Treatment of Visceral Leishmaniasis

8

Begoña Monge-Maillo and Rogelio López-Vélez

8.1 General Treatment Considerations in Visceral Leishmaniasis

Untreated, advanced cases of visceral leishmaniasis (VL) can result in death mainly associated to progressive wasting, superinfection and/or haemorrhage. So, all persons with symptomatic VL should be treated with antileishmanial drugs. Other complementary measures are needed in many cases and can include nutritional support, treatment of other infectious diseases (e.g. tuberculosis, malaria or bacterial or parasitic dysentery) and blood transfusions. The therapeutic options for VL are diverse and depend on different factors, such as geographical area of the infection (Alvar et al. 2006); the *Leishmania* species involved, the development of failure to habitual treatments (Croft et al. 2006; Alvar et al. 2008), the evidence of HIV coinfection or other infections and the presence of malnourishment. The goal of the "best treatment option" is to cure the patient, to minimize the appearance of resistance and to decrease the duration of hospitalization, all at the lower cost (Alvar et al. 2006).

The treatment regimen recommended should also follow national and regional guidelines, if applicable. This drug policy in endemic countries and the therapeutic decisions should be based on the individual benefit–risk ratio of medicines, the health service setting, the availability of antileishmanial medicines and public health considerations, such as the prevention of drug resistance.

B. Monge-Maillo • R. López-Vélez (✉)
National Referral Unit for Tropical Diseases, Infectious Diseases Department, Ramón y Cajal University Hospital, IRICYS, Madrid, Spain
e-mail: rogelio.lopezvelez@salud.madrid.org

F. Bruschi, L. Gradoni (eds.), *The Leishmaniases: Old Neglected Tropical Diseases*, https://doi.org/10.1007/978-3-319-72386-0_8

8.2 Patient Evaluation Before Treatment

Treatment should be given always after confirmation of the infection. While there are several approaches to the diagnosis of VL, it is recommended to use different diagnostic approaches to maximize the likelihood of a positive *Leishmania* results. Methods employed are visualization of the characteristic amastigote in blood smears or aspirates from lymph nodes, bone marrow, liver or spleen (histopathology), parasite isolation by in vitro culture, molecular detection of parasite DNA and serologic testing (Mary et al. 2006; Sundar and Rai 2002a). Persons newly diagnosed with VL should also be assessed for concurrent HIV/AIDS or other causes of cell-mediated immunosuppression.

8.3 Treatment Options

Properties of antileishmanial drugs are shown in Appendix and end of Chap. 9 (Treatment of tegumentary forms of leishmaniasis).

The traditional treatment of VL used to be *pentavalent antimonials*, introduced in the 1940s. However, the development of resistance, especially in India, with failure rates of up to 60%, as well as their potential toxicity, made it necessary to research for new treatment options. Thus and since the 1980s, the use of *amphotericin B deoxycholate* has been introduced, especially in the more developed countries. Progressively, and due to their efficacy and lower toxicity, *lipid formulations of amphotericin B* have been gaining importance, becoming the first-choice treatment established by the US Food and Drug Administration. Nonetheless, their elevated cost reduces its use in less powerful nations. In countries of fewer resources, studies have been carried out demonstrating the efficacy of parenteral *paromomycin* as a cheap treatment with medium toxicity, commercialized in India and available in East Africa.

Within the range of oral treatments *miltefosine* had demonstrated very good cure rates in adults and children in India, Nepal and Bangladesh with VL by *L. donovani* (Sundar et al. 2002, 2006; Bhattacharya et al. 2007; Ritmeijer et al. 2006). However, currently, a high rate of clinical failures has been reported (Rijal et al. 2013; Sundar et al. 2012). Moderate efficacy has been observed in East Africa (Ritmeijer et al. 2006), while more data from Mediterranean countries and Latin America are needed.

Currently, *combination therapies* are considered the best regimens for treating VL in many parts of the world as dosing and duration of treatment are decreased, thereby decreasing toxicity, costs and drug resistance (Monge-Maillo and Lopez-Velez 2013).

8.4 Definition of Healing and Follow-Up

It has been shown that clinical parameters correlate well with parasitological response to VL treatment. Therefore, clinical parameters should be used to monitor the response to the VL treatment and to make the follow-up.

The confirmation of a parasitological response performed by repeating a bone marrow or spleen aspiration is not recommended in a patient with an adequate clinical response. The antibody levels are not useful to monitor the treatment response because they can persist positive for a long time (usually 6–8 months).

The clinical parameters that indicate a response to the VL treatment are the normalization of the temperature, an increase in appetite and weight and a decrease in the liver and spleen size. Blood test must show that the level of leukocytes, haemoglobin and platelets rises (Maru 1979; Kager et al. 1984).

Normally patients responding to treatment become afebrile in 5–7 days while visceromegaly usually resolves slower, within 3–6 months, although some decrease may be seen in approximately 10 days after initiation of treatment (Cascio et al. 2004). Leukopenia and thrombocytopenia generally normalize within a month, but resolution of anaemia may be slower taking from 6 to 12 months to recover (Kager et al. 1984; Berman et al. 1998).

There have been identified several factors associated with a higher risk of death which are immunosuppression, prolonged disease, malnutrition, concomitant infections, gastrointestinal symptoms, mucosal bleeding, jaundice, <1 year of age and laboratory signs such as severe anaemia, neutrophils <500 cells/μL and platelets <50,000 cells/μL (Werneck et al. 2003; Collin et al. 2004; Mueller et al. 2009).

Patients should be clinically evaluated at the end of treatment, at 1 month after and at 6 months after. Therapeutic failure is defined as a return of clinical signs and symptoms of VL in concert with parasitological confirmation. It can occur in patients with no immunodeficiency, and mostly 6–12 months after treatment. However, failure is more likely in those with HIV co-infection or compromised cell-mediated immunity for other reasons representing more of an immunologic failure rather than a drug failure.

8.5 Treatment According to the Country of Infection

The Oxford evidence grading system was applied when reviewing information:

(A) Randomized controlled trials in representative patient groups.
(B) Randomized controlled trials in less homogenous patient groups (small numbers, different species included) as well as cohort trials and case control studies in representative patient.
(C) Cohort trials or case control studies in less homogenous patient groups, as well as case series of representative patient groups.
(D) Case series of less homogenous patient groups and expert opinion were ranked.

8.5.1 The Mediterranean Region (Box 8.1)

VL is hypoendemic in the Mediterranean region, where it is caused by the protozoon *L. infantum*. This parasite is transmitted by the bite of infected

phlebotomine female sandflies of the *Phlebotomus* genus and is maintained in a zoonotic cycle with dogs acting as the main reservoir (World Health Organization (WHO) 2010).

Cases in the Mediterranean region only contribute to 5–6% of the global burden of VL, with an estimated annual incidence of 1200–2000 cases (Alvar et al. 2012).

The incidence of VL has been declining in the last decades, mainly in areas where living standards have improved. VL associated with HIV infection is also declining in the past few years in Europe and the Mediterranean region.

The actual recommended therapeutic regimens for VL caused by *L. infantum* in the Mediterranean region by the World Health Organization are liposomal amphotericin B, up to a total dose of 18–21 mg/kg as first choice; pentavalent antimonials, 20 mg Sb^{v+}/kg per day IM or IV for 28 days as second choice and amphotericin B deoxycholate, 0.75–1.0 mg/kg for a total dose of 2–3 g, as third choice (World Health Organization (WHO) 2010).

In the Mediterranean region, evidence with pentavalent antimonials is not too strong, and therapeutic attitudes even vary from country to country. During the 1990s, antimonials were the first-line treatment in France, Greece, Italy, Malta, Spain, Portugal, Morocco, Algeria and Tunisia, with cure rates of 95% in immunocompetent patients (Gradoni et al. 2008). The information recollected from 11 countries of Southern Europe, Northern Africa and the Middle East in the twenty-first century reflects certain variations in the treatment recommendations (Gradoni et al. 1995): By this way in Morocco, Tunisia, Turkey and Palestine, the antimonials were the first-line treatment. Meanwhile in Portugal, Spain, Greece and Italy, antimonials and amphotericin B preparations were the two options for first-line treatment, even though antimonials were not administered in patients with severe immunosuppression and preparations of liposomal amphotericin B were recommended for the treatment of relapses after antimonials. In France, Italy and Cyprus, liposomal amphotericin B was the first-line treatment, and relapses were treated with different regimens of the same dug. Another study which recollects a total of 1210 cases of VL in children of between 0 and 14 years in Albania from 1995 to 2009 demonstrated that antimonials at a dose of 20 mg Sb^{v+}/kg/day for 21–28 days continue to be effective, with a cure rate of 99% (Petrela et al. 2010).

