Visceral Leishmaniasis

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KEYWORDS

• Visceral leishmaniasis • Neglected disease • Chronic fever
• Human immunodeficiency virus

KEY POINTS

- Visceral leishmaniasis is a neglected but typically fatal vector-borne protozoan disease reported from all continents except Antarctica and Australia.
- The parasite targets the reticuloendothelial system, with infiltration of the spleen, liver, bone marrow and lymph nodes causing organomegaly and pancytopenia.
- Parasitologic diagnosis relies on invasive procedures like spleen or bone marrow aspirate, but most cases can be detected using molecular or serological testing.
- The commonly used drugs for the treatment of visceral leishmaniasis include antimonials, conventional and liposomal amphotericin B and miltefosine.
- Besides the emergence of drug resistance, the increase in VL-HIV coinfection in disease-endemic countries poses an important challenge.

Visceral leishmaniasis (VL), also known as kala-azar, is a disseminated protozoan infection caused by *Leishmania donovani* complex.[1,2] VL is essentially caused by *L donovani* and *Leishmania infantum* (synonym *Leishmania chagasi* in South-America). Exceptionally, visceralization of species typically associated with cutaneous leishmaniasis has been observed. Most commonly, this has been reported with *Leishmania tropicalis* in the Middle East and *Leishmania amazonensis* in South-America. In individuals infected with human immunodeficiency virus (HIV), visceralization of a number of dermatotropic species has been documented as well (see section on VL-HIV coinfection).[3]

EPIDEMIOLOGY AND TRANSMISSION

VL is transmitted through the bite of female hematophageous sand flies from the genus *phlebotomus* in the old world and *Lutzomiya* in the new world. At the global

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level, there are over 10 species of sand flies playing a role in the transmission of VL, with at least 1 species involved per geographic region.[1,2] The adult fly, about 2 to 4 mm long, is most active during dusk and night time. Resting sites are dark, moist places including soil cracks, termite hills, and other shady places. Whereas transmission is predominantly peri-domestic in the Indian subcontinent, it mostly occurs outside villages in East Africa. Rare modes of transmission for VL include intravenous drug use, blood transfusion, organ transplantation, congenital transmission, and laboratory accidents.[4]

Depending on the transmission characteristics, 2 types of VL exist (**Fig. 1**). The zoonotic form, with dogs as main reservoir, occurs in the Mediterranean basin, China, the Middle East, and South America. This form is caused by *L infantum.* At the global level, the antroponotic form, with human-to-human transmission without animal reservoir, is clearly more common. This form is caused by *L donovani* and is prevalent in East Africa, Bangladesh, India, and Nepal.[1,4] Whereas *L infantum* predominantly affects children and immunocompromised individuals, *L donovani* tends to affect all age groups.

VL is reported in over 70 countries from 5 continents, with the exception of Australia and Antarctica, with 200 million people at risk. Overall, it is estimated that around 500,000 new cases occur annually, with and estimated 50,000 deaths, although the real number is probably much higher.[1,5] Ninety percent of all cases occur in 5 countries: India, Bangladesh, Nepal, Sudan, and Brazil (see **Fig. 1**). With an estimated 300,000 cases per year, India carries the largest VL burden.

VL typically affects poor communities in remote, rural areas, although peri-urbanization has been reported in some countries like Brazil.[1,5] Outbreaks occur during massive migration or resettlement of susceptible hosts into endemic areas, or disturbance to the habitat of the sand fly like deforestation for expansion of agricultural sites. The increase in immunosuppressed individuals, related to the HIV epidemic, has additionally contributed to increased case loads in certain areas, predominantly East Africa.

PATHOGENESIS

The parasite exists in 2 distinct forms: a promastigote form found in the vector, and an amastigote form, which develops intracellularly in the susceptible mammalian host.

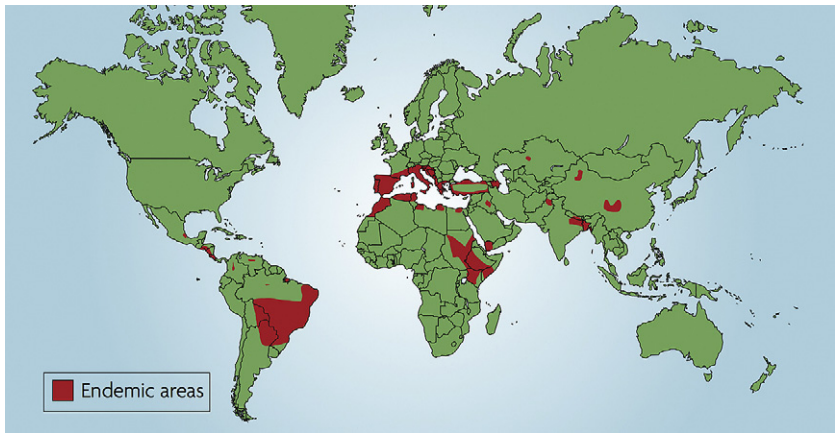

Fig. 1. Geographic distribution of visceral leishmaniasis. (*Reprinted from* Desjeux P. Leishmaniasis. Nat Rev Microbiol 2004;2:692; with permission.)

