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Treatment Options for Visceral Leishmaniasis and HIV Coinfection

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Abstract

Leishmania and HIV coinfection is a major health problem in more than 35 countries worldwide. The impaired immune function of visceral leishmaniasis/HIV-coinfected patients may: (i) favor the reactivation of latent* Leishmania *infection; (ii) induce a more severe presentation of visceral leishmaniasis; (iii) cause a poorer therapeutic response; and (iv) increase the risk of relapse after treatment. One of the major challenges in the management of visceral leishmaniasis/HIV coinfection is developing an effective drug therapy that not only resolves the first episode of visceral leishmaniasis but also prevents relapse. However, scarce evidence and data are available on the optimal therapy for visceral leishmaniasis/HIV coinfection. In our study we reviewed the efficacy of several drugs currently employed for visceral leishmaniasis in HIV patients and current knowledge of secondary prophylaxis. Additionally, we reviewed a set of ongoing clinical trials that are being performed to evaluate the efficacy of new therapeutic regimens for visceral leishmaniasis in patients with and without HIV. Finally, other therapeutic strategies based on immunotherapy, vaccination, or screening for latent leishmaniasis infection in HIV patients are reviewed. Apart from being potentially useful in clinical practice, the results obtained in our study highlight the need for further research on the management of visceral leishmaniasis/HIV coinfection. **(AIDS Rev. 2016;18:32-43)**

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Key words

Leishmania. Visceral leishmaniasis. Therapeutics. HIV. Liposomal amphotericin B. Miltefosine. Sodium stibogluconate. Meglumine antimoniate.

Introduction

Visceral leishmaniasis (VL), also called Kala-Azar, is a widespread protozoal infection prevailingly caused by *Leishmania donovani* and *L. infantum* (also known as *L. chagasi* in Latin America). Visceral leishmaniasis can also be caused by *L. tropica* in the Middle East and by *L. amazonensis* in South America. Leishmaniasis is transmitted by the bite of a female hematophagous sand fly of the genus *Phlebotomus* in the Old World and by *Lutzomyia* in the New World[1].

Two types of VL have been described according to their transmission characteristics: (i) the zoonotic form, mainly caused by *L. infantum,* with dogs being the main reservoir and which occurs in the Mediterranean basin, China, the Middle East, and South America; and (ii) the anthroponotic form, which is not transmitted from an animal reservoir but from human to human and the infection is mainly transmitted by *L. donovani* in East Africa, Bangladesh, India, and Nepal.

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It is estimated that there are 12 million people with VL in the world, with approximately 0.2-0.4 million new cases per year. Six countries account for over 90% of VL cases in the world: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia[2].

Globally, 35 million (33.2-37.2 million) people are currently living with HIV. Sub-Saharan Africa is the most severely affected area as it accounts for nearly 71% of the people living with HIV worldwide[3].

To date, *Leishmania* and HIV coinfection has been reported in more than 35 countries. In the early 1990s, a rapid increase in the incidence of VL/HIV coinfection was noticed in the Mediterranean basin, coinciding with the peak of the HIV epidemic. Thus, 28 (85%) of the 33 countries where the World Health Organization (WHO) detected the first cases of VL/HIV coinfection were in the Mediterranean basin, with Spain in the lead[4]. The number of cases of coinfection reached its peak in 1997 and its incidence plateaued between 1998 and 2001. Since 2001 the incidence of VL/HIV coinfection has decreased significantly mainly due to the administration of antiretroviral treatments (ART) for HIV in the Mediterranean region[5]. Currently, there are other geographical areas (specifically Ethiopia and Sudan) where the rate of VL/HIV coinfection is very high, probably due to the fact that the use of ART is not so widespread. Interestingly, VL/HIV coinfection is increasing in other regions, such as in certain areas of India, where the incidence of HIV is low (< 1%). The likely cause is population movements, and VL/HIV coinfection should be considered an emerging problem in these regions[6,7].

One of the major challenges of VL/HIV coinfection is developing an effective drug therapy that not only resolves the first episode of VL, but also prevents relapse. To date, amphotericin B and its lipid formulations, pentavalent antimonials, paromomycin, and miltefosine, have demonstrated efficacy against VL in immunocompetent patients. However, there is scarce evidence of its efficacy in VL/HIV coinfected patients. Moreover, the efficacy of the different treatments varies depending on the *Leishmania* species and the geographical area where it is acquired.

This study reviews the state of the art of therapies for VL/HIV coinfection. For this purpose, we evaluated the evidence published as well as the data being gathered in ongoing clinical trials with VL/HIV-coinfection therapies and secondary prophylaxis. We mainly focused on drugs on the market and drugs being tested in humans. Apart from being potentially useful in clinical practice, the results obtained highlight the need for further research to better understand the mechanisms of VL/HIV coinfection.

Current antiparasitic options for visceral leishmaniasis in HIV-coinfected patients

Coinfection of VL and HIV hinders therapeutic response and is the cause of frequent relapse, especially in patients with CD4 < 200 cells/µl. Only a few clinical trials have been conducted on the efficacy of some therapies for VL/HIV coinfection, and the majority has been carried out in Europe (infections caused by *L. infantum)* and East Africa. Many questions still remain unanswered such as the optimal drug, dosage, duration of treatment and prophylaxis, and the efficacy of combined therapies for VL/HIV coinfection[8].