In the Mediterranean area there is scare experience with amphotericin B deoxycholate; in fact, the liposomal preparations of amphotericin B are preferred as the first-line drugs in those cases where antimonials had previously failed (Gradoni et al. 1995). A retrospective study of five cases of VL in Tunisia treated with amphotericin B deoxycholate obtained a 100% response (Toumi et al. 2007).

Although there are no randomized clinical trials performed in the Mediterranean region with liposomal amphotericin B, there are a high number of case series that give an important accumulation of evidence about its use. Response rates obtained were superior to 97% with total doses of between 18 and 24 mg/kg in different regimens of administration (Figueras Nadal et al. 2003; Cascio et al. 2004; di

Martino et al. 2004; Kafetzis et al. 2005; Minodier et al. 2005). Therefore, it has been proven that liposomal amphotericin B reduced the average duration of hospital care when compared to antimonials (Kafetzis et al. 2005), and it is effective in those cases where antimonials had previously failed (Minodier and Garnier 2000). For all these reasons, and despite the absence of randomized clinical trials, liposomal amphotericin B is considered a reference treatment in the case of VL in the Mediterranean countries in adults as well as in children (Rosenthal et al. 2009).

There is nearly no experience neither with pentamidine nor with paromomycin for VL in the Mediterranean area.

Reliable data on the efficacy of miltefosine in VL in the Mediterranean region has not been published. However, its oral administration makes it an attractive therapeutic option.

Box 8.1 Therapeutic Options for Visceral Leishmaniasis in the Mediterranean Region

- **Sodium stibogluconate or meglumine antimoniate:** (IM or IV) 20 mg Sb^{v+}/kg/day for 28–30 days [B]
- **Amphotericin B deoxycholate:** (IV) 0.7–1 mg/kg/day, on alternate days, for 15–20 doses [D]
- **Liposomal amphotericin B:** (IV) 3–5 mg/kg/day for 3–10 doses (total dose 18–30 mg/kg in adults and 15 mg/kg in children) [B]
- **Paromomycin:** (IM) 15–20 mg (11–15 mg base) kg/day for 21–28 days [D]
- **Miltefosine:** (oral dosing) for 28 days; 2.5 mg/kg/day in children aged 2–11 years; 50 mg/day in those aged ≥12 years with bodyweight <25 kg; 100 mg/day in those aged ≥12 years with bodyweight ≥25 kg; 150 mg/day in those aged ≥12 years with bodyweight ≥50 kg [D]

8.5.2 The Middle East and Central Asia Region (Box 8.2)

Incidence of visceral leishmaniasis in the Middle East to Central Asia from 2004 to 2008 has been estimated to be between 4500 and 9500 cases per year. The most affected countries are Iraq, China, Georgia and Iran. In the last years, countries with ample resources like Saudi Arabia have been able to take good measures to control the diseases and have manage to reduce the incidence of leishmaniasis. Meanwhile, other countries like Syria and Iraq due to the war they suffer from a lack of access to health care and from a compromised nutritional status putting the exposed population at greater risk of the disease. The *Leishmania* species mainly involved in the VL cases in the Middle East and Central Asia region is *L. infantum* except for Saudi Arabia where it is mostly caused by *L. donovani* (Alvar et al. 2012; Salam et al. 2014).

No randomized clinical trials have been performed in the Middle East and Central Asia evaluating the efficacy of the different therapeutic regimens for

VL. However pentavalent antimonials have been the drug of choice for more than 70 years now. Currently due to the emergence of drug resistance and toxicity of antimonials, liposomal amphotericin B, miltefosine and paromomycin are replacing antimonials. Since the species involved is mainly *L. infantum* as in the Mediterranean region, the treatment recommendations established for the Middle Eastern region could be compared to those for the Mediterranean region.

Box 8.2 Therapeutic Options for Visceral Leishmaniasis in the Middle East and Central Asia Region

- **Sodium stibogluconate or meglumine antimoniate:** (IM or IV) 20 mg Sb^{v+}/kg/day for 28–30 days [C]
- **Amphotericin B deoxycholate:** (IV) 0.7–1 mg/kg/day, on alternate days, for 15–20 doses [D]
- **Liposomal amphotericin B:** (IV) 3–5 mg/kg/day for 3–10 doses (total dose 18–30 mg/kg in adults and 15 mg/kg in children) [D]
- **Paromomycin:** (IM) 15–20 mg (11–15 mg base) kg/day for 21–28 days [D]
- **Miltefosine:** (orally) for 28 days; 2.5 mg/kg/day in children aged 2–11 years; 50 mg/day in those aged ≥12 years with bodyweight <25 kg; 100 mg/day in those aged ≥12 years with bodyweight ≥25 kg; 150 mg/day in those aged ≥12 years with bodyweight ≥50 kg [D]

8.5.3 The Indian Subcontinent and South Asia Region (Box 8.3)

The estimated incidence of visceral leishmaniasis in the Indian subcontinent and South-east between 2006 and 2010 was from 162,100 to 313,600 cases per year. India (where the state of Bihar accounts for the majority of the cases), Bangladesh and Nepal are the most affected countries. Visceral leishmaniasis in this area has an anthroponotic transmission and is caused by *L. donovani* (Alvar et al. 2012).

Many clinical trials have been performed in the Indian subcontinent which confers in most cases a high quality of evidence in the treatment recommendation. Pentavalent antimonials are considered a first-line drug due to their efficacy observed in several clinical trials in Bangladesh and Nepal. However, there are zones where resistance have developed, fundamentally in the state of Bihar (India) where the pentavalent antimonial rates of resistance reaches up to 60% of all cases (Sundar and Rai 2002b), and so they should not be used. In the beginning of the 1990s, the first clinical trials with amphotericin B were performed in India for VL obtaining response rates of 98–100% even in those cases where antimonials had previously failed (Jha et al. 1995; Thakur et al. 1993a).

Due to the high rate of resistance to antimonials in India and the high cure rates obtained with amphotericin B, this is established nowadays as one of the drugs of choice for VL in the Indian subcontinent. In India different regimens of liposomal amphotericin B have been tested with a diverse range of response rates (Sundar et al. 2004). Doses administered for several days at different total doses have been tested and more recently a regimen based on a single dose has demonstrated efficacy (Thakur 2001; Sundar et al. 2010). Therefore, due to is efficacy and because lipid formulation of amphotericin B has less renal toxicity than amphotericin B deoxycholate, liposomal amphotericin B is considered a first-line drug for VL in the Indian subcontinent.

Pentamidine was the second drug tested in VL-endemic areas in India when faced with the need to find alternative treatment to pentavalent antimonials. Already in the 1980s, pentamidine resulted as an efficient therapeutic regimen (Thakur 1984). However, the response rate started to decrease after the decade of the 1990s in certain areas of India (Mishra et al. 1992). Moreover, later studies compared it with other therapeutic options used in the area, as is the case with amphotericin B demonstrating that pentamidine was less effective (Das et al. 2009).

Paromomycin has been also tested in India mostly in Bihar where it presented high cure rates and good tolerance with the exception of an increase in liver function test parameters, which decrease towards baseline over time. So paromomycin had a reasonably safe profile and efficacy tested even in paediatric patients (Jha et al. 1998; Sundar et al. 2007; Sinha et al. 2011a).

Several clinical trials performed for VL in India and Bangladesh showed high cure rates with miltefosine regimens for 28 days (Sundar et al. 2002, 2006; Bhattacharya et al. 2007; Rahman et al. 2011). Also high cure rates have been obtained in paediatric clinical trials performed in India (Sundar et al. 2003; Bhattacharya et al. 2004). These initial results lead to propose miltefosine as a first-line drug for VL in India, Nepal and Bangladesh. However, more recent studies have revealed that after a decade of use of miltefosine in the Indian subcontinent, the relapse rate with miltefosine has increased significantly in these countries, and a development of tolerance and resistance to this drug is suspected. Therefore, the strength of the recommendation of miltefosine for the Indian subcontinent may decrease (Rijal et al. 2013; Dorlo et al. 2014).