Infection occurs after inoculation of promastigotes into the skin following the bite of an infected sand fly. Promastigotes are taken up by macrophages, where they develop into amastigotes and multiply within phagolysosomes (**Fig. 2**). Subsequently, the parasites can disseminate and infect cells of the reticuloendothelial system in various tissues, predominantly infiltrating the spleen, bone marrow, liver, and lymph nodes.[1,2,5]

However, infection does not progress to overt disease in the majority of individuals, and in some highly endemic areas, up to 30% of habitants demonstrate evidence of asymptomatic infection. Asymptomatic infection can be detected early on by serologic tests. The subsequent development of cell-mediated immunity can be revealed by leishmanin skin testing (similar as the purified protein derivative [PPD] skin test for tuberculosis).[1,2,5] Oligosymptomatic, self-limiting forms have been described in South America. Most likely, viable parasites persist after primary infection, leading to reactivation and disease in case of immunosuppression like HIV infection and malnutrition.[3]

Although the determinants of progression to disease after primary infection are only partly understood, parasitic virulence, nutritional status, age, and host genetic factors are thought to contribute. Control of infection relies on activated, leishmanicidal macrophages and an intact T-helper cell type 1 (Th1) response. Overt disease is associated with a mixed Th1/Th2 response. High levels of regulatory T cells are thought to contribute to the pronounced immunosuppression seen during VL.[1,2] Upon successful treatment, increased production of Th1 cytokines and decreased interleukin (IL)-10

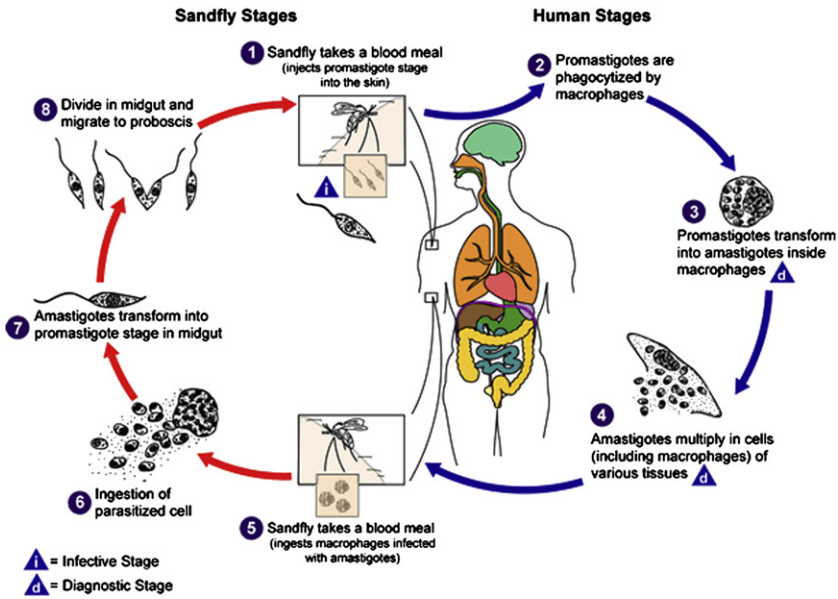

Fig. 2. Life cycle of the leishmania parasite. *Leishmania donovani/infantum* exists in 2 forms: promastigotes in the sandfly, amastigotes localized in macrophages in the mammalian host. After inoculation in the skin following a bite of an infected sandfly, a systemic infection can occur mainly targeting the reticulo-endothelial system in bone marrow, spleen, liver, and lymph nodes. (*From* Centers for Disease Control and Prevention DPDx. Leishmaniasis. Available at: http://www.dpd.cdc.gov/dpdx/HTML/Leishmaniasis.htm.)

levels are seen. Current evidence suggests that after cure of VL, apparent immunity is established.

CLINICAL PRESENTATION

The incubation period for VL typically ranges from 2 to 6 months, but can vary from weeks to several years. Patients present with insidious onset fever, weight loss, and organomegaly that persists for months. Splenomegaly is prominent (**Fig. 3**). It is often soft on palpation and can complicate with infarction or spontaneous subcapsular bleeding. Hepatomegaly is less marked.[2,4] Lymphadenopathy is usually observed in Sudan but is rare in other endemic regions. Darkening of the skin is mainly described in South Asia, where it got the name kala-azar (meaning black fever in Hindi). Anemia, thrombocytopenia, and neutropenia are usually seen, reflecting bone marrow suppression and splenic sequestration. Hyperglobulinemia is common. Mild-to-moderate increases in liver enzymes can be documented as well. The patients may become cachexic and edematous from hypoalbuminemia or congestive heart failure due to anemia. Epistaxis, gum bleeding, petechial, and bleeding from other sites can occur. Hepatic dysfunction, jaundice, and ascitis can occur in advanced disease.[2,4] Patients are at high risk for additional infections like otitis media, gastrointestinal (GI) infections, and pneumonia and complicate easily with sepsis. A raised white blood cell count in the peripheral blood should trigger investigations for concomitant infections. Atypical localizations, like the GI and respiratory tract, have been documented, but seem to be especially more common in VL-HIV coinfection.[3] Without treatment, VL is almost universally fatal.