Pentavalent antimonials

The evidence currently available on the efficacy of pentavalent antimonials in HIV patients has been gathered mainly in European studies. Published series of VL/HIV-coinfected patients treated with 20 mg Sb^{v+}/kg/day for 28-30 days report varying cure rates ranging from 33 to 82%, with high relapse rates[9]. Specifically, two clinical trials have been performed comparing meglumine antimoniate (MA) with amphotericin B deoxycholate (AB) and amphotericin B lipid complex (ABLC). In the first study, cure rates were 65.9% for AB and 62.2% for MA[10]. In the second study, where two different doses of ABLC were compared (15 and 30 mg/kg total dose), with MA reporting cure rates of 33, 42, and 37% respectively. Although the efficacy of ABLC was similar to that of MA, the toxicity of MA was substantially higher[11]. In Ethiopia the cure rate reported for sodium stibogluconate (SSG) was not much better and only 43.5% of HIV-positive patients were cured at six months follow-up[12]. However, better outcomes were observed in two other Ethiopian studies, which reported cure rates of 65.2-78.6%, although the analysis included a high number of non-HIV patients[13,14]. Recently, a retrospective study with SSG for VL/HIV coinfection performed in Ethiopia reported a 43.9% cure rate at the end of treatment, although 21.1% of patients had to discontinue the treatment temporarily or permanently due to toxicity reactions[15]. In India the use of pentavalent antimonials is limited due to the high resistance rates reported, especially in the state of Biha. The HIV infection in VL patients, with lower cure rates and higher relapse rates as compared to immunocompetent patients, could be associated with higher resistance to

antimonials[16]. According to the literature, antimonials should not be used in VL/HIV patients since higher toxicity and mortality rates have been reported for this patient population as compared to non-HIV VL patients[15,17].

Amphotericin B deoxycholate, amphotericin B lipid complex and liposomal amphotericin B regimens

Due to its safety profile, liposomal amphotericin B (LAB) is recommended by the WHO and other international organizations as the preferred treatment for VL/HIV coinfection, although published studies with LAB are scarce.

In Spain several studies have been performed on *L. infantum.* In the first study with AB vs. MA, where AB was administered at a dose of 0.7 mg/kg/day for 28 days (20 mg/kg total dose), similar initial cure rates (62.6% for AB) and relapse rates were reported for AB and antimonials[10]. In another study, a total dose of 30 mg/kg of ABLC proved to be slightly superior to a total dose of 15 mg/kg of ABLC and 20 mg Sb^{v+}/kg/day of MA for 28 days, although the cure rate was only 42%[11].

Several studies have specifically focused on LAB. In a study performed in four European health centers, VL was treated with LAB in HIV patients (40 mg/kg total dose) with a good initial clinical and parasitological response, although all patients who completed follow-up eventually relapsed[18]. In another study performed in Ethiopia, LAB was administered to a cohort of HIV-positive and HIV-negative patients (total dose: 30 mg/kg), reaching a 60% cure rate[17]. Also, in a retrospective study performed in India, LAB was given to recently diagnosed VL/HIV-coinfected patients (20-25 mg/kg total doses); the final cure rate obtained at 1-2 years of follow-up was 85%, and tolerance to the drug was excellent[19]. Finally, in a recent retrospective study carried out in eastern Sudan, a total dose of 30 mg/kg of LAB was administered to a cohort of VL patients. Although the cure rate for non-HIV patients was high, mortality in VL/HIV-coinfected patients was substantial. The specific cure rate for HIV patients is not reported in the study[20].

Miltefosine

Miltefosine has been used for treating patients with VL, but scarce data are available on its efficacy, tolerance, and safety in HIV patients. In Germany a study was performed with miltefosine in HIV patients in whom previous treatment for VL had failed. Initially, the cure rate was 64%, but almost all patients finally relapsed when the miltefosine treatment was discontinued. Also, this study revealed that miltefosine was well tolerated even in long-term treatment periods, although interactions with ART were not reported. The study concluded that clinical relapse could be either treated by administering repeated courses of miltefosine or prevented with miltefosine in combination with other anti-leishmania drugs[21]. Another published study performed in Spain reported the administration of miltefosine to four coinfected patients who were severely immunosuppressed and who had not responded to a previous treatment with AB or MA for VL. Initially, all patients responded clinically but, when treatment was discontinued, all patients relapsed[22]. In Ethiopia, a randomized, open-label clinical trial was performed with oral miltefosine 100 mg/day for 28 days versus SSG 20 mg Sb^{v+}/kg/day for 30 days in a population where HIV is highly prevalent. In this case, miltefosine was observed to be safer for HIV-infected patients, but less effective than SSG[13].

