of the need of "off-label" prescription. In the absence of specific local regulatory indications, a variety of L-AmB-based regimens were used. The data from the present study show a 98.4% efficacy of L-Amb in immunocompetent patients, in good agreement with most trials reporting 90 to 98% efficacy, with a total dose of 18 to 21 mg/kg (33, 34). In our sample, the mean cumulative dose of L-AmB used in immunocompetent patients was significantly lower than in HIV-infected patients, which is in line with other studies and most international guidelines, recommending an extended course of L-AmB and a total dose of up to 40 mg/kg in HIV/VL coinfection (14, 33, 34). Indeed, although no severe adverse events were reported, higher doses and longer treatments of L-AmB were associated with a modest increase in low-grade toxicity in HIV-negative patients. We therefore suggest that, in view of the high success rates and limited adverse reactions observed both in the present and in other published investigations, regulatory Italian authorities should register the best schedules of L-AmB for treatment of VL, even in the absence of further studies, to avoid unnecessary over dosage of L-Amb-B in this setting (16, 33–35). In conclusion, our study adds evidence to the efficacy and appropriateness of current treatment schedules of L-AmB for VL in both immunocompetent and immunocompromised patients.

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REFERENCES

1. **Dujardin JC, Campino L, Cañavate C, Dedet JP, Gradoni L, Soteriadou K, Mazeris A, Ozbel Y, Boelaert M.** 2008. Spread of vector-borne diseases and neglect of leishmaniasis in Europe. Emerg. Infect. Dis. **14:**1013–1018. http://dx.doi.org/10.3201/eid1407.071589.
2. **Antinori S, Schifanella L, Corbellino M.** 2012. Leishmaniasis: new insights from an old and neglected disease. Eur. J. Clin. Microbiol. Infect. Dis. **31:**109–118. http://dx.doi.org/10.1007/s10096-011-1276-0.
3. **Cascio A, Gradoni L, Scarlata F, Gramiccia M, Giordano S, Russo R, Scalone A, Camma C, Titone L.** 1997. Epidemiologic surveillance of visceral leishmaniasis in Sicily, Italy. Am. J. Trop. Med. Hyg. **57:**75–78.
4. **Gaeta GB.** 2004. Clinical signs of visceral leishmaniasis in adults: is the manner of presentation changing? Parasitologia **46:**225–226.
5. **Sagnelli C, Di Martino F, Coppola N, Crisci A, Sagnelli E.** 2012. Acute liver failure: a rare clinical presentation of visceral leishmaniasis. New Microbiol. **35:**93–95.
6. **Russo R, Nigro L, Panarello G, Montineri A.** 2003. Clinical survey of leishmania/HIV coinfection in Catania, Italy: the impact of highly active antiretroviral therapy (HAART). Ann. Trop. Med. Parasitol. **97:**149–155. http://dx.doi.org/10.1179/000349803225002624.
7. **Russo R, Laguna F, López-Vélez R, Medrano FJ, Rosenthal E, Cacopardo B, Nigro L.** 2003. Visceral leishmaniasis in those infected with HIV: clinical aspects and other opportunistic infections. Ann. Trop. Med. Parasitol. **97:**99–105. http://dx.doi.org/10.1179/000349803225002570.
8. **Bassetti M, Pizzorni C, Gradoni L, Del Bono V, Cutolo M, Viscoli C.** 2006. Visceral leishmaniasis infection in a rheumatoid arthritis patient treated with adalimumab. Rheumatology **45:**1446–1448. http://dx.doi.org/10.1093/rheumatology/kel235.
9. **Prigione I, Castagnola E, Imberti L, Gambini C, Gradoni L, Dianzani U, Ramenghi U, Giacopelli F, Moretta A, Moretta L, Plebani A, Fischer A, Pistoia V.** 2009. Multiple relapses of visceral leishmaniasis in an adolescent with idiopathic CD4+ lymphocytopenia associated with novel immunophenotypic and molecular features. Pediatr. Infect. Dis. J. **28:**161–163. http://dx.doi.org/10.1097/IFN.0b013e318185513d.
10. **Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BP, Nakamura CV.** 2011. Recent advances in leishmaniasis treatment. Int. J. Infect. Dis. **15:**e525–32. http://dx.doi.org/10.1016/j.ijid.2011.03.021.
11. **Omollo R, Alexander N, Edwards T, Khalil EA, Younis BM, Abuzaid AA, Wasunna M, Njoroge N, Kinoti D, Kirigi G, Dorlo TP, Ellis S, Balasegaram M, Musa AM.** 2011. Safety and efficacy of miltefosine alone and in combination with sodium stibogluconate and liposomal amphotericin B for the treatment of primary visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. Trials **12:**166. http://dx.doi.org/10.1186/1745-6215-12-166.
12. **Cota GF, de Sousa MR, Rabello A.** 2011. Predictors of visceral leishmaniasis relapse in HIV-infected patients: a systematic review. PLoS Negl. Trop. Dis. **5:**e1153. http://dx.doi.org/10.1371/journal.pntd.0001153.
13. **Gradoni L, Soteriadou K, Louzir H, Dakkak A, Toz SO, Jaffe C, Dedet JP, Campino L, Cañavate C, Dujardin JC.** 2008. Drug regimens for visceral leishmaniasis in Mediterranean countries. Trop. Med. Int. Health **13:**1272–1276. http://dx.doi.org/10.1111/j.1365-3156.2008.02144.x.
14. **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 of visceral leishmaniasis. Clin. Infect. Dis. **43:**917–924. http://dx.doi.org/10.1086/507530.
15. **Laguna F.** 2003. Treatment of leishmaniasis in HIV-positive patients. Ann. Trop. Med. Parasitol. **97:**135–142. http://dx.doi.org/10.1179/000349803225002606.
16. **Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson AD.** 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment and a proposed research and development agenda. Lancet Infect. Dis. **2:**494–501. http://dx.doi.org/10.1016/S1473-3099(02)00347-X.
17. **Davidson RN, di Martino L, Gradoni L, Giacchino R, Gaeta GB, Pempinello R, Scotti S, Cascio A, Castagnola E, Maisto A, Gramiccia M, di Caprio D, Wilkinson RJ, Bryceson AD.** 1996. Short-course treatment of visceral leishmaniasis with liposomal amphotericin B (AmBisome). Clin. Infect. Dis. **22:**938–943. http://dx.doi.org/10.1093/clinids/22.6.938.
18. **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.