Post-kala-azar dermal leishmaniasis (PKDL) is a chronic skin rash that appears after effective treatment of VL due to *L donovani* (**Fig. 4**).[6] It is considered a sign of immune reconstitution against the parasite, with recovery of the cell-mediated immunity. PKDL is very common (50%–60%) in Sudan occurring during or within 6 months of VL treatment, while it is less frequent (5%–10%) in India and usually occurs years after treatment. Exceptionally, it can occur in patients without history of VL. It starts with erythematous macules and papules around the perinasal areas and often progresses to plaques and nodules, subsequently spreading to the shoulders, the trunk, and extremities. Since the parasite can be detected in the skin lesions, such patients can potentially have a role in the transmission of the disease, acting as reservoirs. Whereas most forms are self-healing in Sudan, this is not the case in India.[6]

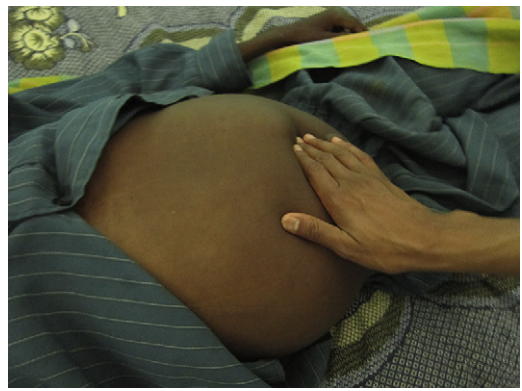

Fig. 3. A patient with visceral leishmaniasis and massive splenomegaly (Gondar, Ethiopia).

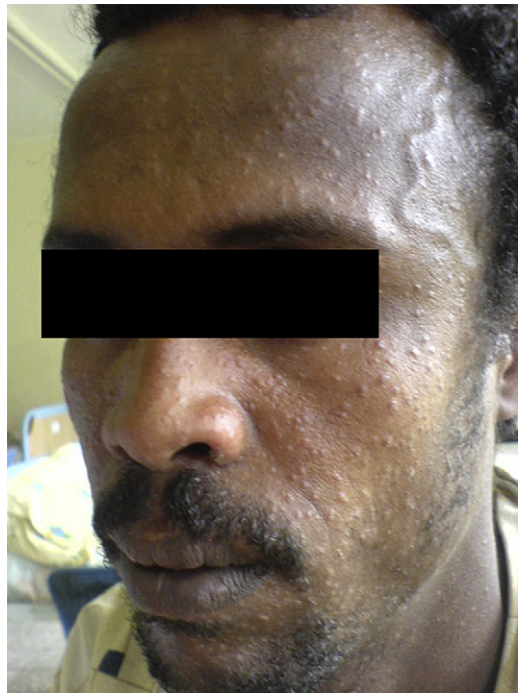

Fig. 4. A patient with post-kala-azar dermal leishmaniasis (PKDL) (Gondar, Ethiopia). PKDL typically starts with erythematous macules and papules around the perinasal areas, which often progresses to plaques and nodules and spreads to the shoulders, the trunk, and extremities.

DIAGNOSIS

Parasitologic Diagnosis

The current gold standard for diagnosis relies on the visualization of the amastigote form of the parasite within macrophages by microscopic examination of tissue aspirates (spleen, bone marrow, or lymph nodes) after Giemsa staining (**Fig. 5**).[1,7] Whereas in Europe bone marrow aspiration is most commonly done, spleen aspiration is predominantly used in Africa and Asia, although the later is associated with a risk of life-threatening bleeding of around 0.1%. Specificity of microscopy is high, but its sensitivity varies between spleen (93%–99%), bone marrow (52%–85%), and lymph node (52%–58%) aspirates. Culture can additionally improve sensitivity, but requires special media (Novy-MacNeal-Nicolle [NNN] media) and is not widely available in most disease-endemic regions. Growth can take up to several weeks. Promising findings have been reported with a microculture method using peripheral blood samples, with high sensitivities and shorter growth duration obtained.[7] Species identification is usually not needed for patient management.

Serologic Diagnosis

Several serologic tests have been developed. High diagnostic accuracy has been reported with indirect fluorescence antibody (IFA) tests, enzyme-linked immunosorbent assays (ELISA), and immunoblotting. Although test performance varies across different studies, more recent studies have reported high sensitivities (96%–100%)

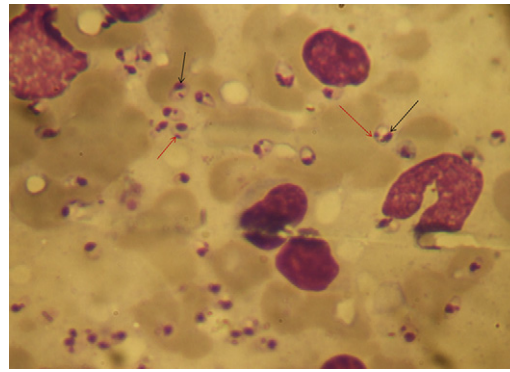

Fig. 5. Leishmania amastigotes (small purple bodies) in spleen tissue from a patient with visceral leishmaniasis (Gondar, Ethiopia). *Red arrows* show the kinetoplast, and the *black arrows* show the marginalized nucleus.