Recent studies performed with immunocompetent patients have revealed that after a decade of use of miltefosine in the Indian subcontinent, the relapse rate has increased significantly in India, Bangladesh, and Nepal. Thus, around 7-10% of VL patients treated with miltefosine relapsed within six months after treatment discontinuation, and 13-20% relapsed after a 12-month follow-up[23-26]. A study revealed that relapse was more frequent in patients < 12-15 years old[26]. This could be due to the fact that children have a different immune response or distinct pharmacokinetic characteristics, which suggests that the miltefosine dosing regimen proposed for children may need to be increased[25]. Moreover, a series of *in vitro* studies have found a correlation between the accumulation of miltefosine within the parasite and its efficacy. Also, there is evidence that susceptibility to *Leishmania in vitro* is significantly higher pre-treatment than post-treatment. However, such differences were not associated with clinical outcomes[23-27]. Therefore, the rapid response and common gastrointestinal adverse events associated with miltefosine generally result in premature treatment discontinuation; these, added to the long elimination half-life of miltefosine, have been identified as risk factors for the development of tolerance and resistance to this drug. In anthroponotic foci like the Indian subcontinent, the causes described above could explain the increase observed in refractory parasites. If we consider these factors concerning HIV patients, who show higher relapse rates and more persistent asymptomatic parasitemia than non-HIV patients[28], then VL/HIV-coinfected

patients could be a group at higher risk of developing resistance or tolerance to miltefosine.

Other drugs

There is scarce data available about the efficacy of second-line drugs such as pentamidine, paromomycin, or fluconazole for VL/HIV coinfection. Most of the published studies were clinical cases where these drugs were mostly administered in combination with other drugs to patients resistant to first-line treatment[29,30]. Finally, paromomycin should be used with caution because patients rapidly develop resistance, which risk can increase due to VL/HIV coinfection[29].

Combination therapy

Many experts recommend the administration of combination therapies to coinfected patients with multiple relapses[5]. Combination therapies can increase the efficacy of a treatment and may also reduce the reservoirs, transmission, and emergence of resistant parasites. Moreover, combination therapies have been associated with lower treatment duration, dosage, toxicity, and costs and better compliance[31].

In vitro studies have shown that some synergism exists between LAB and miltefosine and between LAB and paromomycin[32]. However, no clinical trials have been published that assess the effectiveness of these combinations in VL/HIV-coinfected patients, and the data available is based on case series or case reports.

In Spain a study was performed in 11 VL/HIV-coinfected patients due to *L. infantum*. Meglumine antimoniate was combined with allopurinol and good results were obtained in the patients who received the combined therapy for at least four weeks[33]. Also in Spain, another case was reported of a coinfected patient who did not respond to LAB, MA, miltefosine, or even to a combined therapy of miltefosine plus LAB. Finally, clinical and parasitological response was achieved after the administration of a combined therapy of MA 20 mg Sb^{v+}/kg/day plus parenteral paromomycin 16 mg/kg/day for 30 days; then, maintenance therapy with itraconazole 400 mg/day plus miltefosine 150 mg/day was given with a schedule of one month on, two months off until CD4 cell count was 350 cells/mm^3 for 3-6 months[34]. A case reported in Italy described a coinfected patient who received treatment with LAB (40 mg/kg/day total dose) and the growth factor of rHuGM-CSF colonies (150 mcg subcutaneously twice weekly for 12 consecutive weeks). After a two-year follow-up, the patient was free from relapse and no side effects were reported[35]. A German HIV-positive patient acquired VL after visiting several southern European countries. He had previously relapsed after receiving LAB and miltefosine, and he finally developed end-stage renal failure. A novel combination therapy with intravenous pentamidine (300 mg/day) and oral fluconazole (200 mg/day) was administered for three weeks, with no clinical signs of relapse after five months of treatment[30]. Another case has been reported of a VL/HIV-coinfected patient from Eritrea who did not respond to LAB and was re-treated with SSG 20 mg/kg/day plus miltefosine 100 mg/day for 30 days, with good tolerance and response[36].

A retrospective study was recently carried out in India in a clinical cohort of 102 VL/HIV-coinfected patients. The treatment administered was LAB (30 mg/kg total dose) in six equal dose infusions administered in combination with miltefosine 100 mg/day (dose for 12-25 kg 50 mg/day) on alternate days for 14 days. All patients were encouraged to start or continue on ART: the overestimated cumulative incidence rates of poor outcome for VL treatment at 6, 12, and 18 months follow-up were 13.9, 18.4, and 27.2%, respectively. Of the 100 patients discharged after initial cure, eight relapsed during follow-up, with a median time to relapse of 11 months (IQR 4-15)[37].

Secondary prophylaxis

Secondary prophylaxis is needed after the patient has completed and responded to initial treatment. Thus, a meta-analysis that included 1,017 coinfected patients reported that secondary prophylaxis reduces the rate of relapse of VL significantly (OR 0.228). However, scarce data is available that determines the most effective drug, dose, and regimen to be administered[38].

The only randomized clinical trial with a maintenance therapy was performed in Spain. Patients were allocated to receive maintenance therapy with ABLC at a dose of 3-5 mg/kg/day intravenously every three weeks for 12 months, while controls did not receive any maintenance therapy. The results demonstrated that maintenance therapy reduced the relapse rate from 22 to 50%[39]. Another prospective study evaluated the effectiveness of maintenance therapy with LAB at 4 mg/kg/day for five days and then once a week for five more weeks (total 10 doses); up to 80% of patients were reported to be free of disease after a 12-month follow-up[40].