In India, several studies published about combined therapy have obtained favourable results in patients. Combinations tested have been pentamidine and antimonials (Thakur et al. 1991), pentamidine and allopurinol (Das et al. 2001) or paromomycin and antimonials (Thakur et al. 1992, 2000). Several studies have demonstrated the efficacy of combinations of liposomal amphotericin B at a single dose followed by different regimens of oral miltefosine (Sundar et al. 2008). Another comparative study performed in India tested a single dose liposomal amphotericin B plus miltefosine or paromomycin or miltefosine plus paromomycin. These combined therapies were non-inferior to the standard treatment (amphotericin B for 30 days) and resulted in fewer adverse events than those assigned standard treatment (Sundar et al. 2011).

Box 8.3 Therapeutic Options for Visceral Leishmaniasis in the Indian Subcontinent and South-East Asian Region

- **Sodium stibogluconate or meglumine antimoniate**: (IM or IV) 20 mg Sb^{v+}/kg/day for 28–30 days
- Nepal and Bangladesh [A], India [not recommended]
- **Amphotericin B deoxycholate**: (IV) 0.7–1 mg/kg/day, daily on alternate days, for 15–20 doses [A]
- **Liposomal amphotericin B**: (IV) 5–10 mg/kg for 1–2 doses (up to total dose of 10 mg/kg) or 3–5 mg/kg/day for 3–5 doses (up to total dose of 15 mg/kg) [A]
- **Pentamidine isethionate**: (IM or IV) 4 mg/kg/day, on alternate days or three times a week, for 15–20 doses [not recommended]
- **Paromomycin**: (IM) 15 mg (11 mg base)/kg/day for 21 days [A]
- **Miltefosine**: (orally) for 28 days; 2.5 mg/kg/day in children aged 2–11 years; 50 mg/day in those aged ≥12 years with bodyweight <25 kg; 100 mg/day in those aged ≥12 years with bodyweight ≥25 kg; 150 mg/day in those aged ≥12 years with bodyweight ≥50 kg [A]
- **Combination therapy**:
  - **Liposomal amphotericin B** (IV), 5 mg/kg single dose + **Miltefosine** (oral), for 7–14 day; 2.5 mg/kg/day in children 2–11 years; 50 mg/day in ≥12 years old with weight < 25 kg; 100 mg/day in ≥12 years with body weight ≥ 25 kg; 150 mg/day in ≥12 years with body weight ≥ 50 kg [A]
  - **Liposomal amphotericin B** (IV), 5 mg/kg single dose + **Paromomycin** (IM), 15 mg (11 mg base)/kg/day for 10 days [A]
  - **Miltefosine** (oral), for 10 days, as above + **Paromomycin** (IM), 15 mg (11 mg base)/kg/day for 10 days [A]

8.5.4 East Africa Region (Box 8.4)

East Africa is one of the most affected regions by VL, only surpassed by the Indian subcontinent, with an estimated annual incidence rate of 29,400–56,700 cases (Alvar et al. 2012). The countries most affected are Sudan, South Sudan and Ethiopia. With much lower VL burden, endemic foci of VL are also found in Eritrea, Somalia, Kenya and Uganda (Alvar et al. 2012). Leishmaniasis affects mostly to poor communities that live in remote areas and that have poor health-care infrastructure. Visceral leishmaniasis in East Africa is caused by *L. donovani*.

Currently treatment in these countries is mostly provided by international organizations such as Médecins Sans Frontières (MSF), Drugs for Neglected Diseases initiative (DNDi) and the World Health Organization (WHO).

In Africa, the first trials with pentavalent antimonials were realized in Kenya in 1983 (Anabwani et al. 1983). Further on, few new studies have been done since then. In the 1990s in Sudan, sodium stibogluconate combined with paromomycin showed higher cure rates than pentavalent antimonials alone (Seaman et al. 1993). Other studies in Kenya and Sudan have analysed the efficacy of generic sodium

stibogluconate versus patented versions, without observing any significant differences and with the advantage of a lower cost (Veeken et al. 2000; Moore et al. 2001). In Uganda, a comparative study was done between amphotericin B deoxycholate and reported a 95% cure rate with antimonials (Mueller et al. 2008). A new study, developed in Ethiopia, demonstrated differences in the cure rate after 6 months in patients from the North versus patients from the South (80% vs. 100%), which was justified by the different rates of confection by HIV (46.4% of the patients from the North were HIV positive, while no case was detected among the patients from the South). Thus, the efficacy of antimonials in Ethiopia in immunocompetent patients seems to be very high.

Experience with amphotericin B deoxycholate was obtained from a study where it was administrated at a dose of 1 mg/kg on alternate days for a period of 30 days, reaching similar cure rates than with antimonials and without any difference in the appearance of severe side effects (Mueller et al. 2008).

There is very little experience of liposomal amphotericin B in Eastern Africa, and the recommendations are based on results obtained in India. In Sudan, total doses of 20 mg/kg were tested with cure rates of 88%, but lower doses of 12 mg/kg only obtained a 50% response rate (Seaman et al. 1995). A reduced clinical trial in phase II in Kenya demonstrated that the efficacy of a total dose 14 mg/kg was higher that of 6 or 10 mg/kg (Berman et al. 1998). A randomized multicentre clinical trial conducted in Eastern Africa showed that a single dose on amphotericin B is not a suitable regimen for VL treatment across Eastern Africa (Khalil et al. 2014; Edwards et al. 2011). Thus, it is expected that, in Eastern Africa, higher doses are needed than in India.

In Eastern Africa, there is scare evidence on pentamidine, and it has proved to be effective in the treatment of patients in Sudan when pentavalent antimonials had previously failed (Khalil et al. 1998). However, in the Indian subcontinent, pentamidine for VL due to *L. donovani* had low cure rates.

In East Africa, the majority of studies executed are based on a comparison between paromomycin and antimonials, or on a combination of both. Good cure rates were obtained with regimens of paromomycin during 21 or 28 days in Kenya and Ethiopia with a lower response in Sudan (Seaman et al. 1993; Melaku et al. 2007; Musa et al. 2010).

A clinical trial performed in Ethiopia with miltefosine in immunocompetent patients registered a 75.6% cure rate (Ritmeijer et al. 2006).

About combined therapy, in Eastern Africa, more concretely in Sudan, two studies were carried out that demonstrated that paromomycin associated with antimonials increased the response rate in comparison with antimonials in monotherapy (Seaman et al. 1993; Melaku et al. 2007). In Kenya, a non-randomized trial drew a comparison between paromomycin and antimonials in monotherapy versus the combination of both, the latter being the most effective option (Chunge et al. 1990). Another clinical trial performed in Sudan, Ethiopia and Kenya demonstrated that combined therapy with paromomycin and antimonials was a safe regimen and just as efficient as antimonials on their own, thus being a good option for treatment in Eastern Africa (Musa et al. 2012). A further clinical trial has been performed in

East Africa to assess whether a short combination of antimonials plus a single dose of liposomal amphotericin B, miltefosine plus a single dose of liposomal amphotericin B and miltefosine alone, were effective in treating VL. None of the regimens tested showed cure rates sufficiently high to develop a phase III trial and to consider these regimens optimal for VL in East Africa (Wasunna et al. 2016).

Box 8.4 Therapeutic Options for Visceral Leishmaniasis in the East Africa Region

- **Sodium stibogluconate or meglumine antimoniate:** (IM or IV) 20 mg Sb^{v+}/kg/day for 28–30 days [A]
- **Amphotericin B deoxycholate:** (IV) 0.7–1 mg/kg/day, on alternate days, for 15–20 doses [C]
- **Liposomal amphotericin B:** (IV) 3–5 mg/kg/day for 6–10 doses (up to total dose of 30 mg/kg) [B]
- **Pentamidine isethionate:** (IM or IV) 4 mg/kg/day, on alternate days or three times a week, for 15–20 doses [not recommended]
- **Paromomycin:** (IM) 15–20 mg (11–15 mg base) kg/day for 21–28 days [B]
- **Miltefosine:** (orally) for 28 days; 2.5 mg/kg/day in children aged 2–11 years; 50 mg/day in those aged ≥12 years with bodyweight <25 kg; 100 mg/day in those aged ≥12 years with bodyweight ≥25 kg; 150 mg/day in those aged ≥12 years with bodyweight ≥50 kg [B]
- **Combination therapy:**
  - **Sodium stibogluconate or meglumine antimoniate** (IM or IV) 20 mg Sb^{v+}/kg/day for 17 day plus **Paromomycin** (IM) 15 mg (11 mg base)/kg/day for 17 days [A]
  - **Liposomal amphotericin B** (IV) 10 mg/kg single dose plus **Sodium stibogluconate** 20 mg/kg/day for 10 days [not recommended]
  - **Liposomal amphotericin B** (IV) 10 mg/kg single dose plus **miltefosine** (orally) 2.5 mg/kg/day for 10 days [not recommended]
  - **Miltefosine** (orally) 2.5 mg/kg/day for 28 days [not recommended]

8.5.5 Latin America Region (Box 8.5)

Visceral leishmaniasis in the Latin America affects mainly zones in the north-east of Brazil where 3000–5000 cases appear every year, usually in the early ages (Jeronimo et al. 2004; Wasunna et al. 2016; Hailu et al. 2010a; Musa et al. 2010). It is a zoonotic infection produced by *L. infantum/chagasi* that causes a high percentage of asymptomatic patients, as opposed to VL in India.