and specificities (96%–98%).[7] However, all suffer from 2 limitations. The fact that antibody response tends to persist (albeit at lower levels) after cure hampers the use of serologic tests to diagnose relapse. Moreover, asymptomatic infections—with positive serologic tests—are common in disease-endemic regions. Since they are technologically demanding, these tests are not routinely available in most disease-endemic areas. Two tests have been specifically developed for field use and have undergone substantial validation.[1,7] The direct agglutination test (DAT) is a semiquantitative test. Agglutination is observed after overnight incubation of dilutions of patient's serum and killed parasites in microtiter plates. Sensitivity has been estimated at 95%, with specificity of 86%. An rK39-based immunochromatographic (ICT) strip test has been developed as well, with sensitivity and specificity estimated at 94% and 95%, respectively, although sensitivity was lower in East Africa.[1] This test currently has an increasing role in VL control programs, since it is easy to perform, cheap (approximately 1$/test), and rapid (10–20 minutes), making it ideal for the remote settings where most VL cases occur.

Antigen Detection Tests

Antigen tests have been explored as well, given their theoretical potential to differentiate active from previous infections and their potential use as noninvasive markers of treatment response (test of cure).[1] The most studied test is the kala-azar latex agglutination test (KAtex), detecting a heat-stable leishmania antigen in the urine of VL patients. Whereas specificity was excellent, sensitivity was low (48%–87%) and variable.[1,7] Although the test correlated well with treatment response,[8] it is currently not sufficiently accurate to serve as a test of cure. Attempts to improve the sensitivity and the format of the test are ongoing.

Molecular Diagnosis

Polymerase chain reaction (PCR)-based assays to detect parasite DNA are being increasingly used in high-income countries, particularly in Europe.[9] High sensitivity and specificity have been reported both on peripheral blood and bone marrow aspirates. PCR on peripheral blood has been recommended as a noninvasive first-line screening test, for both immunocompetent and immunocompromised patients.[10] The different techniques have only poorly been standardized. A point-of-care test

adapted to field conditions is still lacking. The frequent demonstration of PCR-positive tests in asymptomatic infected individuals in disease-endemic regions obviously hampers their clinical use in these settings. Real-time PCR now also allows quantification of parasite burden, which could help in determining active disease and enhance its use as a noninvasive prognostic marker.[11] This has been especially explored in the long-term monitoring of HIV-infected patients, as a way to reduce the need of invasive investigations.[12]

Diagnosis of PKDL relies on microscopic demonstration of parasites in skin specimens (biopsy or slit skin samples).[6] However, sensitivity is low in mild clinical cases. The highest yield can be expected from nodular lesions. Molecular testing of blood and skin samples has been explored in some areas to increase sensitivity.

TREATMENT

Traditionally, treatment of VL has relied on the use of pentavalent antimonials (Sb^{5+}), introduced in the 1940s.[2,13] From the 1980s on, conventional amphotericin B deoxycholate has been increasingly used in high-income countries. Subsequently, different lipid formulations of amphotericin B, most notably liposomal amphotericin B, have been developed, which combine a high efficacy with low toxicity.[14] Liposomal amphotericin B is the only treatment approved by the US Food and Drug Administration (FDA). Several studies have been conducted in low- and middle-income countries with paromomycin, a cheap and effective parenteral drug with an acceptable toxicity profile that can easily be administered in an ambulatory way by intramuscular injection.[15] The development of miltefosine, the first oral drug for VL, has been a major breakthrough.[16,17] This drug is the pillar of the recently launched VL elimination plan in the Indian subcontinent. Both paromomycin and miltefosine are rarely available or used in the United States and Europe. Although other compounds are in the pipeline, these previously mentioned drugs, all belonging to chemically unrelated classes, will probably constitute the main therapeutic options for VL for the next years to come.[13,18] Although the mechanism of action remains poorly defined for some of these, all are thought to have distinct targets. All of these drugs face a number of important disadvantages (**Table 1**).

Clear differences in clinical efficacy of antileishmanials have been observed between different geographic areas, which have to be taken into consideration in treatment decisions.[13,19] In line with this, current World Health Organization (WHO) treatment recommendations differ according to the geographic region (**Box 1**).[20] These differences are thought to be at least partially explained by differences in parasite susceptibility.[13,19]

Individual Drugs

The pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) have been the cornerstone for first-line treatment of VL over the last 70 years. This is now slowly changing due to the availability of alternative treatment options and the emergence of resistance in India over the last 20 years, with treatment failure now observed in up to 60% of cases in certain areas of India.[13,21] Antimonials can be given via intravenous or intramuscular – injections. A major concern is cumulative toxicity, particularly pancreatitis and cardiotoxicity.[22] Pancreatic enzyme elevations are seen frequently, but clinical pancreatitis is uncommon. Whereas mild electrocardiogram (ECG) changes (T-wave flattening or inversion) are seen in around 50% of the patients, serious, but potentially life-threatening, cardiotoxicity is uncommon, occurring in less than 9% of cases.[23] Features of dangerous cardiotoxicity include