Pentavalent antimonials were also evaluated as maintenance therapy administered every 3-4 weeks.

The relapse rate decreased more significantly in the study group than in the patients who either did not receive any treatment or received allopurinol as secondary prophylaxis after a 23-month follow-up[41].

A study has also been performed with_pentamidine administered at a dose of 4 mg/kg/day every 2-4 weeks. No relapses were reported during the follow-up period[42].

In a study performed in Portugal, miltefosine was administered as maintenance therapy in three patients for 21, 14, and 12 months, respectively. The patients remained free of relapse for a median period of 20 months. The authors concluded that miltefosine could be an effective drug for prophylaxis due to its long half-life and oral administration[43].

Azole drugs could be effective as maintenance therapy, but there are no clinical trials to support this theory. Data is based on case series where itraconazole was given at a dose of 600 mg/day for up to 24 months of treatment, without any relapses. The advantage of these drugs is their good tolerance and low toxicity, although there is a risk of developing resistant fungal infection[44]. Itraconazole or fluconazole combined with allopurinol could be a therapeutic option[45,46].

Another relevant aspect to be considered is the duration of maintenance therapy. According to different authors, prophylaxis should be suspended when: (i) patients recover their immune function after administration of ART; (ii) VL is quiescent; and (iii) the $CD4^+$ count is maintained > 200 cells/µl for more than six months[47,48].

Ongoing clinical trials with new therapeutic options for visceral leishmaniasis in HIV-coinfected patients

More effective therapies should be developed for VL/HIV coinfection that also reduce relapse rates without increasing drug toxicity; in addition, such therapies should prevent the development of drug resistance. Currently, combination therapies are gaining popularity as the best strategy to meet these objectives, not only in VL/HIV patients but also in VL patients without HIV infection[31].

An ongoing clinical trial sponsored by the Drugs for Neglected Diseases initiative (DNDi) is currently recruiting patients to compare the efficacy of LAB alone versus LAB in combination with miltefosine in VL/HIV-coinfected patients in Ethiopia (Table 1). It includes adults suffering a first episode or relapse of VL[49].

The DNDi also sponsors another ongoing clinical trial that is being conducted in Ethiopia (Table 1) to evaluate secondary prophylaxis with pentamidine in VL/HIV-coinfected patients. The study includes adults who are being treated for VL relapse or primary VL and who are receiving or will receive ART[50].

Other clinical trials are being performed with different therapies in VL patients without HIV. Cure and response rates seem to differ significantly between VL patients and VL/HIV-coinfected patients. The results of these clinical trials, however, could lead to the development of new therapies that can later be tested in coinfected patients. The main therapeutic options evaluated in ongoing clinical trials are combination regimens: LAB alone vs. LAB with miltefosine or paromomycin or miltefosine and paromomycin[51]; LAB and SSG vs. LAB and miltefosine vs. miltefosine alone[52]; MA alone vs. LAB alone vs. AB alone vs. LAB and MA[53]. There is a study where LAB alone is being evaluated. All studies include pediatric and adult patients[51-54]. Combination therapies are being evaluated in East Africa (Sudan and Kenya)[52], Bangladesh[51], and Brazil[53] and LAB is being tested in Ethiopia[54].

Other ongoing studies are focused on the development of novel drugs. A single-arm trial is being performed in Sudan to evaluate the efficacy of fexinidazole[55]. Fexinidazole is a 2 substituted 5-nitromidazole formulated for oral administration that has demonstrated *in vitro* and *in vivo* efficacy against *L. donovani* in a mouse model. The dose given in this clinical trial was determined by the dose administered in a phase II clinical trial performed for the treatment of African trypanosomiasis in humans[56]. Sitamaquine, an 8-aminoquinoline analog developed within the framework of an antimalarial program, has been reported to be a promising oral drug against VL in India and Africa. The first phase II trial was performed in Kenya with good results, which led to further trials in India and Kenya. These studies showed that sitamaquine was a well-tolerated drug with good cure rates for VL. However, further studies led to the rejection of sitamaquine as a therapeutic option due to its latterly observed nephrotoxicity[57] (Table 2).

In vitro and animal models have also demonstrated the potential effectiveness of another drug called imipramine. Imipramine is a tricyclic antidepressant that has been shown to be highly active against both antimony sensitive and resistant *L. donovani* infection in an infected model of hamster[58]. Animal experimentation showed additional interactions between nitazoxanide, AB, MA, and miltefosine when combined. The authors concluded that further research should be performed to evaluate these therapeutic combinations[59].