19. **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. http://dx.doi.org/10.1086/377542.
20. **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. http://dx.doi.org/10.1086/380971.
21. **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. http://dx.doi.org/10.1056/NEJMoa0903627.
22. **Sinha PK, Roddy P, Palma PP, Kociejowski A, Lima MA, Rabi Das VN, Gupta J, Kumar N, Mitra G, Saint-Sauveur JF, Seena S, Balasegaram M, Parreño F, Pandey K.** 2010. Effectiveness and safety of liposomal amphotericin B for visceral leishmaniasis under routine program conditions in Bihar, India. Am. J. Trop. Med. Hyg. **83:**357–364. http://dx.doi.org/10.4269/ajtmh.2010.10-0156.
23. **Sinha PK, van Griensven J, Pandey K, Kumar N, Verma N, Mahajan R, Kumar P, Kumar R, Das P, Mitra G, Flevaud L, Ferreyra C, Remartinez D, Pece M, Palma PP.** 2011. Liposomal amphotericin B for visceral leishmaniasis in human immunodeficiency virus-coinfected patients: 2-year treatment outcomes in Bihar, India. Clin. Infect. Dis. **53:**e91–e98. http://dx.doi.org/10.1093/cid/cir521.
24. **WHO.** 2010. WHO health topics: leishmaniases. World Health Organization, Geneva, Switzerland. http://www.who.int/topics/leishmaniasis/en/.
25. **Davidson RN, Di Martino L, Gradoni L, Giacchino R, Russo R, Gaeta GB, Pempinello R, Scott S, Raimondi F, Cascio A, Prestileo T, Caldeira L, Wilkinson RJ, Bryceson AD.** 1994. Liposomal amphotericin B (AmBisome) in Mediterranean visceral leishmaniasis: a multi-centre trial. Q. J. Med. **87:**75–81.
26. **Meyerhoff, A.** 1999. U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. Clin. Infect. Dis. **28:**42–51.
27. **Syriopoulou V, Daikos GL, Theodoridou M, Pavlopoulou I, Manolaki AG, Sereti E, Karamboula A, Papathanasiou D, Krikos X, Saroglou G.** 2003. Two doses of a lipid formulation of amphotericin B for the treatment of Mediterranean visceral leishmaniasis. Clin. Infect. Dis. **36:**560–566. http://dx.doi.org/10.1086/367843.
28. **Herrero M, Orfanos G, Argaw D, Mulugeta A, Aparicio P, Parreño F, Bernal O, Rubens D, Pedraza J, Lima MA, Flevaud L, Palma PP, Bashaye S, Alvar J, Bern C.** 2009. Natural history of a visceral leishmaniasis outbreak in highland Ethiopia. Am. J. Trop. Med. Hyg. **81:**373–377.
29. **López-Vélez R.** 2003. The impact of highly active antiretroviral therapy (HAART) on visceral leishmaniasis in Spanish patients who are coinfected with HIV. Ann. Trop. Med. Parasitol. **97:**143–147. http://dx.doi.org/10.1179/000349803225002615.
30. **De La Rosa R, Pineda JA, Delgado J, Macías J, Morillas F, Mira JA, Sánchez-Quijano A, Leal M, Lissen E.** 2002. Incidence of and risk factors for symptomatic visceral leishmaniasis among human immunodeficiency virus type 1-infected patients from Spain in the era of highly active antiretroviral therapy. J. Clin. Microbiol. **40:**762–767. http://dx.doi.org/10.1128/JCM.40.3.762-767.2002.
31. **López-Vélez R, Casado JL, Pintado V.** 2001. Decline of a visceral leishmaniasis epidemic in HIV-infected patients after the introduction of highly active antiretroviral therapy (HAART). Clin. Microbiol. Infect. **7:**394–395. http://dx.doi.org/10.1046/j.1198-743x.2001.00270.x.
32. **De Souza GF, Biscione F, Greco DB, Rabello A.** 2012. Slow clinical improvement after treatment initiation in Leishmania/HIV coinfected patients. Rev. Soc. Bras Med. Trop. **45:**147–150. http://dx.doi.org/10.1590/S0037-86822012000200001.
33. **Sundar S, Chakravarty J.** 2010. Liposomal amphotericin B and leishmaniasis: dose and response. J. Glob Infect. Dis. **2:**159–166. http://dx.doi.org/10.4103/0974-777X.62886.
34. **Gradoni L, Gramiccia M, Scalone A.** 2003. Visceral leishmaniasis treatment, Italy. Emerg. Infect. Dis. **9:**1617–1620. http://dx.doi.org/10.3201/eid0912.030178.
35. **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.