Table 1
The main drugs currently used for treatment of visceral leishmaniasis

Drugs	Regimen	Marketing[a]	Clinical Efficacy	Resistance	Toxicity	Cost/Course	Issues
Pentavalent antimonials	20 mg/kg iv or im daily for 28–30 days	Albert David (SSG); GSK (Pentostam) Sanofi Aventis (Glucantime)	35%–95% (depending on geographic area)	As high as 60% (Bihar, India)	Frequent, potentially severe; Cardiac toxicity, Pancreatitis, Nephro + hepatotoxicity	Generic ~ $53 Branded ~ $70	Quality control Length of treatment Painful injection Toxicity Resistance in India
Amphotericin B	0.75–1 mg/kg iv for 15–20 doses (daily or alternate days)	Bristol Meyers Squibb (Fungizone) Generic companies	>97% all regions	Not documented	Frequent Infusion-related reactions, Nephrotoxicity (in-patient care needed)	Generic price: ~ $21	Need for slow iv infusion Dose-limiting nephrotoxicity Heat stability
Liposomal Amphotericin B	10–30 mg/kg Total dose iv; usually 3–5 mg/kg/dose Single dose (10 mg/kg) in India	Gilead (AmBisome)	Europe and Asia: >95%; Africa: not fully established (higher dose required?)	Not documented	Uncommon and mild; Nephrotoxicity (limited)	Preferential price: $280 (20 mg/kg total dose) Commercial price: ~ 10x	Price Need for slow iv infusion Heat stability (stored <25° C)
Miltefosine	2–2.5 mg/kg/d orally daily over 28 days	Paladin (Impavido)	Asia: 94% (India) Africa: single field study (93% in HIV(-)	Readily obtained in laboratory isolates	Common, usually mild and transient; gastro-intestinal (20%–55%), Nephro + hepatotoxicity Possibly teratogenic	Preferential price: ~ $74 Commercial price: ~ $150	Price Possibly teratogenic Potential for resistance (half-life) Patient compliance
Paromomycin sulfate	15 mg/kg im daily for 21 days (India only)	IOWH/Gland Pharma	Asia: 95% (India) Africa: 15 mg/kg: 64% (Sudan <50%) 20 mg/kg: 80% (Sudan)	Readily obtained in laboratory isolates	Uncommon, Nephrotoxicity Ototoxicity Hepatotoxicity	~ $15	Efficacy variable between and within regions Potential for resistance (?)

Abbreviations: im, intramuscular; iv, intravenous; SSG, sodium stibugluconate.

[a] Marketing authorization holder.

Data from Drugs for Neglected Diseases initiative (DNDi), from data provided during presentation of DNDi during Fourth World Congress on Leishmaniasis (3–7 February, 2009).

Box 1
Treatment recommendations for visceral leishmaniasis per geographic region, as recommended by the World Health Organization (in order of preference)

L donovani—Indian subcontinent

1. Liposomal amphotericin B: 3–5 mg/kg/d intravenously over 3 to 5 days for a total dose of 15 mg/kg or 10 mg/kg intravenously single dose
2. Combination regimens (sequential coadministration)
   a. Liposomal amphotericin B (5 mg/kg intravenously single dose) plus miltefosine (dosage as below) for 7 days
   b. Liposomal amphotericin B (5 mg/kg intravenously single dose) plus paromomycin (dosage as below) for 10 days
   c. Paromomycin plus miltefosine (dosages as below) for 10 days
3. Amphotericin B deoxycholate 0.75–1 mg/kg/d intravenously, daily or on alternate days, for 15 to 20 doses
4. Miltefosine: children 2 to 11 years: 2.5 mg/kg/d; 12 years and older and less than 25 kg body weight: 50 mg/day; 25 to 50 kg: 100 mg/d; over 50 kg: 150 mg/d, orally for 28 days
5. Paromomycin 15 mg (11 mg base)/kg/d intramuscularly for 21 days
6. Pentavalent antimonials: 20 mg Sb^{5+}/kg/d intramuscularly or intravenously for 30 days in areas where they remain effective (including Nepal, Bangladesh, and certain areas in India)
7. Rescue treatment in case of nonresponse: conventional amphotericin B deoxycholate or liposomal amphotericin B at higher doses

L donovani—East Africa

1. Combination therapy: pentavalent antimonials plus paromomycin for 17 days (dosages as above)
2. Pentavalent antimonials monotherapy as above
3. Liposomal amphotericin B 3–5 mg/kg/d intravenously over 6 to 10 days for a total dose of 30 mg/kg
4. Amphotericin B deoxycholate as above
5. Miltefosine as above

L infantum

1. Liposomal amphotericin B 3–5 mg/kg/d intravenously in 3 to 6 doses for a total dose of 18–21 mg/kg
2. Pentavalent antimonials 20 mg/kg Sb5+/kg/d intramuscularly or intravenously for 28 days
3. Amphotericin B deoxycholate 0.75–1 mg/kg/d intravenously, daily or on alternate days for 20 to 30 doses, total dose of 2 to 3 g

a concave ST segment and prolongation of the corrected QT interval. ECG monitoring is warranted while on treatment, and particular attention should be given to those with pre-existing cardiac conditions.[23,24] Cardiac effects are usually reversible within days to weeks after treatment discontinuation. Within the United States, the drug is not licensed, but requests can be addressed to the Centers for Disease Control and Prevention (CDC).