Other marketed drugs, mainly antibiotics such as azithromycin or co-trimoxazole, have been observed to

Table 1. Ongoing clinical trials for visceral leishmaniasis/HIV-coinfected patients

Clinical trial Name/Number Date started/Date estimated primary completion	Design of study	Outcomes	Drugs and regimens
Efficacy trial of Ambisome given alone and Ambisome given in combination with miltefosine for the treatment of VL/HIV-positive Ethiopian patients. NTC02011958/Ethiopia July 2014/January 2016	Phase III randomized, open-label, parallel assignment. Treatment as the primary end point.	Primary outcome: Initial parasitological cure at day 29. Secondary outcomes: Relapse-free survival at day 390. Other outcomes: – Safety endpoint: adverse events and serious adverse events. – Response to ART: measure of CD4. – Pharmacokinetics drug interaction between VL treatment and antiretroviral drugs.	Group 1: Liposomal amphotericin B 40 mg/kg total dose iv infusion 5 mg/kg/day on days 1-5, 10, 17, 24. Group 2: Liposomal amphotericin B 30 mg/kg total dose iv infusion 5 mg/kg per day on days 1, 3, 5, 7, 9, 11 and miltefosine orally every day for 28 days (50 mg/day if < 25 kg weight; 100/day if > 25 kg weight)
Prophylaxis of visceral leishmaniasis relapses in HIV-coinfected patients with pentamidine: a cohort study. NTC01360762/Ethiopia November 2011/August 2015	Phase III, cohort, open-label study with single group assignment. Prevention as the primary purpose.	Primary outcome: Time to relapse or death during 12 months follow-up. Secondary outcomes: – Adverse events during the first year of pentamidine administration. – Number of treatment discontinuations and interruptions. – Number of required additional interventions/therapeutic procedures.	Pentamidine isethionate 300 mg one vial intramuscular or iv during 12 months plus an extended treatment period from 0 to 6 months depending on immunological status. Patients are receiving ART.

Ambisome: liposomal Amphotericin B.
ART: antiretroviral therapy; VL: visceral leishmaniasis; iv: intravenous.

have activity against *Leishmania* infection. However, further research is needed to determine their clinical utility.

Antiretroviral therapy

Visceral leishmaniasis worsens with the immunosuppression caused by HIV infection. Consequently, for VL to be controlled, HIV has to be controlled first. The recovery of immunity by the administration of ART can prevent progression from asymptomatic leishmaniasis to an active disease and can reduce the risk of relapse after treatment, as has been observed in southern Europe[4,38].

Although ART favors VL progression in HIV-coinfected patients, anti-*Leishmania* treatments are not still as effective in coinfected patients as in non-HIV patients. In fact, between 28 and 70% of coinfected patients who receive ART relapse during the 24-month follow-up period. Relapse occurs regardless of whether the CD4 count increases or not and even when the viral load is undetectable. However, ART seems to improve the evolution of VL/HIV-coinfected patients as it reduces the average time to relapse, which is seven months longer than in patients who receive ART[60]. It has also been observed that the response of HIV to ART is also negatively affected by *Leishmania* infection, and although a good viral suppression is achieved, CD4 recovery is usually poor.

In order to improve the effectiveness of these therapies in coinfected patients, different lines of treatment have been explored. One of the approaches investigated is the optimization of HIV ART based on the evidence that HIV protease inhibitors (PI) seem to have a direct antiparasitic effect against *Leishmania*[61]. The first *in vitro* study that demonstrated the activity of PIs against Leishmania species was performed in 2005 with indinavir and saquinavir against *L. infantum* and *L. major*. Subsequently, the activity of PIs against *L. donovani* was also investigated, showing that lopinavir and ritonavir seem to have limited antiparasitic activity[62].

The mechanism of activity of PIs against *Leishmania* has been described from a different perspective. It has