Classically, the treatment of VL in Brazil was based on the use of antimonials, with a cure rate of up to 95% (Santos et al. 2002). In fact, the Pan American Guide for the treatment of infectious diseases established as first-line treatment pentavalent antimonials at a dose of 20 mg Sb^{v+}/kg/day IM or IV for 20–28 days; if there is no response, they propose pentamidine, and if the patient is still not cured, amphotericin B should be used (Organización Panamericana de la Salud 2004). Evidence in Latin America with liposomal amphotericin B is very scarce. In Brazil, total dose

of 20 mg/kg has proven to be effective (Berman et al. 1998). In Colombia two cases were published where the treatment with antimonials had failed but who responded to liposomal amphotericin B (Velez et al. 2009).

Although pentamidine is recommended by the Pan American Guide for the treatment of infectious diseases, there is little literature about its use in Latin America. Moreover a decrease of its efficacy has been observed which added to its serious and sometimes irreversible toxicity, and the development of other drugs has made that it is practically abandoned.

There is no data about paromomycin for visceral leishmaniasis in Latin America.

Box 8.5 Therapeutic Options for Visceral Leishmaniasis in the Latin America Region

- **Sodium stibogluconate or meglumine antimoniate:** (IM or IV) 20 mg Sb^{v+}/kg/day for 28–30 days [B]
- **Amphotericin B deoxycholate:** (IV) 0.7–1 mg/kg/day, on alternate days, for 15–20 doses [C]
- **Liposomal amphotericin B:** (IV) 3–5 mg/kg/day for 6–10 doses (up to total dose of 30 mg/kg) [C]
- **Pentamidine isethionate:** (IM or IV) 4 mg/kg/day, on alternate days or three times a week, for 15–20 doses [not recommended]
- **Miltefosine:** (orally) for 28 days; 2.5 mg/kg/day in children aged 2–11 years; 50 mg/day in those aged ≥12 years with bodyweight <25 kg; 100 mg/day in those aged ≥12 years with bodyweight ≥25 kg; 150 mg/day in those aged ≥12 years with bodyweight ≥50 kg [C]

8.6 Treatment of Failures and Relapses

There is scare experience to give a strong evidence recommendation of a therapeutic option for a VL infection that has initially failed to respond or that has relapse. They can be treated with another drug, or use the same drug in a different dose or for longer periods, or a combination therapy can be administrated. The selection of the drug must be based on the *Leishmania* species involved, on the immune situation of the patient and on the prevalence of therapeutic failure rates in the geographic area of acquisition.

8.7 Treatment of Visceral Leishmaniasis Under Special Conditions

8.7.1 Visceral Leishmaniasis and HIV Co-Infection

Leishmania and HIV co-infection have been reported in more than 35 countries. In the early 1990s, a rapid increase in the incidence of VL/HIV co-infection was noticed in the Mediterranean basin, coinciding with the peak of the HIV epidemic. The 85% of the countries where the WHO detected the first cases of co-infections

were in the Mediterranean basin, with Spain in the lead (Alvar et al. 1997). The number of cases of co-infection reached its peak in 1997, and its incidence plateaued between 1998 and 2001. Since 2001, the incidence of VL/HIV co-infection has decreased significantly mainly due to the administration of antiretroviral treatments (ARTs) for HIV in the Mediterranean region (Alvar et al. 2008). On our days there are other geographical areas, mostly Ethiopia and Sudan, where the rate of VL/HIV co-infection is very high, probably due to the fact that ARTs are not so widespread. Interestingly, VL/HIV co-infection 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 co-infection should be considered an emerging problem in these regions (Diro et al. 2014b; Singh 2014).

Patients with VL and HIV co-infection have usually a worse therapeutic response presenting frequent relapses especially among those patients with CD <200 cell/μL. Only a few clinical trials have been conducted on the efficacy of some drugs for VL/HIV co-infection, and the majorities have 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 co-infection (Cota et al. 2013).

8.7.1.1 The Mediterranean Region

The evidence currently available on the efficacy of pentavalent antimonials in HIV patients has been gathered mainly in European studies reporting varying cure rates ranging from 33 to 82%, with high relapse rates (Pintado and Lopez-Velez 2001). Specifically, two clinical trials have been performed comparing meglumine antimoniate with amphotericin B deoxycholate and amphotericin B lipid complex. The efficacy between pentavalent antimonials and amphotericin in the two evaluated presentation were similar. However, the toxicity of pentavalent antimonials was substantially higher (Laguna et al. 1999, 2003).

Experience with liposomal amphotericin B is based on studies performed in four European health centres, where VL was treated with liposomal amphotericin B in HIV patients, with a good initial clinical and parasitological response, although all patients who completed follow-up eventually relapsed (Russo et al. 1996).

In Germany, a study was performed with miltefosine in HIV patients in whom other previous treatment for VL had failed. Initially, the cure rates were high, but almost all patients finally relapsed when miltefosine was discontinued. However, miltefosine was well tolerated even in long-term treatment periods, suggesting that clinical relapse could be either treated by administering repeated courses of miltefosine or prevented with miltefosine in combination with other antileishmanial drugs (Sindermann et al. 2004). Another study performed in Spain described four cases of co-infected patients who were severely immunosuppressed and who had not responded to a previous treatment with amphotericin B or pentavalent antimonials and that where treated with miltefosine. Initially, all patients responded clinically but, when treatment was discontinued, all patients relapsed (Troya et al. 2008).

Combination therapy has been tested in several studies in the Mediterranean region for VL/HIV co-infected patients. In Spain, a study performed with 11 VL/

HIV co-infected patients due to *L. infantum*, meglumine antimoniate was combined with allopurinol, and good results were obtained (Laguna et al. 1994). Also in Spain, another case was reported of a co-infected patient who did not respond to previous monotherapies and who finally responded to a combined therapy of meglumine antimoniate plus paromomycin followed by maintenance therapy with itraconazole plus miltefosine given 1 month on and 2 months off until CD4 cell count was 350 cells/mm^3 for 3–6 months (Barragan et al. 2010). A case reported in Italy described a co-infected patient who received treatment with liposomal amphotericin B and the growth factor of rHuGM-CSF colonies (Mastroianni 2004). A German HIV-positive patient who had acquired VL after visiting several southern European countries did not response to liposomal amphotericin B and to miltefosine. He finally responded to a combination therapy with intravenous pentamidine and oral fluconazole for 3 weeks (Rybniker et al. 2010).

8.7.1.2 The Indian Subcontinent and South-East Asian Region

In India, the use of pentavalent antimonials is limited due to the high resistance rates reported, especially in the state of Bihar. VL infection in HIV patients—with lower cure rates and higher relapse rates as compared to immunocompetent patients—could be associated with higher resistance to antimonial drug (Chakravarty and Sundar 2010).

In a retrospective study performed in India, liposomal amphotericin B was given to recently diagnosed VL/HIV co-infected patients with a final cure rate obtained at 1- to 2-year follow-up of 85%, and the tolerance to the drug was excellent (Sinha et al. 2011b).

There are no specific studies performed in the Indian subcontinent for patients with VL and HIV co-infection treated with miltefosine. However 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 and several risk factors for the development of tolerance and resistance to this drug have been identified. Therefore, HIV patients, who show higher relapse rates and more persistent asymptomatic parasitaemia than non-HIV patients (van Griensven et al. 2014), could be a group at a higher risk of developing resistance or tolerance to miltefosine in this area with anthroponotic transmission.

A retrospective study was carried out in India in a clinical cohort of 102 VL/HIV co-infected patients. The treatment administered was liposomal amphotericin B in combination with miltefosine. Cure rates at 6, 12 and 18 months did not reach 30% (Mahajan et al. 2015).

8.7.1.3 East African Region

Studies in East Africa with pentavalent antimonials for VL and HIV co-infected were performed mainly in Ethiopia, reporting heterogeneous cure rates, but most of them did not reach the 50% of patients cured (Ritmeijer et al. 2001, 2006; Hailu et al. 2010b). Moreover toxicity reached 21.1% of patients in some studies which made patients having to discontinue the treatment temporarily or permanently (Diro et al. 2014a).