Conventional amphotericin B is an effective treatment option, with toxicity and need of prolonged hospitalization as major disadvantages. Traditionally it has been most often used as second-line or rescue treatment.[13] Liposomal amphotericin B has enhanced tissue distribution and longer tissue half-life, resulting in less toxicity and

less demanding treatment regimens.[14] Whereas liposomal amphotericin B is currently the preferential treatment regimen in high-income countries, it is increasingly being explored in low-income countries as well. Recommended treatment regimens vary between geographic regions, and the optimal treatment schedule remains to be determined. Traditionally, a total dose of 18 to 21 mg/kg of liposomal amphotericin B has been recommended, with varying treatment schedules being used.[14] FDA recommendations, based on studies with confirmed or presumed *L infantum,* propose a total dose of 21 mg/kg (3 mg/kg at days 1–5, 14 and 21).[25] Two consecutive doses of 10 mg/kg have been used for treatment of children in Europe. More recent evidence suggests that lower total doses could suffice in the Indian subcontinent, where even a single dose of 10 mg/kg of liposomal amphotericin B has been proved effective.[26] Probably, higher doses might be needed in East Africa, at least in some areas.[27]

Paromomycin is a novel option for treatment of antroponotic VL, which can entirely be given in an ambulatory way.[28] It is now an option for first-line treatment in the Indian subcontinent, and in combination with antimonials in East Africa. Whereas high efficacy of paromomycin has been consistently documented in India, treatment response has been lower in East Africa, and higher doses have been required. Of interest, within this region, clear differences in efficacy have been seen in between and within different countries.[29] Paromomycin is hardly used or available outside Africa and the Indian subcontinent. No clinical trials have been conducted with *L infantum*. Similarly, most data on miltefosine come from areas with antroponotic VL. The long terminal half-life (~150 hours) combined with the observed poor treatment compliance when given as self-administered outpatient therapy has raised concerns of rapid emergence of drug resistance in disease-endemic countries.[30] Miltefosine is potentially teratogenic, requiring effective contraception until several months after its use. Miltesosine is licensed in a limited number of countries, including Germany, Colombia, and India. Special FDA regulations exist for miltefosine use in the United States.

Combination Therapy

Combination therapy has increasingly been explored, particularly in highly endemic regions, aiming to identify a short, cheap, well-tolerated combination regimen that can preferably be given in an ambulatory way and requiring minimal clinical monitoring. Combination therapy could also help to delay the emergence of resistance and increase the therapeutic lifespan of the respective drugs, as has been used for diseases like malaria, tuberculosis, and HIV.[30] A 17-day combination of antimonials with paromomycin was found effective in East Africa (93% efficacy). Combination regimens including liposomal amphotericin B (5 mg/kg single dose), paromomycin and/or miltefosine were also found highly effective (98%–99%) and safe, and are now included in WHO recommendations for the Indian subcontinent (see **Box 1**).[20]

Treatment Monitoring

In routine clinical practice, treatment response is usually assessed clinically (resolution of fever, weight gain and splenomegaly, and improvement in hematological abnormalities), with parasitologic evaluation performed on clinical indication. Clinical improvement is typically seen within 7 to 10 days after treatment initiation. Splenomegaly might need several months to disappear completely. In some centers, tissue aspiration or PCR on peripheral blood is performed routinely at the end of treatment besides clinical evaluation, particularly in coinfected patients. Close follow-up for relapse for at least 6 months is recommended. In general, over 90% to 95% of immunocompetent patients demonstrate a good clinical response to treatment, with treatment unresponsiveness, death, or severe toxicity seen in less than 5% to 10% of patients.[2,13]

However, treatment outcomes vary widely between different geographic regions and depending on severity of disease and the presence of coinfections. Up to 5% to 10% of immunocompetent individuals with apparent cure develop relapse, most commonly within 6 months after treatment. Ideally, patients should also be monitored for the occurrence of PKDL.

Special Situations: PKDL

With most cases resolving without treatment in East Africa, treatment is only indicated for severe, complicated, or persisting cases.[6] On the other hand, treatment is generally recommended for all in the Indian subcontinent, except perhaps for those with very mild disease. Limited data are available to guide treatment of PKDL. In East Africa, prolonged administration of antimonials (20 mg Sb^{5+}/kg/d for 30–60 days) or liposomal amphotericin B (2.5 mg/kg/d for 20 days) has been recommended. For the Indian subcontinent, conventional amphotericin B (1 mg/kg/d for 20 days, to be repeated up to 3–4 times with 20-day intervals) or miltefosine for 12 weeks is currently recommended by WHO.[20] Promising findings have been reported with therapeutic vaccination in Sudan.[31]

PREVENTIVE MEASURES FOR TRAVELERS

No vaccine or chemoprophylaxis to prevent infection currently exists. Preventive measures are particularly important for individuals traveling in rural areas and in more primitive conditions, although the parasite has now also spread to peri-urban areas in some regions. Other people at risk include soldiers. Preventive measures aiming at reducing contact with sand flies include the use of (ideally insecticide-treated) bed nets and the use of insecticide sprays in the sleeping room. It is advised to avoid outdoor activities, especially from dusk to dawn, when activity of sand flies is highest. Chances of bites can be reduced by wearing protective clothing and applying insect repellent. Spraying of clothing with permethrin-containing insecticides can additionally be considered. The CDC Web site can be consulted for more information (http://www.dpd.cdc.gov/dpdx/HTLM/Leishmaniasis.htm).