Table 2. Ongoing clinical trials for visceral leishmaniasis treatment

Clinical trial Name/Number/Country Date started/Date estimated primary completion	Design of study	Outcomes	Drugs and regimens
A phase III open label, randomized, study of three short-course combination regimens (Ambisome, miltefosine, paromomycin) compared with Ambisome alone for the treatment of visceral leishmaniasis in Bangladesh. NTC01122771/Bangladesh[51] May 2010/December 2011	Phase III randomized, open-label, parallel assignment with treatment as the primary purpose.	Primary outcome: Definitive cure at 6 months post-treatment defined as no clinical signs or symptoms and at least one of: improved hemoglobin or spleen regression if the spleen was palpable on admission, in absence of clinical signs or symptoms at any time during the 6 months follow-up. Secondary outcomes: – Initial cure defined as no signs or symptoms at day 45 and at least one of the following: improved hemoglobin and spleen regression if the spleen was palpable on admission. – Adverse events during treatment and follow-up period.	Group 1: Liposomal amphotericin B 15 mg/kg total dose infusion of 5 mg/kg/day iv on days 1, 3 and 5. Group 2: Liposomal amphotericin B 5 mg/kg/day total dose in a single dose iv + miltefosine 1.5-2.5 mg/kg/day for 10 days (days 1-10). Group 3: Liposomal amphotericin B 5 mg/kg total dose in a single dose + miltefosine 1.5-2.5 mg/kg/day for 10 days (days 1-10) + paromomycin base 11 mg/kg/day im for 10 days (days 2-11). Group 3: miltefosine 1.5-2.5 mg/kg/day for 10 days (days 1-10) + paromomycin base 11 mg/kg/day im for 10 days (days 1-10).
Open-label, sequential step, safety and efficacy study to determine the optimal single dose of Ambisome for patients with visceral leishmaniasis. NTC00832208/Ethiopia[54] April 2009/June 2011	Phase II/III Randomized, open-label, parallel assignment with treatment as primary purpose.	Primary outcomes: Efficacy as parasitological clearance with no relapse at 6 months post treatment assessed by clinical status and confirmed by splenic or bone marrow aspiration. Secondary outcomes: – Parasitological clearance at day 30.	Group 1: Liposomal amphotericin B total dose 21 mg/kg iv; 3 mg/kg/day on days 1-5, 14 and 21. Group 2: Liposomal amphotericin B iv as a single dose at 7.5 mg/kg increasing to 10, 12.5 and 15 mg/kg depending on the results of the interim analyses.
Clinical trial to assess the safety and efficacy of sodium stibogluconate and Ambisome combination, miltefosine and Ambisome, and miltefosine alone for the treatment of visceral leishmaniasis in Eastern Africa. NTC01067443/Sudan and Kenya[52] March 2010/June 2011	Phase II randomized open-label parallel assignment with treatment as primary purpose.	Primary outcome: Initial cure at day 28. Secondary outcome: – Final cure at day 210 – Adverse events and serious adverse events occurring up to day 60.	Group 1: Liposomal amphotericin B 10 mg/kg total dose on one day iv on day 1 + SSG at 20 mg/kg/day iv/im from days 2-11. Group 2: Liposomal amphotericin B 10 mg/kg total dose in one dose on day 1 + miltefosine 2.5 mg/kg/day orally from days 2-11. Group 3: Miltefosine 2.5 mg/kg/day orally from days 1-28.
Efficacy and safety study of drugs for treatment of visceral leishmaniasis in Brazil. NTC0130738/Brazil[53] February 2011/November 2014	Phase IV randomized, open-label, parallel assignment with treatment as primary purpose.	Primary outcome: Cure rate defined as complete remission of clinical signs and symptoms, 3 months after treatment plus normal hematological lab and no relapse at 6 months follow-up. Secondary outcome: – Improvement rate at 30 days defined as fever disappearing, stable or improving hematological lab abnormalities plus any spleen size reduction. – Relapse rate 6 months after treatment. – Adverse event rate and intensity.	Group 1: Antimoniate of N-methylglucantime 20 mg/kg/day iv for 20 days. Group 2: Liposomal amphotericin B 21 mg/kg total dose given in 3 mg/kg/day iv for 7 consecutive days. Group 3: Amphotericin B deoxycholate 14 mg/kg total dose given in 1 mg/kg/day iv for 14 consecutive days. Group 4: Liposomal amphotericin B 10 mg/kg total dose given iv in a single dose (day 1) + antimoniate N-methylglucamine 20 mg/kg/day for 10 days (days 2-10).

(Continue)

Table 2. Ongoing clinical trials for visceral leishmaniasis treatment *(Continued)*

Clinical trial Name/Number/Country Date started/Date estimated primary completion	Design of study	Outcomes	Drugs and regimens
Trial to determine efficacy of fexinidazole in VL in patients in Sudan. NTC01980199/Sudan[55] November 2013/November 2014	Phase II single group assignment open-label with treatment as primary purpose.	Primary outcome: Initial cure at day 28 defined as absence of parasites in tissue aspirate. Secondary treatment: – Final cure at day 210 defined as patients with initial cure at day 28 with no further sign or symptoms of VL at day 210.	Fexinidazole (comp 600 mg) 1,800 mg/day once a day for 4 days continued by 1200 mg once a day for 6 days.

Ambisome: liposomal Amphotericin B.
VL: visceral leishmaniasis; iv: intravenous; im: intramuscular.

been observed that PIs can inhibit a parasite enzyme (the aspartyl peptidase) and that parasites exposed to PIs can produce metabolic changes that can reduce the activity of aspartyl peptidases and so decrease the patient's susceptibility to the treatment[63]. Other authors have observed that the PI nelfinavir can induce oxidative stress in *Leishmania* amastigotes, which could lead to apoptosis. It has been further suggested that oxidative stress could cause cross-resistance with other drugs such as antimonials; this aspect, however, requires further research[64]. A recent study in mice showed that the PIs lopinavir/ritonavir and atazanavir can influence innate defense mechanisms in VL/HIV coinfection through different intracellular pathways that are key to the control of both HIV and *Leishmania* infection[65].

Recent studies have demonstrated that CCR5, a coreceptor for HIV-1 entry expressed on the surface of $CD4^+$ and $CD8^+$ T-cells, plays a significant role in the entry and establishment of the *Leishmania* parasite in the monocytes and macrophages. Therefore, it is postulated that the use of ART based on CCR5 inhibitors may also be useful for the control of the *Leishmania* infection[66].

Patients who initiate ART should be monitored in order to detect any toxicity secondary to the treatment. The immune reconstitution inflammatory syndrome (IRIS) must be controlled, although leishmaniasis associated with IRIS seems to be relatively uncommon[67]. Several case reports have described cases of symptomatic VL associated with ART and, in other cases, dermatologic manifestations have been observed to predominantly develop as diffuse patterns resembling post-kala-azar[68].

Immunotherapies and vaccines for visceral leishmaniasis in HIV-coinfected patients

It is known that following *Leishmania* infection, the disease progresses when the immune response of the host is suboptimal or excessive. Therefore, an immunotherapy that could modulate immune response might be a prophylactic or therapeutic option for VL and VL/HIV-coinfected patients. In other cases, immunotherapy could be administered in combination with a conventional therapy so that the drug dose needed is reduced and its efficacy is improved, thus reducing toxicity and the emergence of resistant strains[69,70]. A vaccine could also be developed to prevent the infection or its clinical manifestations. For this purpose, the protective antigens that induce an effective T-cell response need to be identified[71,72].