In another study performed in Ethiopia liposomal amphotericin B was administered to a cohort of HIV-positive and HIV-negative patients reaching a 60% cure rate (Ritmeijer et al. 2011). In a retrospective study carried out in eastern Sudan, liposomal amphotericin B was administered to a cohort of VL patients. Although the cure rate for non-HIV patients was high, mortality in VL/HIV co-infected patients was substantial. The specific cure rate for HIV patients is not specifically reported in the study (Salih et al. 2014).

In Ethiopia, a randomized, open-label clinical trial was performed with oral miltefosine versus pentavalent antimonials 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 pentavalent antimonials (Ritmeijer et al. 2006).

Box 8.6 Therapeutic Regimens for Visceral Leishmaniasis and HIV Co-Infected Patients

- **Mediterranean region**
  - Sodium stibogluconate or meglumine antimoniate: (IM or IV) 20 mg/Sb^{v+}/kg/day for 28 days [B]
  - Amphotericin B: (IV) 0.7 mg/kg/day for 28 days [A]
  - Amphotericin B lipid complex: (IV) total dose 30 mg/kg [B]
- **Indian Subcontinent and Central Asia region**
  - Liposomal Amphotericin B: (IV) total dose 20–30 mg/kg [C]
- **East Africa region**
  - Sodium stibogluconate or meglumine antimoniate: (IM or IV) 20 mg/Sb^{v+}/kg/day for 28 days [B]
  - Miltefosine (orally) 100 mg/day for 28 days [B]

8.7.1.4 Secondary Prophylaxis for VL in HIV Co-Infection (Box 8.7)

In VL/HIV co-infected patients after the patient has finished and response to the initial treatment for VL, there is some times the need to establish a secondary prophylaxis. There is a meta-analysis that included 1017 co-infected patients that reported that secondary prophylaxis reduces significantly the relapse rate of VL (OR 0.228). However, there is scarce information that can determine which is the best drug, the dose to be given and which is the most effective regimen (Cota et al. 2011).

The only randomized clinical trial performed took place in Spain, and maintenance therapy with amphotericin B lipid complex was compared with no maintenance therapy. Results demonstrated how maintenance therapy reduced the relapse rates from 22 to 50% (Lopez-Velez et al. 2004). Another prospective study evaluated the effectiveness of maintenance therapy with liposomal amphotericin B and reported up to 80% of patients free of diseases after 12 months follow-up (Molina et al. 2007). In another study maintenance therapy with pentavalent antimonials were evaluated, and the relapse rate reduced significantly more than in those patients who either did not receive any treatment or who received allopurinol as secondary prophylaxis (Ribera et al. 1996). Pentamidine was also evaluated, and

there were no relapses during the follow-up period (Perez-Molina et al. 1996). Miltefosine was evaluated in Portugal as a maintenance therapy in three patients remaining free of relapse for a median period of 20 months (Marques et al. 2008).

Another oral drug such as azoles has been found effective but based only on a series of cases where itraconazole or a combination of itraconazole or fluconazole with allopurinol were evaluated (Lafeuillade et al. 1992; Raffi et al. 1995). The advantage of these drugs is their good tolerance and low toxicity, although there is a risk of developing resistant fungal infections (Angarano et al. 1998; Torrus et al. 1996).

There is not clear data about until when maintenance therapy should be kept. According to different authors, once the patients have recovered their immune function with ART and the VL is quiescent, suspension of the prophylaxis could then be considered when the CD4+ count is maintained >200 cells/μL for more than 6 months (Berenguer et al. 2000; Soriano et al. 2000).

Box 8.7 Therapeutic Regimens of Secondary Prophylaxis for Leishmaniasis and HIV Co-Infected Patients

- **Mediterranean region**
  - Amphotericin B lipid complex (IV) 3–5 mg/kg/day every 3 weeks [A]
  - Meglumine antimoniate (IM or IV) 850 mg Sb^{v+} every 4 weeks [B]
  - Pentamidine isethionate (IV) 4 mg/kg/day every 2–4 weeks [C]

8.7.1.5 Follow-Up and Detection of Relapse of VL and HIV Co-Infection

There are several factors that have been identified as possible risk factors for VL relapse among HIV patients: (a) CD 4 cell count <100 cells/mm^3 when VL is diagnosed, (b) a low scarce increase in the CD 4 cell count in response to ART and (c) absence of secondary prophylaxis and history of previous episodes of relapse (Cota et al. 2011). Relapse may occur even among those patients who have been treated correctly and are receiving ART and even with secondary prophylaxis, so probably these measures only partially protect the patients (Cota et al. 2011). Hence these patients have to be monitored, indefinitely identifying clinical data that can suggest a relapse which should be parasitologically confirmed. It has been reported that the evidence of only a positive nonquantitative polymerase chain reaction (PCR) for *Leishmania* is not enough to determinate a VL relapse. However, the use of an ultrasensitive quantitative *Leishmania* PCR to monitor the parasite load seems useful to predict the risk of relapse in VL/HIV co-infected patients (Molina et al. 2013).

8.7.2 Visceral Leishmaniasis and Pregnancy

There is little experience on the treatment of VL in pregnancy, and most of the published information is based on clinical cases, most of them from East Africa (Mueller et al. 2006; Adam et al. 2009).

Undoubtedly not treating pregnant women with VL can pose a risk for the health of the mother and the foetus much greater than the possible toxicity of the treatment. Fatal outcomes of VL during pregnancy have been described such as spontaneous abortion, small-for-birth date and congenital leishmaniasis (Nyakundi et al. 1988; Eltoum et al. 1992).

Among the different therapeutic options, amphotericin B and its lipid formulations seem to be the most indicated. No congenital transmission and no spontaneous abortion have been described during amphotericin B treatment regimens on pregnant women (Thakur et al. 1993b; Dereure et al. 2003; Mueller et al. 2006). Pentavalent antimonials do not seem to be safe during pregnancy due to its potential teratogenic effect (Paumgartten and Chahoud 2001). Moreover, although pentavalent antimonials have been described as efficient for VL in pregnant women and able to avoid vertical transmission, relapse and therapeutic failures of VL have also been described (Utili et al. 1995). Paromomycin is an aminoglycoside able to cross the placental barrier and can accrue in the foetus plasma and amniotic fluid. There are no data about its use for VL in pregnant women, but as other aminoglycosides, its use could cause ototoxicity to the foetus, so it should not be administrated during pregnancy (Davidson et al. 2009). Pentamidine is also contraindicated during pregnancy as well as miltefosine because they are both potentially embryotoxic and teratogenic. In fact, women in child-bearing age should be tested for pregnancy before administrating any of these teratogenic drugs, and in the case of miltefosine, contraception should be administrated during and for 3 months after treatment (Monge-Maillo and Lopez-Velez 2015).

References

Adam GK, Abdulla MA, Ahmed AA, Adam I. Maternal and perinatal outcomes of visceral leishmaniasis (kala-azar) treated with sodium stibogluconate in eastern Sudan. Int J Gynaecol Obstet. 2009;107(3):208–10.

Alvar J, Canavate C, Gutierrez-Solar B, Jimenez M, Laguna F, Lopez-Velez R, et al. Leishmania and human immunodeficiency virus coinfection: the first 10 years. Clin Microbiol Rev. 1997;10(2):298–319.

Alvar J, Croft S, Olliaro P. Chemotherapy in the treatment and control of leishmaniasis. Adv Parasitol. 2006;61:223–74.

Alvar J, Aparicio P, Aseffa A, Den Boer M, Canavate C, Dedet JP, et al. The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev. 2008;21(2):334–59.

Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671.

Anabwani GM, Ngira JA, Dimiti G, Bryceson AD. Comparison of two dosage schedules of sodium stibogluconate in the treatment of visceral leishmaniasis in Kenya. Lancet. 1983;1(8318):210–3.

Angarano G, Maggi P, Coppola SL, Cavaliere RL. Itraconazole as maintenance therapy for visceral leishmaniasis in HIV-infected patients. Eur J Clin Microbiol Infect Dis. 1998;17(5):365–7.

Barragan P, Lopez-Velez R, Olmo M, Podzamczer D. Visceral Leishmaniasis treated with antimonials/paromomycin followed by itraconazole/miltefosine after standard therapy failures in a human immunodeficiency virus-infected patient. Am J Trop Med Hyg. 2010;83(1):10–2.