VL-HIV COINFECTION

VL has emerged as an important opportunistic infection in VL-endemic areas in the era of HIV. The HIV epidemic has significantly influenced the epidemiology, the clinical manifestations, and course of VL. In return, VL also accelerates HIV disease progression by increasing the viral replication, leading to further immunosuppression. HIV contributed to the re-emergence of VL in Europe in the 1990s, with 50% to 60% of all VL cases coinfected at some point.[3] Subsequently, VL-HIV coinfection was reported from 35 countries. The highest prevalence of HIV among VL patients is reported from northwest Ethiopia, ranging from 20% to 30%.

In the presence of HIV coinfection, VL tends to be more severe and manifest atypically, particularly with advanced HIV disease.[3] Patients tend to have less organomegaly, while nonreticulo-endothelial organs are commonly involved. The disease may present with concomitant cutaneous, GI, or other tissue involvement.[32] Patients can even present without fever or without splenomegaly. Resemblance of VL clinical features with several other HIV-associated opportunistic conditions leads to additional diagnostic difficulties.

In general, serologic tests have lower performance among HIV patients. The sensitivity of rK39 ELISA was found to be as low as 22% in Spain.[33] However, detailed studies on the performance of the different methods and how sensitivity varies across regions

and populations are lacking. On the other hand, parasite load in peripheral blood seems to be higher in coinfected individuals. Consequently, sensitivity of PCR-based methods, microscopy of peripheral blood, and antigen-based tests seems to be higher in this population. In some centers in Europe, the first diagnostic step consists of PCR or microscopy on peripheral blood.[3]

Treatment of VL in coinfected patients is also challenging. HIV coinfection is associated with poor treatment responses, higher initial failure and relapse rates, more drug toxicity, and higher treatment-associated mortality.[3,34] Current WHO guidelines recommend liposomal amphotericin B at a high dose (40 mg/kg), although lower doses might suffice in some regions.[35] Toxicity of antimonials is enhanced in HIV coinfection. In an Ethiopian study, miltefosine was found safer but less effective than antimonials.[36] The role of combination therapy in VL-HIV coinfection is currently being explored.

Antiretroviral treatment should be initiated as soon as antileishmanial drugs are tolerated, usually within the second week after VL treatment initiation. Although widespread use of antiretroviral treatment has resulted in dramatic reductions in the incidence of VL-HIV coinfection in southern Europe, it appears to have only a partially protective effect against relapses. Repeated relapses tend be become progressively less acute, more atypical, and less responsive to treatment. Even while on highly active antiretroviral therapy (HAART), 1-year relapse rates of 30% to 60% have been reported.[3] Consequently, secondary prophylaxis is currently given in Europe, after achieving parasitologic cure. Administration of liposomal amphotericin B, antimonials, or pentamidine every 3 to 4 weeks has been most commonly used. Experience with miltefosine for secondary prophylaxis is limited. In the Mediterranean, transmission is essentially zoonotic, although transmission through needles among intravenous drug users has probably occurred as well. It is currently unclear whether and how prophylaxis can be safely implemented in areas with antroponotic transmission, where the risk of rapid spread of drug-resistant strains is a concern. Additional information can be found at http://www.cdc.gov/mmwr/pdf/rr/rr5804.pdf.

SUMMARY

VL is one of the major neglected infectious diseases. Whereas the development of rapid diagnostic tests has been a significant progress, non-invasive cheap tests to assess treatment response and diagnose relapse are currently lacking. Molecular testing is increasingly used in high-income countries. Significant progress has been made in terms of treatment, including the development of combination therapy. The emergence of drug resistance in disease-endemic countries is concerning and should be closely monitored. VL-HIV coinfection is increasing worldwide and brings additional challenges in terms of diagnosis and treatment. Concerted efforts from scientists, implementers and funding agents will be required to ensure access to VL diagnosis and treatment at the global level and achieve improved VL control. This should go hand in hand with VL prevention efforts.

REFERENCES

1. Chappuis F, Sundar S, Hailu A. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol 2007;5:873–82.
2. Murray HW, Berman JD, Davies CR, et al. Advances in leishmaniasis. Lancet 2005;366:1561–77.