Follow-up and relapse detection

When VL is diagnosed, several factors have been identified as possible risk factors for VL relapse among HIV patients with a CD4 cell count < 100 cells/mm^3 as follows: (i) a low, slight increase in CD4 cell count in response to ART; (ii) the absence of secondary prophylaxis; and (iii) a history of previous episodes of relapse[38]. Relapse may occur in patients who have been treated with LAB and ART and even with secondary prophylaxis; therefore, it seems that these measures may only partially protect patients from relapse[38]. Hence, lifelong follow-up of VL/HIV-coinfected patients should be performed for any clinical manifestations of

relapse that can be parasitologically confirmed. The mere evidence of a positive non-quantitative polymerase chain reaction (PCR) for *Leishmania* is not enough for determining a diagnosis of VL relapse. However, monitoring the parasite load by ultrasensitive quantitative *Leishmania* PCR has been shown to be useful in predicting the risk of relapse after a VL episode in HIV-infected patients[73].

Relapsed patients should receive treatment. Patients who have been previously treated with amphotericin formulations can receive re-treatment with amphotericin as relapse does not seem to be related to a failed drug therapy[74]. In other cases, other alternative drugs such as LAB plus miltefosine can be administered alone or in combination.

Screening and treatment strategies for visceral leishmaniasis in HIV-coinfected patients

Since infection in immunocompetent individuals is generally controlled by the immune system, no specific measures seem to be necessary for asymptomatic infected patients. However, HIV-infected patients are at a higher risk of progression to VL after *Leishmania* infection. Moreover, as previously described, VL therapies are not sufficiently effective in VL/HIV patients who have been associated with lower initial cure rates and higher relapse rates during follow-up. Toxicity to treatment is also higher in VL/HIV-coinfected patients. Therefore, the question is inevitably raised about the usefulness of screening for latent *Leishmania* infection in HIV-coinfected patients and the effectiveness of administering pre-emptive therapies to this patient population. Several authors have posed this question regarding a region where VL is endemic and with a high prevalence of HIV like Ethiopia[75]. The objective proposed is to screen for latent *Leishmania* infection as well as for other opportunistic infections such as *Pneumocystis jiroveci* or *Cryptococcus spp* in HIV patients.

However, screening for latent *Leishmania* infection in HIV patients has several limitations. First of all, asymptomatic or latent VL infection is difficult to detect and even if a sensitive and specific diagnostic test was performed in HIV patients, it is not clear that a positive result would have any clinical and prognostic implications. Several diagnostic techniques have been employed to detect latent *Leishmania* infection in HIV patients. Thus, the *Leishmania* skin test is performed by intradermal inoculation of a suspension of promastigotes, yet there is no consensus on the optimal type and dose of antigen for HIV patients. Several antibody detection tests have been developed with high sensitivity and specificity for identifying latent *Leishmania* infection in immunocompetent patients; however, their applicability in HIV patients is a matter of controversy. Serum antibodies may not be detectable in patients with severe immunosuppression by standard techniques[76]. Antigen detection techniques, such as the latex agglutination test in urine, have been demonstrated to have high sensitivity for the *Leishmania* antigen in VL/HIV-coinfected patients. However, the specificity of the test decreases in asymptomatic patients[77]. Molecular techniques such as PCR have also been developed. These techniques have been shown to have more sensitivity and specificity than other diagnostic tests[76]. PCR can be performed on a wide range of clinical samples such as serum, tissue aspirates, and urine. However, its applicability as a screening tool for *Leishmania* in asymptomatic HIV and non-HIV patients has not been demonstrated. In fact, in regions where *Leishmania* is endemic, positive serum PCR results have been obtained for *Leishmania* in asymptomatic healthy individuals. Quantitative PCR tests have also been developed for *Leishmania,* which may be useful for detecting VL relapse after treatment; however, its effectiveness in detecting VL in asymptomatic HIV patients has not been demonstrated.

Secondly, there is another important limitation to the development of a screening and pre-emptive strategy for VL in HIV-coinfected patients: the best therapeutic option for primary prophylaxis has not been identified yet. Experience is based on secondary prophylaxis used to prevent relapse in areas where zoonotic transmission is prevalent. However, there is no data on any pre-emptive therapy that is not strongly associated with a higher risk of developing drug resistance, especially in areas where transmission is prevailingly anthroponotic[75].

In Spain, where *L. infantum* is endemic, several studies have been undertaken where HIV patients were screened. In the first study, 291 HIV-infected patients were screened by bone marrow aspiration, regardless of their symptoms. *Leishmania* amastigotes were detected in 32/291 (11%) and 13 of the 32 (41%) were asymptomatic. Consequently, nearly 4.5% of HIV-infected patients had asymptomatic VL. The authors concluded that VL is highly prevalent, and frequently subclinical, in HIV-infected patients in Spain[78].