Berenguer J, Cosin J, Miralles P, Lopez JC, Padilla B. Discontinuation of secondary anti-leishmania prophylaxis in HIV-infected patients who have responded to highly active antiretroviral therapy. AIDS. 2000;14(18):2946–8.

Berman JD, Badaro R, Thakur CP, Wasunna KM, Behbehani K, Davidson R, et al. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. Bull World Health Organ. 1998;76(1):25–32.

Bhattacharya SK, Jha TK, Sundar S, Thakur CP, Engel J, Sindermann H, et al. Efficacy and tolerability of miltefosine for childhood visceral leishmaniasis in India. Clin Infect Dis. 2004;38(2):217–21.

Bhattacharya SK, Sinha PK, Sundar S, Thakur CP, Jha TK, Pandey K, et al. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. J Infect Dis. 2007;196(4):591–8.

Cascio A, di Martino L, Occorsio P, Giacchino R, Catania S, Gigliotti AR, et al. A 6 day course of liposomal amphotericin B in the treatment of infantile visceral leishmaniasis: the Italian experience. J Antimicrob Chemother. 2004;54(1):217–20.

Chakravarty J, Sundar S. Drug resistance in leishmaniasis. J Glob Infect Dis. 2010;2(2):167–76.

Chunge CN, Owate J, Pamba HO, Donno L. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. Trans R Soc Trop Med Hyg. 1990;84(2):221–5.

Collin S, Davidson R, Ritmeijer K, Keus K, Melaku Y, Kipngetich S, et al. Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. Clin Infect Dis. 2004;38(5):612–9.

Cota GF, de Sousa MR, Rabello A. Predictors of visceral leishmaniasis relapse in HIV-infected patients: a systematic review. PLoS Negl Trop Dis. 2011;5(6):e1153.

Cota GF, de Sousa MR, Fereguetti TO, Rabello A. Efficacy of anti-leishmania therapy in visceral leishmaniasis among HIV infected patients: a systematic review with indirect comparison. PLoS Negl Trop Dis. 2013;7(5):e2195.

Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev. 2006;19(1):111–26.

Das VN, Ranjan A, Sinha AN, Verma N, Lal CS, Gupta AK, et al. A randomized clinical trial of low dosage combination of pentamidine and allopurinol in the treatment of antimony unresponsive cases of visceral leishmaniasis. J Assoc Physicians India. 2001;49:609–13.

Das VN, Siddiqui NA, Pandey K, Singh VP, Topno RK, Singh D, et al. A controlled, randomized nonblinded clinical trial to assess the efficacy of amphotericin B deoxycholate as compared to pentamidine for the treatment of antimony unresponsive visceral leishmaniasis cases in Bihar, India. Ther Clin Risk Manag. 2009;5(1):117–24.

Davidson RN, den Boer M, Ritmeijer K. Paromomycin. Trans R Soc Trop Med Hyg. 2009;103(7):653–60.

Dereure J, Duong Thanh H, Lavabre-Bertrand T, Cartron G, Bastides F, Richard-Lenoble D, et al. Visceral leishmaniasis. Persistence of parasites in lymph nodes after clinical cure. J Infect. 2003;47(1):77–81.

di Martino L, Gramiccia M, Occorsio P, Di Muccio T, Scalone A, Gradoni L. Infantile visceral leishmaniasis in the Campania region, Italy: experience from a Paediatric Referral Centre. Parassitologia. 2004;46(1–2):221–3.

Diro E, Lynen L, Mohammed R, Boelaert M, Hailu A, van Griensven J. High parasitological failure rate of visceral leishmaniasis to sodium stibogluconate among HIV co-infected adults in Ethiopia. PLoS Negl Trop Dis. 2014a;8(5):e2875.

Diro E, Lynen L, Ritmeijer K, Boelaert M, Hailu A, van Griensven J. Visceral Leishmaniasis and HIV coinfection in East Africa. PLoS Negl Trop Dis. 2014b;8(6):e2869.

Dorlo TP, Rijal S, Ostyn B, de Vries PJ, Singh R, Bhattarai N, et al. Failure of miltefosine in visceral leishmaniasis is associated with low drug exposure. J Infect Dis. 2014;210(1):146–53.

Edwards T, Omollo R, Khalil EA, Yifru S, Musa B, Musa A, et al. Single-dose liposomal amphotericin B (AmBisome(R)) for the treatment of visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. Trials. 2011;12:66.

Eltoum IA, Zijlstra EE, Ali MS, Ghalib HW, Satti MM, Eltoum B, et al. Congenital kala-azar and leishmaniasis in the placenta. Am J Trop Med Hyg. 1992;46(1):57–62.

Figueras Nadal MC, Garcia de Miguel MJ, Asensi Botet F, Velasco Bernardo R, Canals Baeza A, Ausin Aoiz I. Short course treatment for visceral leishmaniasis with liposomal amphotericin B in immunocompetent patients. An Pediatr (Barc). 2003;59(6):535–40.

Gradoni L, Bryceson A, Desjeux P. Treatment of Mediterranean visceral leishmaniasis. Bull World Health Organ. 1995;73(2):191–7.

Gradoni L, Soteriadou K, Louzir H, Dakkak A, Toz SO, Jaffe C, et al. Drug regimens for visceral leishmaniasis in Mediterranean countries. Trop Med Int Health. 2008;13(10):1272–6.

Hailu A, Musa A, Wasunna M, Balasegaram M, Yifru S, Mengistu G, et al. Geographical variation in the response of visceral leishmaniasis to paromomycin in East Africa: a multicentre, open-label, randomized trial. PLoS Negl Trop Dis. 2010a;4(10):e709.

Hailu W, Weldegebreal T, Hurissa Z, Tafes H, Omollo R, Yifru S, et al. Safety and effectiveness of meglumine antimoniate in the treatment of Ethiopian visceral leishmaniasis patients with and without HIV co-infection. Trans R Soc Trop Med Hyg. 2010b;104(11):706–12.

Jeronimo SM, Duggal P, Braz RF, Cheng C, Monteiro GR, Nascimento ET, et al. An emerging peri-urban pattern of infection with Leishmania chagasi, the protozoan causing visceral leishmaniasis in northeast Brazil. Scand J Infect Dis. 2004;36(6–7):443–9.

Jha TK, Giri YN, Singh TK, Jha S. Use of amphotericin B in drug-resistant cases of visceral leishmaniasis in north Bihar, India. Am J Trop Med Hyg. 1995;52(6):536–8.

Jha TK, Olliaro P, Thakur CP, Kanyok TP, Singhania BL, Singh IJ, et al. Randomised controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. BMJ. 1998;316(7139):1200–5.

Kafetzis DA, Velissariou IM, Stabouli S, Mavrikou M, Delis D, Liapi G. Treatment of paediatric visceral leishmaniasis: amphotericin B or pentavalent antimony compounds? Int J Antimicrob Agents. 2005;25(1):26–30.

Kager PA, Rees PH, Manguyu FM, Bhatt KM, Wellde BT, Hockmeyer WT, et al. Clinical, haematological and parasitological response to treatment of visceral leishmaniasis in Kenya. A study of 64 patients. Trop Geogr Med. 1984;36(1):21–35.

Khalil EA, el Hassan AM, Zijlstra EE, Hashim FA, Ibrahim ME, Ghalib HW, et al. Treatment of visceral leishmaniasis with sodium stibogluconate in Sudan: management of those who do not respond. Ann Trop Med Parasitol. 1998;92(2):151–8.

Khalil EA, Weldegebreal T, Younis BM, Omollo R, Musa AM, Hailu W, et al. Safety and efficacy of single dose versus multiple doses of AmBisome for treatment of visceral leishmaniasis in eastern Africa: a randomised trial. PLoS Negl Trop Dis. 2014;8(1):e2613.

Lafeuillade A, Chaffanjon P, Delbeke E, Quilichini R. Maintenance itraconazole for visceral leishmaniasis in HIV infection. Am J Med. 1992;92(4):449.

Laguna F, Lopez-Velez R, Soriano V, Montilla P, Alvar J, Gonzalez-Lahoz JM. Assessment of allopurinol plus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV. J Infect. 1994;28(3):255–9.

Laguna F, Lopez-Velez R, Pulido F, Salas A, Torre-Cisneros J, Torres E, et al. Treatment of visceral leishmaniasis in HIV-infected patients: a randomized trial comparing meglumine antimoniate with amphotericin B. Spanish HIV-Leishmania Study Group. AIDS. 1999;13(9):1063–9.