3. Alvar J, Aparicio P, Aseffa A, et al. The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev 2008;21:334–59.
4. Herwaldt BL. Leishmaniasis. Lancet 1999;354:1191–9.
5. Guerin PJ, Olliaro P, Sundar S, et al. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect Dis 2002;2:494–501.
6. Zijlstra EE, Musa AM, Khalil EA, et al. Post-kala-azar dermal leishmaniasis. Lancet Infect Dis 2003;3:87–98.
7. Srivastava P, Dayama A, Mehrotra S, et al. Diagnosis of visceral leishmaniasis. Trans R Soc Trop Med Hyg 2011;105:1–6.
8. Sundar S, Agrawal S, Pai K, et al. Detection of leishmanial antigen in the urine of patients with visceral leishmaniasis by a latex agglutination test. Am J Trop Med Hyg 2005;73:269–71.
9. Reithinger R, Dujardin JC. Molecular diagnosis of leishmaniasis: current status and future applications. J Clin Microbiol 2007;45:21–5.
10. Antinori S, Calattini S, Longhi E, et al. Clinical use of polymerase chain reaction performed on peripheral blood and bone marrow samples for the diagnosis and monitoring of visceral leishmaniasis in HIV-infected and HIV-uninfected patients: a single-center, 8-year experience in Italy and review of the literature. Clin Infect Dis 2007;44:1602–10.
11. Mary C, Faraut F, Drogoul MP, et al. Reference values for *Leishmania infantum* parasitemia in different clinical presentations: quantitative polymerase chain reaction for therapeutic monitoring and patient follow-up. Am J Trop Med Hyg 2006;75:858–63.
12. Riera C, Fisa R, Ribera E, et al. Value of culture and nested polymerase chain reaction of blood in the prediction of relapses in patients co-infected with leishmania and human immunodeficiency virus. Am J Trop Med Hyg 2005;73:1012–5.
13. Alvar J, Croft S, Olliaro P. Chemotherapy in the treatment and control of leishmaniasis. Adv Parasitol 2006;61:223–74.
14. Bern C, Adler-Moore J, Berenguer J, et al. Liposomal amphotericin B for the treatment of visceral leishmaniasis. Clin Infect Dis 2006;43:917–24.
15. Sundar S, Chakravarty J. Paromomycin in the treatment of leishmaniasis. Expert Opin Investig Drugs 2008;17:787–94.
16. Berman JD. Development of miltefosine for the leishmaniases. Mini Rev Med Chem 2006;6:145–51.
17. Croft SL, Engel J. Miltefosine–discovery of the antileishmanial activity of phospholipid derivatives. Trans R Soc Trop Med Hyg 2006;100(Suppl 1):S4–8.
18. Maltezou HC. Visceral leishmaniasis: advances in treatment. Recent Pat Antiinfect Drug Discov 2008;3:192–8.
19. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev 2006;19:111–26.
20. World Health Organization. Control of the leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva (Switzerland): World Health Organization; 2010. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf. Accessed March 7, 2012.
21. Sundar S. Drug resistance in Indian visceral leishmaniasis. Trop Med Int Health 2001;6:849–54.
22. Sundar S, Chakravarty J. Antimony toxicity. Int J Environ Res Public Health 2010;7:4267–77.
23. Chulay JD, Spencer HC, Mugambi M. Electrocardiographic changes during treatment of leishmaniasis with pentavalent antimony (sodium stibogluconate). Am J Trop Med Hyg 1985;34:702–9.

24. Herwaldt BL, Berman JD. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. Am J Trop Med Hyg 1992;46:296–306.
25. Meyerhoff A. U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. Clin Infect Dis 1999;28:42–8.
26. Sundar S, Jha TK, Thakur CP, et al. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. Clin Infect Dis 2003;37:800–4.
27. Mueller M, Ritmeijer K, Balasegaram M, et al. Unresponsiveness to AmBisome in some Sudanese patients with kala-azar. Trans R Soc Trop Med Hyg 2007;101: 19–24.
28. Davidson RN, Den BM, Ritmeijer K. Paromomycin. Trans R Soc Trop Med Hyg 2009;103:653–60.
29. Hailu A, Musa A, Wasunna M, 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 2010;4:e709.
30. van Griensven J, Balasegaram M, Meheus F, et al. Combination therapy for visceral leishmaniasis. Lancet Infect Dis 2010;10:184–94.
31. Musa AM, Khalil EA, Mahgoub FA, et al. Immunochemotherapy of persistent post-kala-azar dermal leishmaniasis: a novel approach to treatment. Trans R Soc Trop Med Hyg 2008;102:58–63.
32. Rosenthal E, Marty P, Del GP, et al. HIV and leishmania coinfection: a review of 91 cases with focus on atypical locations of leishmania. Clin Infect Dis 2000;31: 1093–5.
33. Medrano FJ, Canavate C, Leal M, et al. The role of serology in the diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type-1. Am J Trop Med Hyg 1998;59:155–62.
34. Pintado V, Martin-Rabadan P, Rivera ML, et al. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients. A comparative study. Medicine (Baltimore) 2001;80:54–73.
35. Sinha PK, van Griensven J, Pandey K, et al. Liposomal amphotericin B for visceral leishmaniasis in human immunodeficiency virus-coinfected patients: 2-year treatment outcomes in bihar, India. Clin Infect Dis 2011;53:e91–8.
36. Ritmeijer K, Dejenie A, Assefa Y, 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;43:357–64.