In another study, screening was performed by indirect immunofluorescence antibody test (IFAT) excluding all patients with a history of visceral or cutaneous

leishmaniasis. The patients with a significant IFAT titter underwent a bone marrow aspiration or tissue biopsy for direct visualization of the parasite and culture in Novy-McNeal-Nicolle´s medium, PCR testing, and/or *Leishmania* antigen test in urine. A total of 179 HIV patients were included, of whom only six (3%) had significant IFAT anti-*Leishmania* titters. None of them presented fever, splenomegaly, or anemia. Parasites were visually detected in only two of the six patients, who were classified as having subclinical VL infection. These patients were treated with five doses of LAB followed by a monthly dose of LAB as secondary prophylaxis after 48 months without any clinical manifestation of VL relapse. The other four with a positive IFAT but with no visualization of parasites were classified as having latent VL. These patients did not receive any treatment and did not present any symptoms of *Leishmania* infection during follow-up, the duration of which ranged from 24 to 36 months. Finally, there was a patient with a negative IFAT who developed symptoms for VL a month after the screening. The authors concluded that the results of the study did not support the use of IFAT as a screening method for *Leishmania* in HIV patients living in areas where *Leishmania* was endemic[79].

In another study, screening for *Leishmania* was performed by enzyme-linked immunosorbent assay (ELISA). The patients with a positive result and evidence of pancytopenia or hepatosplenomegaly underwent a bone marrow aspiration. Of the 187 patients screened, serological test for *L. infantum* was positive in only 7 (3.7%). As none of them had any symptoms or analytical results suggestive of VL, no further studies were performed[80].

Conclusions

Visceral leishmaniasis is a major public health problem that affects over 35 countries worldwide. VL/HIV coinfection has decreased in regions such as the Mediterranean basin due to the introduction of ART. However, the prevalence of VL/HIV coinfection is still very high in some countries in East Africa, whereas coinfection is rare in countries of the Indian subcontinent with a high prevalence of VL but a low prevalence of HIV. However, the spread of HIV in these countries has made VL/HIV coinfection an emerging disease.

The impaired immune function in HIV-coinfected patients may favor the reactivation of latent *Leishmania* infection, which has been associated with a more severe VL presentation, worse therapeutic response, and a higher risk of relapse after treatment. Thus, VL in HIV-coinfected patients is a life-threatening infection; unfortunately, scant experience and data are available about the best therapeutic option for these patients. In our review, we observed that AB, and specifically its lipid formulations, seems to be the most effective option. On the other hand, there is evidence that standard treatment with antimonials should not be administered to VL/HIV patients as it has been associated with high toxicity and mortality rates. Also, since miltefosine is administered orally, it has been suggested as a very good option, but patients who initially respond relapse once treatment is discontinued. There is insufficient data about other treatments such as pentamidine, paromomycin, or fluconazole to recommend them for coinfected patients. Nor have any studies been performed in HIV patients that evaluate the efficacy of immunotherapies for VL. Experts suggest that to increase the efficacy of VL treatments in HIV-coinfected patients, the best option is probably based on a combination of therapeutic regimens. However, only a clinical trial is being performed to evaluate the efficacy of liposomal amphotericin B plus miltefosine in VL/HIV-coinfected patients in Africa.

Another difficulty that is hard to handle in coinfected patients is the high rate of relapse. It is difficult to know in advance which patients will relapse, although ultrasensitive quantitative *Leishmania* PCR may be a good option. Moreover, little is known about which drugs, dose, and duration are the best for secondary prophylaxis. The few clinical trials performed have demonstrated that maintenance treatment with meglumine antimoniate and with amphotericin B lipid complex seem to reduce relapse rates. However, it should be taken into account that prolonged prophylaxis may favor the development of resistant strains, especially in areas where transmission is anthroponotic. Currently, a clinical trial is being conducted in Ethiopia to evaluate the efficacy of pentamidine as secondary prophylaxis for VL in HIV patients. An association has been reported between improved immunity by the administration of ART and lower relapse rates, although this method does not prevent recurrence definitely.

Highlights box

- Visceral leishmaniasis in HIV infected patients may lead to more severe VL presentation, may cause a worse therapeutic response and higher toxicity, and may increase the relapse rate after treatment, especially in patients with CD4 < 200 cells/µl.

- Amphotericin B lipid formulations seem to be the most effective therapeutic option for VL/HIV-coinfected patients, and antimonials should be avoided due to the high toxicity and mortality rates observed in HIV patients.
- It has been suggested that combined drug regimens are probably the best option for VL/HIV-coinfected patients in order to increase the efficacy and reduce the toxicity. A good option may probably be liposomal amphotericin B plus miltefosine due to its observed synergism.
- Secondary prophylaxis has been proven to significantly reduce the relapse rate of VL in HIV patients; also, it should be initiated after the end of the initial treatment course. However, there is no conclusive evidence on the best drug and regimen for this type of patient.
- Follow-up must be performed indefinitely for any clinical manifestation of relapse, which must be parasitologically confirmed. The parasite load determined by ultrasensitive quantitative *Leishmania* PCR may be useful to predict the risk of relapse.
- The implementation of antiretroviral therapy for HIV infection can improve the immunity and decrease the progression from asymptomatic leishmaniasis infection to active disease and reduce relapse rates after treatment.

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