Laguna F, Videla S, Jimenez-Mejias ME, Sirera G, Torre-Cisneros J, Ribera E, et al. Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study. J Antimicrob Chemother. 2003;52(3):464–8.

Lopez-Velez R, Videla S, Marquez M, Boix V, Jimenez-Mejias ME, Gorgolas M, et al. Amphotericin B lipid complex versus no treatment in the secondary prophylaxis of visceral leishmaniasis in HIV-infected patients. J Antimicrob Chemother. 2004;53(3):540–3.

Mahajan R, Das P, Isaakidis P, Sunyoto T, Sagili KD, Lima MA, et al. Combination treatment for visceral Leishmaniasis patients co-infected with human immunodeficiency virus in India. Clin Infect Dis. 2015;61(8):1255–62.

Marques N, Sa R, Coelho F, Oliveira J, Saraiva Da Cunha J, Melico-Silvestre A. Miltefosine for visceral leishmaniasis relapse treatment and secondary prophylaxis in HIV-infected patients. Scand J Infect Dis. 2008;40(6–7):523–6.

Maru M. Clinical and laboratory features and treatment of visceral leishmaniasis in hospitalized patients in Northwestern Ethiopia. Am J Trop Med Hyg. 1979;28(1):15–8.

Mary C, Faraut F, Drogoul MP, Xeridat B, Schleinitz N, Cuisenier B, et al. Reference values for Leishmania infantum parasitemia in different clinical presentations: quantitative poly-

merase chain reaction for therapeutic monitoring and patient follow-up. Am J Trop Med Hyg. 2006;75(5):858–63.
Mastroianni A. Liposomal amphotericin B and rHuGM-CSF for treatment of visceral leishmaniasis in AIDS. Infez Med. 2004;12(3):197–204.
Melaku Y, Collin SM, Keus K, Gatluak F, Ritmeijer K, Davidson RN. Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. Am J Trop Med Hyg. 2007;77(1):89–94.
Minodier P, Garnier JM. Childhood visceral leishmaniasis in Provence. Arch Pediatr. 2000;7(Suppl 3):572s–7s.
Minodier P, Robert S, Noel G, Blanc P, Retornaz K, Garnier JM. First-line liposomal amphotericin B for pediatric visceral leishmaniasis in southern France. Arch Pediatr. 2005;12(7):1102–8.
Mishra M, Biswas UK, Jha DN, Khan AB. Amphotericin versus pentamidine in antimony-unresponsive kala-azar. Lancet. 1992;340(8830):1256–7.
Molina I, Falco V, Crespo M, Riera C, Ribera E, Curran A, et al. Efficacy of liposomal amphotericin B for secondary prophylaxis of visceral leishmaniasis in HIV-infected patients. J Antimicrob Chemother. 2007;60(4):837–42.
Molina I, Fisa R, Riera C, Falco V, Elizalde A, Salvador F, et al. Ultrasensitive real-time PCR for the clinical management of visceral leishmaniasis in HIV-infected patients. Am J Trop Med Hyg. 2013;89(1):105–10.
Monge-Maillo B, Lopez-Velez R. Therapeutic options for visceral Leishmaniasis. Drugs. 2013;73(17):1863–88.
Monge-Maillo B, Lopez-Velez R. Miltefosine for visceral and cutaneous leishmaniasis: drug characteristics and evidence-based treatment recommendations. Clin Infect Dis. 2015;60(9):1398–404.
Moore E, O'Flaherty D, Heuvelmans H, Seaman J, Veeken H, de Wit S, et al. Comparison of generic and proprietary sodium stibogluconate for the treatment of visceral leishmaniasis in Kenya. Bull World Health Organ. 2001;79(5):388–93.
Mueller M, Balasegaram M, Koummuki Y, Ritmeijer K, Santana MR, Davidson R. A comparison of liposomal amphotericin B with sodium stibogluconate for the treatment of visceral leishmaniasis in pregnancy in Sudan. J Antimicrob Chemother. 2006;58(4):811–5.
Mueller Y, Nguimfack A, Cavailler P, Couffignal S, Rwakimari JB, Loutan L, et al. Safety and effectiveness of amphotericin B deoxycholate for the treatment of visceral leishmaniasis in Uganda. Ann Trop Med Parasitol. 2008;102(1):11–9.
Mueller Y, Mbulamberi DB, Odermatt P, Hoffmann A, Loutan L, Chappuis F. Risk factors for in-hospital mortality of visceral leishmaniasis patients in eastern Uganda. Trop Med Int Health. 2009;14(8):910–7.
Musa AM, Younis B, Fadlalla A, Royce C, Balasegaram M, Wasunna M, et al. Paromomycin for the treatment of visceral leishmaniasis in Sudan: a randomized, open-label, dose-finding study. PLoS Negl Trop Dis. 2010;4(10):e855.
Musa A, Khalil E, Hailu A, Olobo J, Balasegaram M, Omollo R, et al. Sodium stibogluconate (SSG) & paromomycin combination compared to SSG for visceral leishmaniasis in East Africa: a randomised controlled trial. PLoS Negl Trop Dis. 2012;6(6):e1674.
Nyakundi PM, Muigai R, Were JB, Oster CN, Gachihi GS, Kirigi G. Congenital visceral leishmaniasis: case report. Trans R Soc Trop Med Hyg. 1988;82(4):564.
Organización Panamericana de la Salud. Guía para el tratamiento de las enfermedades infecciosas. Washington: OPS; 2004. http://www.ops-oms.org/common/Display.asp?Lang=S&RecID=9629.
Paumgartten FJ, Chahoud I. Embryotoxicity of meglumine antimoniate in the rat. Reprod Toxicol. 2001;15(3):327–31.
Perez-Molina JA, Lopez-Velez R, Montilla P, Guerrero A. Pentamidine isethionate as secondary prophylaxis against visceral leishmaniasis in HIV-positive patients. AIDS. 1996;10(2):237–8.
Petrela R, Kuneshka L, Foto E, Zavalani F, Gradoni L. Pediatric visceral leishmaniasis in Albania: a retrospective analysis of 1,210 consecutive hospitalized patients (1995–2009). PLoS Negl Trop Dis. 2010;4(9):e814.

Pintado V, Lopez-Velez R. HIV-associated visceral leishmaniasis. Clin Microbiol Infect. 2001;7(6):291–300.

Raffi F, Merrien D, Le Pape P, Reliquet V. Use of an Itraconazole/allopurinol combination for the treatment of visceral leishmaniasis in a patient with AIDS. Clin Infect Dis. 1995;21(5):1338–9.

Rahman M, Ahmed BN, Faiz MA, Chowdhury MZ, Islam QT, Sayeedur R, et al. Phase IV trial of miltefosine in adults and children for treatment of visceral leishmaniasis (kala-azar) in Bangladesh. Am J Trop Med Hyg. 2011;85(1):66–9.

Ribera E, Ocana I, de Otero J, Cortes E, Gasser I, Pahissa A. Prophylaxis of visceral leishmaniasis in human immunodeficiency virus-infected patients. Am J Med. 1996;100(5):496–501.

Rijal S, Ostyn B, Uranw S, Rai K, Bhattarai NR, Dorlo TP, et al. Increasing failure of miltefosine in the treatment of kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or noncompliance. Clin Infect Dis. 2013;56(11):1530–8.

Ritmeijer K, Veeken H, Melaku Y, Leal G, Amsalu R, Seaman J, et al. Ethiopian visceral leishmaniasis: generic and proprietary sodium stibogluconate are equivalent; HIV co-infected patients have a poor outcome. Trans R Soc Trop Med Hyg. 2001;95(6):668–72.

Ritmeijer K, Dejenie A, Assefa Y, Hundie TB, Mesure J, Boots G, et al. A comparison of miltefosine and sodium stibogluconate for treatment of visceral leishmaniasis in an Ethiopian population with high prevalence of HIV infection. Clin Infect Dis. 2006;43(3):357–64.

Ritmeijer K, ter Horst R, Chane S, Aderie EM, Piening T, Collin SM, et al. Limited effectiveness of high-dose liposomal amphotericin B (AmBisome) for treatment of visceral leishmaniasis in an Ethiopian population with high HIV prevalence. Clin Infect Dis. 2011;53(12):e152–8.

Rosenthal E, Delaunay P, Jeandel PY, Haas H, Pomares-Estran C, Marty P. Liposomal amphotericin B as treatment for visceral leishmaniasis in Europe, 2009. Med Mal Infect. 2009;39(10):741–4.

Russo R, Nigro LC, Minniti S, Montineri A, Gradoni L, Caldeira L, et al. Visceral leishmaniasis in HIV infected patients: treatment with high dose liposomal amphotericin B (AmBisome). J Infect. 1996;32(2):133–7.

Rybniker J, Goede V, Mertens J, Ortmann M, Kulas W, Kochanek M, et al. Treatment of visceral leishmaniasis with intravenous pentamidine and oral fluconazole in an HIV-positive patient with chronic renal failure—a case report and brief review of the literature. Int J Infect Dis. 2010;14(6):e522–5.

Salam N, Al-Shaqha WM, Azzi A. Leishmaniasis in the middle East: incidence and epidemiology. PLoS Negl Trop Dis. 2014;8(10):e3208.

Salih NA, van Griensven J, Chappuis F, Antierens A, Mumina A, Hammam O, et al. Liposomal amphotericin B for complicated visceral leishmaniasis (kala-azar) in eastern Sudan: how effective is treatment for this neglected disease? Trop Med Int Health. 2014;19(2):146–52.

Santos MA, Marques RC, Farias CA, Vasconcelos DM, Stewart JM, Costa DL, et al. Predictors of an unsatisfactory response to pentavalent antimony in the treatment of American visceral leishmaniasis. Rev Soc Bras Med Trop. 2002;35(6):629–33.

Seaman J, Pryce D, Sondorp HE, Moody A, Bryceson AD, Davidson RN. Epidemic visceral leishmaniasis in Sudan: a randomized trial of aminosidine plus sodium stibogluconate versus sodium stibogluconate alone. J Infect Dis. 1993;168(3):715–20.

Seaman J, Boer C, Wilkinson R, de Jong J, de Wilde E, Sondorp E, et al. Liposomal amphotericin B (AmBisome) in the treatment of complicated kala-azar under field conditions. Clin Infect Dis. 1995;21(1):188–93.

Sindermann H, Engel KR, Fischer C, Bommer W, Miltefosine Compassionate Use Program. Oral miltefosine for leishmaniasis in immunocompromised patients: compassionate use in 39 patients with HIV infection. Clin Infect Dis. 2004;39(10):1520–3.

Singh S. Changing trends in the epidemiology, clinical presentation, and diagnosis of Leishmania-HIV co-infection in India. Int J Infect Dis. 2014;29C:103–12.

Sinha PK, Jha TK, Thakur CP, Nath D, Mukherjee S, Aditya AK, et al. Phase 4 pharmacovigilance trial of paromomycin injection for the treatment of visceral leishmaniasis in India. J Trop Med. 2011a;2011:645203.

Sinha PK, van Griensven J, Pandey K, Kumar N, Verma N, Mahajan R, et al. Liposomal amphotericin B for visceral leishmaniasis in human immunodeficiency virus-coinfected patients: 2-year treatment outcomes in Bihar, India. Clin Infect Dis. 2011b;53(7):e91–8.

Soriano VF, Dona CF, Rodriguez-Rosado RF, Barreiro PF, Gonzalez-Lahoz J. Discontinuation of secondary prophylaxis for opportunistic infections in HIV-infected patients receiving highly active antiretroviral therapy. AIDS. 2000;14(4):383–6.

Sundar S, Rai M. Laboratory diagnosis of visceral leishmaniasis. Clin Diagn Lab Immunol. 2002a;9(5):951–8.

Sundar S, Rai M. Advances in the treatment of leishmaniasis. Curr Opin Infect Dis. 2002b;15(6):593–8.

Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, et al. Oral miltefosine for Indian visceral leishmaniasis. N Engl J Med. 2002;347(22):1739–46.

Sundar S, Jha TK, Sindermann H, Junge K, Bachmann P, Berman J. Oral miltefosine treatment in children with mild to moderate Indian visceral leishmaniasis. Pediatr Infect Dis J. 2003;22(5):434–8.

Sundar S, Mehta H, Suresh AV, Singh SP, Rai M, Murray HW. Amphotericin B treatment for Indian visceral leishmaniasis: conventional versus lipid formulations. Clin Infect Dis. 2004;38(3):377–83.

Sundar S, Jha TK, Thakur CP, Bhattacharya SK, Rai M. Oral miltefosine for the treatment of Indian visceral leishmaniasis. Trans R Soc Trop Med Hyg. 2006;100(Suppl 1):S26–33.

Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. N Engl J Med. 2007;356(25):2571–81.

Sundar S, Rai M, Chakravarty J, Agarwal D, Agrawal N, Vaillant M, et al. New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. Clin Infect Dis. 2008;47(8):1000–6.

Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. N Engl J Med. 2010;362(6):504–12.

Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, et al. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet. 2011;377(9764):477–86.

Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clin Infect Dis. 2012;55(4):543–50.

Thakur CP. Epidemiological, clinical and therapeutic features of Bihar kala-azar (including post kala-azar dermal leishmaniasis). Trans R Soc Trop Med Hyg. 1984;78(3):391–8.

Thakur CP. A single high dose treatment of kala-azar with Ambisome (amphotericin B lipid complex): a pilot study. Int J Antimicrob Agents. 2001;17(1):67–70.

Thakur CP, Kumar M, Pandey AK. Comparison of regimes of treatment of antimony-resistant kala-azar patients: a randomized study. Am J Trop Med Hyg. 1991;45(4):435–41.

Thakur CP, Olliaro P, Gothoskar S, Bhowmick S, Choudhury BK, Prasad S, et al. Treatment of visceral leishmaniasis (kala-azar) with aminosidine (= paromomycin)-antimonial combinations, a pilot study in Bihar, India. Trans R Soc Trop Med Hyg. 1992;86(6):615–6.

Thakur CP, Sinha GP, Pandey AK, Barat D, Sinha PK. Amphotericin B in resistant kala-azar in Bihar. Natl Med J India. 1993a;6(2):57–60.

Thakur CP, Sinha GP, Sharma V, Barat D. The treatment of kala-azar during pregnancy. Natl Med J India. 1993b;6(6):263–5.

Thakur CP, Kanyok TP, Pandey AK, Sinha GP, Zaniewski AE, Houlihan HH, et al. A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis. Trans R Soc Trop Med Hyg. 2000;94(4):429–31.

Torrus D, Boix V, Massa B, Portilla J, Perez-Mateo M. Fluconazole plus allopurinol in treatment of visceral leishmaniasis. J Antimicrob Chemother. 1996;37(5):1042–3.

Toumi A, Kilani B, Ammari L, Tiouiri H, Kanoun F, Belhadj S, et al. Demographic, clinical and therapeutic features of adult visceral leishmaniasis at the Rabta hospital in Tunis (Tunisia) from 1983 to 2002. Bull Soc Pathol Exot. 2007;100(4):282–6.

Troya J, Casquero A, Refoyo E, Fernandez-Guerrero ML, Gorgolas M. Long term failure of miltefosine in the treatment of refractory visceral leishmaniasis in AIDS patients. Scand J Infect Dis. 2008;40(1):78–80.

Utili R, Rambaldi A, Tripodi MF, Andreana A. Visceral leishmaniasis during pregnancy treated with meglumine antimoniate. Infection. 1995;23(3):182–3.

van Griensven J, Carrillo E, Lopez-Velez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect. 2014;20(4):286–99.

Veeken H, Ritmeijer K, Seaman J, Davidson R. A randomized comparison of branded sodium stibogluconate and generic sodium stibogluconate for the treatment of visceral leishmaniasis under field conditions in Sudan. Trop Med Int Health. 2000;5(5):312–7.

Velez ID, Colmenares LM, Munoz CA. Two cases of visceral leishmaniasis in Colombia resistant to meglumine antimonial treatment. Rev Inst Med Trop Sao Paulo. 2009;51(4):231–6.

Wasunna M, Njenga S, Balasegaram M, Alexander N, Omollo R, Edwards T, Dorlo TP, Musa B, Ali MH, Elamin MY, Kirigi G, Juma R, Kip AE, Schoone GJ, Hailu A, Olobo J, Ellis S, Kimutai R, Wells S, Khalil EA, Strub Wourgaft N, Alves F, Musa A. Efficacy and safety of AmBisome in combination with sodium stibogluconate or miltefosine and miltefosine monotherapy for African visceral leishmaniasis: phase II randomized trial. PLoS Negl Trop Dis. 2016;10(9):e0004880.

Werneck GL, Batista MS, Gomes JR, Costa DL, Costa CH. Prognostic factors for death from visceral leishmaniasis in Teresina, Brazil. Infection. 2003;31(3):174–7.

World Health Organization (WHO). Control of the leishmaniases: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. Geneva: WHO; 2010. WHO technical report series; no. 949. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf.