Visceral Leishmaniasis Recent Advances in Diagnostics and Treatment Regimens

Johan van Griensven, MD, MSc, PhD*, Ermias Diro, MD, PhD

INTRODUCTION

Visceral leishmaniasis (VL) is a disseminated protozoan infection caused by Leishmania donovani and Leishmania infantum. Exceptionally, dermatotropic species can visceralize and cause VL, particularly in immunosuppressed patients. Transmission occurs via the bite of phlebotome sand flies. Blood transfusion, intravenous drug use, organ transplantation, and congenital and laboratory accidents constitute exceptional modes of transmission. The zoonotic form, caused by L infantum, occurs in the Mediterranean basin, China, the Middle East, and South America, and has dogs as the main reservoir. The anthroponotic form, caused by L donovani, is prevalent in eastern Africa, Bangladesh, India, and Nepal. Globally, an estimated 500,000 new cases occur annually. Currently, the Indian subcontinent and eastern Africa carry more than 70% of the VL burden.

The parasite exists in 2 forms. The promastigote form is found in the vector; the amastigote form is found in the host and targets the reticulo-endothelial system in various tissues, predominantly infiltrating the spleen, bone marrow, liver, and lymph nodes. The spectrum of disease ranges from asymptomatic infection to

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* Department of Clinical Sciences, Institute of Tropical Medicine, Nationalestraat 155, Antwerp 2000, Belgium; † Department of Internal Medicine, University of Gondar, Post Office Box 196, Gondar, Ethiopia

* Corresponding author.

E-mail address: jvangriensven@itg.be


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life-threatening illness. Activation of macrophages and an intact T-helper cell type 1 (Th1) response contribute to immune control. Consequently, immunosuppression is a strong risk factor for VL. Most commonly, the incubation period for symptomatic VL ranges from 2 to 6 months. Viable parasites may persist for decades, even after apparently successful treatment, and in the case of immunosuppression, these reactivate and cause disease. Typical manifestations include chronic fever, weight loss, and hepatosplenomegaly, with pancytopenia on blood examination. Without treatment, VL is almost universally fatal. After apparently effective treatment of VL caused by *L. donovani*, post-kala-azar dermal leishmaniasis (PKDL), a chronic skin rash, can develop. Whereas 25% to 50% of the patients in Sudan develop PKDL, it is clearly less common, and occurring longer after treatment, in the Indian subcontinent.

CURRENT DIAGNOSTIC TOOLS AND STRATEGIES

Direct Parasitologic Diagnosis (Microscopy/Culture)

Traditionally, direct visualization of the parasite via microscopy (after Giemsa staining) or culture on invasive samples (spleen, bone marrow or lymph node aspirates, or liver biopsy) has been the gold standard diagnosis (Fig. 1). Spleen aspiration is only done routinely in eastern Africa and the Indian subcontinent, while bone marrow aspiration is more commonly done in Europe, Brazil, and the United States. Although specificity is high, sensitivity is not perfect, with the best performance for spleen aspirates (93%–99%), followed by bone marrow aspiration/biopsy (53%–86%). Lymph node aspiration has a fair sensitivity (53%–65%), but enlarged lymph nodes are relatively rare in VL patients, except in Sudan. Correct diagnosis requires well-trained staff, which is more challenging for countries where VL is only exceptionally seen. Culture reportedly further increases sensitivity on top of microscopy, but is cumbersome and only done in selected laboratories. Although microculture gives more rapid results, this generally still leads to a diagnostic delay of several days (to weeks). Microscopy or culture on noninvasive samples (peripheral blood, buffy coat, peripheral blood mononuclear cells [PBMCs]) has been evaluated also. Although microscopy on these samples tended to have a low sensitivity, microculture using buffy coat and PMBCs had a sensitivity of 85% and 91%, respectively, in an Indian study, with results available within 5 to 15 days and 3 to 7 days, respectively.

![Fig. 1. 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.](image-url)
Historically, several serologic methods have been used, including the enzyme linked immunosorbent assay (ELISA), the indirect fluorescent antibody test (IFAT), the indirect hemagglutination assay (IHA), and immunoblotting, predominantly used in high-income countries. Although performance varies across studies, methodology, and antigen used, most of these combine a fair sensitivity (80%–100%), with a fair specificity (80%–100%). With the advent of new tests, some of these are currently rarely used in routine practice. Although initially crude parasite antigens were mainly used for serologic diagnosis, several recombinant proteins have been evaluated more recently. Currently, in North American reference diagnostic laboratories, the rK39 rapid diagnostic test (RDT), ELISA on crude antigen, and/or the direct agglutination test (DAT) are in use. In Latin America and Spain, IFAT remains in use, but other tests such as the rK39 RDT/ELISA and DAT are increasingly being studied with promising results.

**Development of rapid diagnostic test and enzyme linked immunosorbent assay against recombinant proteins**

One important step has been the development of the rK39-based RDT. The K39 protein contains 39 amino acids originating from a highly conserved kinesin region from a Brazilian *L. infantum/chagasi* strain. This cheap and easy to use test performs well in the Indian subcontinent and constitutes a cornerstone of the current regional VL elimination program. In a recent systematic review, sensitivity of the rK39 RDT in the Indian subcontinent was estimated at 97%, while it was only 85% in eastern Africa. Intermediate values have been reported from Latin America (Table 1). In Europe, the limited available data suggest relatively poor sensitivity (83%) in human immunodeficiency virus (HIV)-negative patients. Importantly, performance of the rK39 RDT varies to some extent according to the brand used, contributing to the heterogeneity observed across studies. Only 2 rK39-based RDTs are sufficiently validated for clinical use: the Kalazar Detect (Inbios, Seattle, WA), and the IT-LEISH (BioRad, Marnes-la-Coquette, France).

The more recently developed rK28 RDT maintained high sensitivity in the Indian subcontinent, and also displayed improved sensitivity (above 95%) in east Africa. Data from Latin America remain limited. Several other recombinant proteins have been evaluated (eg, rKE16 and rK26), but these generally did not outperform rK28 or rK39 tests, at least not in all continents.

**The direct agglutination test**

The DAT was developed as a serologic test that could be deployed in VL endemic areas with limited laboratory infrastructure. As most tests using whole parasite antigens, the test can be falsely positive (usually with low titers) in a number of other diseases such as Chagas disease, brucellosis, and malaria.

Data suggest good sensitivity and specificity in the Indian subcontinent and Brazil (see Table 1). In eastern Africa, DAT was also found substantially more sensitive than the rK39 RDT. Data from Europe are limited, but the largest and most recent study suggested only moderate diagnostic accuracy. One of the disadvantages is the overnight incubation, precluding same-day results.

Serologic tests have the disadvantage in that they can be positive in patients with an asymptomatic *Leishmania* infection or those with a history of VL (precluding diagnosis of VL relapse). This has fueled interest in noninvasive tests directly detecting components of the parasite.
<table>
<thead>
<tr>
<th>Test Country/Region</th>
<th>Sensitivity (%) (95% Confidence Interval)</th>
<th>Specificity (%) (95% Confidence Interval)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K39 RDT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cochrane meta-analysis (2014)</td>
<td>17</td>
<td>97 (90–99.9)</td>
<td>90.2 (76.1–97.7)</td>
</tr>
<tr>
<td>WHO-TDR multicontinent study (2012)</td>
<td>99.6 (97.8–99.9)</td>
<td>96.0 (92.8–97.8)</td>
<td>88.6–98.1</td>
</tr>
<tr>
<td><strong>DAT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systematic review (2006)</td>
<td>90.1</td>
<td>93.2 (89.1–95.3)</td>
<td>96.1 (89.2–98.6)</td>
</tr>
<tr>
<td>India</td>
<td>97.6 (94.8–98.9)</td>
<td>96.0 (92.8–97.8)</td>
<td>88.6–98.1</td>
</tr>
<tr>
<td>Eastern Africa</td>
<td>86.3 (81.7–90.9)</td>
<td>96.1 (89.2–98.6)</td>
<td>96.0 (92.8–97.8)</td>
</tr>
<tr>
<td>Brazil</td>
<td>87.3 (84.6–89.8)</td>
<td>96.1 (89.2–98.6)</td>
<td>96.0 (92.8–97.8)</td>
</tr>
<tr>
<td><strong>Europe: largest study - Spain (2018)</strong></td>
<td>84.2 (74.3–94.2)</td>
<td>100 (99.6–100)</td>
<td>96.8 (93.9–98.4)</td>
</tr>
</tbody>
</table>

Abbreviation: TDR, the special program for research and training in tropical diseases.
Urine Antigen Tests

There is 1 latex antigen test commercially available (KAtex, Kalon Biological, United Kingdom). Although in a Cochrane meta-analysis, specificity was high (93%), sensitivity was low (64%). Consequently, it is currently rarely used in clinical practice. Sensitivity was slightly better (84%–86%) in HIV patients.\(^{28,29}\) Several new urine antigen tests have been developed in ELISA format, displaying better sensitivity.\(^{30}\) Commercially available RDTs based on these antigens are not available yet. Potentially, antigen tests could be of value as a test of cure.\(^{30}\)

Molecular Tests

Molecular tests are increasingly in use for VL diagnosis. A recent systematic review demonstrated high sensitivity (>95%) of polymerase chain reaction (PCR) on bone marrow, peripheral blood, or buffy coat samples, without clear differences in performance on blood between \(L\) \textit{donovani} in Asia and east Africa and \(L\) \textit{infantum} in the Mediterranean.\(^{31}\) Data on PCR on blood from cases infected by \(L\) \textit{infantum} in Latin America were limited but do suggest a similar performance in this continent. Similarly, the limited data on real-time PCR suggest performance comparable to conventional PCR. PCR on blood could thus be an interesting first noninvasive step in the diagnostic work-up. However, specificity was low (63%–76%) in the better designed studies (Table 2). On the 1 hand, this could indicate a suboptimal gold standard used (with true cases being missed); on the other hand it also indicates that in endemic areas a substantial proportion of individuals without VL are PCR positive (asymptomatic \textit{Leishmania} infection). This indicates that a positive test should be interpreted in combination with a sound clinical case definition, and ideally other tests such as serology. High parasite loads in quantitative PCR give additional weight to VL diagnosis.

To allow implementation in field settings, loop-mediated isothermal amplification (LAMP) has been explored. As accuracy looks comparable to conventional methods, molecular tests are likely to be increasingly used in resource-constrained endemic areas also.\(^{22,31–33}\) Both qualitative and quantitative tests are also being evaluated to assess cure, as they usually turn negative after treatment.\(^{32}\)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Pooled estimates from a meta-analysis of the diagnostic performance of molecular methods to diagnoses visceral leishmaniasi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Study &amp; Sample</td>
<td>Number of Studies</td>
</tr>
<tr>
<td>All studies (case control &amp; consecutive studies combined)</td>
<td></td>
</tr>
<tr>
<td>PCR blood</td>
<td>19</td>
</tr>
<tr>
<td>PCR bone marrow</td>
<td>8</td>
</tr>
<tr>
<td>Consecutive studies</td>
<td></td>
</tr>
<tr>
<td>PCR blood</td>
<td>4</td>
</tr>
<tr>
<td>PCR bone marrow</td>
<td>5</td>
</tr>
<tr>
<td>HIV/VL – all studies combined</td>
<td></td>
</tr>
<tr>
<td>PCR buffy coat cells</td>
<td>4</td>
</tr>
<tr>
<td>PCR bone marrow</td>
<td>5</td>
</tr>
</tbody>
</table>

Although diagnosis and treatment vary by the geographic origin of the patient and the causative species, species identification is rarely required for patient management unless there is geographic overlap of *L. infantum* and *donovani* (which is uncommon), there is doubt on the exact region of exposure (eg, exposure in different regions), or in immunosuppressed individuals in whom species not typically causing VL can visceralize (eg, *L. tropica*). Species identification is typically done using enzyme electrophoresis, or increasingly by molecular methods. However, there is a range of molecular methods at hand, potentially leading to variable results between laboratories.

**Diagnosis of Visceral Leishmaniasis in Immunocompromised Patients**

Diagnosis of VL in immunocompromised patients (particularly HIV patients) can be challenging for several reasons. First, atypical presentations are not uncommon, whereby parasites are only found in atypical locations (eg, intestinal, oral ulcers), and bone marrow or spleen aspiration can consequently be negative.3 Second, serologic tests tend to be less sensitive in immunocompromised patients.34 Hence, negative results have to be interpreted carefully, and using more than 1 serologic test if available might be useful. In a meta-analysis from 2013, DAT and Western blotting were the best-performing serologic tests (Table 3).35 However, the authors (Cota and colleagues35) acknowledged that overall evidence is still limited. For example, for DAT, there were only 4 studies in total, and no studies from the Indian subcontinent or Latin America were included. All included studies on IFAT, Western blotting, and ELISA were from Europe. Only 2 studies on the rK39 RDT were included (1 from India, 1 from Ethiopia).

Since then, several interesting studies on the rK39 RDT and DAT in HIV patients have been published. One study from Brazil reported on the rK39 RDT (sensitivity: 45.6%–46.2%; specificity: 97.0%–98.4%), DAT (sensitivity: 87.8%–89.7%; specificity: 82.3%–86.4%), and IFAT (sensitivity: 60.9%–61.5%; specificity: 87.1%–89.5%).35 The better sensitivity of DAT compared with the rK39 RDT in HIV patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of Studies for Sensitivity/Specificity</th>
<th>Pooled Sensitivity in Random-Effects Model (%)</th>
<th>Pooled Specificity in Random-Effects Model (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>4/3</td>
<td>81 (61–95)</td>
<td>90 (66–100)</td>
</tr>
<tr>
<td>IFAT</td>
<td>21/4</td>
<td>51 (43–58)</td>
<td>93 (81–99)</td>
</tr>
<tr>
<td>rK39 RDT</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ELISA</td>
<td>5/3</td>
<td>66 (40–88)</td>
<td>90 (77–98)</td>
</tr>
<tr>
<td>Western Blotting</td>
<td>8/4</td>
<td>84 (75–91)</td>
<td>82 (65–94)</td>
</tr>
<tr>
<td>Molecular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR whole blood</td>
<td>8/3</td>
<td>92 (83–98)</td>
<td>96 (80–100)</td>
</tr>
<tr>
<td>PCR bone marrow</td>
<td>3/0</td>
<td>98 (93–100)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available (no or too few studies available).

is in line with observations in eastern Africa (DAT: 89%, rK39: 77%). Similar observations were made recently in Europe evaluating DAT (sensitivity 91.3%, specificity 83.3%) and rK39 RDT (sensitivity: 67.3% specificity: 100%). The fair sensitivity of DAT would thus be contributing to ruling out VL; the better specificity of the rK39 RDT would be useful to confirm VL.

In non-HIV immunosuppressive conditions, serologic tests seem to be less compromised. In a systematic review, the sensitivity of IFAT was 93% in transplant patients but only 48% in HIV patients. The same review suggested a sensitivity of microscopy on bone marrow aspiration of 81% in HIV patients and 98% in transplant patients.

In the meta-analysis by Cota and colleagues, molecular methods clearly outperformed serologic methods, with PCR on whole blood and bone marrow displaying high sensitivity. In a more recent meta-analysis, PCR on bone marrow and buffy coat had a high sensitivity in HIV patients (see Table 2). Because of the paucity of (quality) studies, no pooled estimate on peripheral blood was provided in this review. As to peripheral blood microscopy, using buffy coat or PBMCs was found to be relatively sensitive in HIV patients; sensitivity reached 56% with PBMCs, compared with 29% in HIV-negative patients.

PCR has also been proposed to assess treatment response and for prediction or early detection of relapse. Importantly, leishmanial DNA can be detected in HIV patients after treatment, without clinical significance, indicating care should be taken to diagnose VL relapse solely on a positive PCR test. For this reason, quantitative PCR thresholds of parasite load have been proposed predicting relapse. However, these are highly dependent on the specific PCR protocol used and hence might not be easily generalizable. Nevertheless, progressively increasing PCR levels after successful treatment should raise concern about (the risk of) VL relapse.

**Diagnostic Approach**

The diagnostic work-up should take into consideration the (likely) origin of the *Leishmania* infection, especially if rK39 testing is done, and the immunocompetency of the patient. If invasive samples are not to be collected for other indications (eg, to rule out hematological conditions), a step-wise approach could be taken whereby initial testing with PCR and serology (1 or 2 different tests) on peripheral blood (and possibly complemented with microscopy and/or culture) is done. Particularly in immunocompromised patients, using more than 1 serologic tests might be useful. If the diagnosis cannot be reliably ruled out or confirmed, invasive testing with PCR and microscopy (and possibly culture) is done on tissue samples (eg, bone marrow, lymph nodes). Doubtful test results like low level positive results on serology and PCR (if quantitative) would also be an indication for invasive testing. Indeed, as specificity of some tests is suboptimal, not every positive *Leishmania* test equals VL, but the pretest probabilities together with the results of various tests should be integrated to arrive to a high probability of ruling in or ruling out the disease. In case of a high clinical suspicion with negative initial *Leishmania* tests, repeating tests has been found useful also (with an initial negative bone marrow aspiration being positive when repeated).

For patients presenting with a possible VL relapse, serologic tests are of no use, as antibodies remain positive for prolonged periods after VL treatment. PCR (ideally quantitative) and direct parasitologic tests are of value here.

**VISCERAL LEISHMANIASIS TREATMENT UPDATE**

Untreated symptomatic VL is almost always fatal. Thus, all symptomatic patients with VL need treatment with antileishmanial drugs. Additional ancillary medications
and other supportive care may also be needed. The effectiveness of antileishmanial drugs varies with the host immune status and the geographic location where the *Leishmania* infection was acquired. Thus, treatment recommendations are based on this classification. To date, liposomal amphotericin B (L-AmB) and miltefosine are the only US Food and Drug administration (FDA)-approved drugs for VL treatment (Table 4).

**Treatment of Visceral Leishmaniasis in Immunocompetent Hosts**

L-AmB is currently recommended first line antileishmanial drug based on the available efficacy data from different VL endemic regions of the world. The FDA-approved dosage regimen is 3 mg/kg/d intravenously on days 1 to 5, 14, and 21 (total dose, 21 mg/kg). A large retrospective cohort study in India has shown 99% initial cure rate with 20 mg/kg of L-AmB with good safety profile and low relapse and PKDL rates. The use of single-dose L-AmB (10 mg/kg) was also found effective, with a cure rate greater than 95%, and it is used in the national leishmaniasis elimination program in India. Most of the experience for *L. infantum* transmission regions came from the Mediterranean basin area that demonstrated good efficacy and safety. Unfortunately, the efficacy of L-AmB in East Africa and Brazil was lower than expected, and higher doses may be needed (30–40 mg/kg). Although the high dose has cost implications for most of the endemic regions, L-AmB remains to be the preferred choice for its safety and short regimen.

Antimonials continued to be effective in all VL endemic regions, except in the Indian subcontinent (Bihar and the neighboring regions), where parasite susceptibility to antimonials has decreased. Multicenter trials in eastern Africa have shown high efficacy and decreased hospitalization rates with combination therapy of antimonials and paromomycin. Thus, the combination of sodium stibogluconate 20 mg/kg intravenously or intramuscularly and paromomycin 15 mg/kg intramuscularly both for 17 days is recommended as the preferred first-line regimen over 30 days of antimonial alone for VL in this region. However, both of these drugs are associated with several serious adverse effects and still require prolonged hospitalization compared with the L-AmB regimen.

Other alternative antileishmanial agents are miltefosine, the only available oral antileishmanial drug in use to date, and paromomycin. Although these drugs have also been found to be highly effective in the Indian subcontinent as monotherapy, rapid emergence and spread of resistance are concerns. Use of these drugs in combination regimens has helped to overcome the challenge and maintain their effectiveness. A sequential combination therapy of liposomal amphotericin B (5 mg/kg single dose) followed by 10 days of 11 mg/kg/d paromomycin or 7 days of 50 mg/kg/d miltefosine have been found to be highly effective (98%–99%) and safe, and are now included in World Health Organization (WHO) recommendations for the Indian subcontinent. The limited experiences with these drugs in eastern Africa have shown lesser effectiveness, variable performance, and need for higher doses. The linear dosage of miltefosine based on body weight was found to have lower steady state serum concentration and was associated with higher treatment failure among children. Allometric dosing results in optimal steady state concentration in both adults in children and is the preferred dosing algorithm for miltefosine.

About 5% to 10% of primary VL cases may not show response, or may relapse after some months of the initial treatment. Nonresponding and relapsing patients can be treated by prolonging the duration of therapy, increasing dose of L-AmB, or shifting to alternative drugs. The use of conventional amphotericin has decreased over time because of its high nephrotoxicity and hypokalemia. Pentamidine is also differed
<table>
<thead>
<tr>
<th>Drugs and Mechanism of Action</th>
<th>Regimen</th>
<th>Marketing*</th>
<th>Clinical Efficacy</th>
<th>Toxicity</th>
<th>Monitoring</th>
<th>Cost/Course</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>20 mg/kg iv or im daily for 28–30 d</td>
<td>GlaxoSmithKline (Pentostam) Sanofi Aventis (Glucantime) Available via CDC in USA and Special access Program in Canada</td>
<td>35%–95% (depending on geographic area)</td>
<td>Frequent, potentially severe; cardiac toxicity, pancreatitis, Nephro + hepatotoxicity</td>
<td>Baseline and weekly, CBC, transaminases, lipase, amylase, serum creatinine, potassium and ECG</td>
<td>Generic ~ $64.5 Branded ~ $85.6</td>
<td>Quality control issues with generic rugs Length of treatment Painful injection Toxicity Resistance in India</td>
</tr>
<tr>
<td>- Virtually unknown mechanism; inhibition of several metabolic pathways leading to impaired macromolecule (DNA, RNA, proteins) synthesis</td>
<td></td>
<td></td>
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<tr>
<td>Amphotericin B deoxycholate</td>
<td>0.75–1 mg/kg iv for 15–20 doses (daily or alternate days)</td>
<td>Bristol-Myers Squibb (Fungizone®)</td>
<td>&gt; 97% all regions</td>
<td>Frequent Infusion-related reactions, nephrotoxicity, hypokalemia (in-patient care needed)</td>
<td>Baseline and weekly serum creatinine, BUN, potassium and urinalysis</td>
<td>Generic price: ~ $21</td>
<td>Need for slow iv infusion Dose-limiting Nephrotoxicity Potassium replacement Heat stability</td>
</tr>
<tr>
<td>- Plasma membrane inhibitor</td>
<td></td>
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<tr>
<td>Liposomal Amphotericin B</td>
<td>10–30 mg/kg total dose iv; usually 3–5 mg/kg/dose Single dose (10 mg/kg) in India</td>
<td>Gilead via Astellas (Ambisome) FDA approved The Liposome company (Abelcet) Not FDA approved for VL</td>
<td>Europe and Asia: &gt; 95%; lower in East Africa and Brazil (higher dose required) Uncommon and mild; Nephrotoxicity</td>
<td>Baseline serum creatinine, blood urea nitrogen and potassium</td>
<td>Preferential price: $378 (21 mg/kg total dose) Commercial price: ~ 10x</td>
<td>Price Need for slow iv infusion Heat stability (stored &lt;25°C)</td>
<td></td>
</tr>
<tr>
<td>- Plasma membrane inhibitor</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- Enhanced tissue distribution, longer half-life, less toxicity</td>
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<table>
<thead>
<tr>
<th>Drugs and Mechanism of Action</th>
<th>Regimen</th>
<th>Marketing*</th>
<th>Clinical Efficacy</th>
<th>Toxicity</th>
<th>Monitoring</th>
<th>Cost/Course</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miltefosine</td>
<td>2–2.5 mg/kg/d orally daily over 28 d (India only)</td>
<td>Knight Therapeutics (Impavidio) via Profounda in the United States, via Special Access Program in Canada FDA approved</td>
<td>Asia: &gt;90% but relapse rate increasing; Africa: single field study (93% in non-HIV patients)</td>
<td>Common, usually mild and transient; gastro-intestinal (20%–55%), Nephro + hepatotoxicity Possibly teratogenic</td>
<td>Baseline and weekly liver and renal functions. Pregnancy test before treatment</td>
<td>Preferential price: approximately $80.49 Commercial price: ~ $22,000 – $34,000</td>
<td>Need for effective double contraception up to 3 months post treatment. Allometric dosing Easy resistance development Adverse effects affect compliance</td>
</tr>
</tbody>
</table>

| Paromomycin Sulfate           | 15 mg/kg intramuscularly daily for 21 d (India only) | IOWH/Gland Pharma | Asia: 95% (India) Africa: 15 mg/kg: 64% (Sudan <50%) 20 mg/kg: 80% (Sudan) | Uncommon Nephrotoxicity Ototoxicity Hepatotoxicity Painful injections | Baseline and weekly renal function and audiometry | ~ $23.8 | Efficacy variable between and within regions Potential for easy resistance development Useful in combination therapy |

Abbreviation: CBC, complete blood count.

* Marketing authorization holder.
from routine use in VL treatment because of its adverse effects such as diabetes mel-ititus (Table 5).

**Visceral Leishmaniasis**

**Visceral Leishmaniasis Treatment in Immunosuppressed Hosts**

**Visceral leishmaniasis/human immunodeficiency virus coinfection**

In the presence of HIV coinfection, the existing antileishmanial drugs are associated with poor treatment responses (higher initial failure and relapse rates), more adverse events, and added toxicities and higher mortality. A review of different regimens used for VL treatment in HIV showed higher toxicity and mortality with antimonials other than L-AmB. Current guidelines recommend L-AmB at a high dose (total of 40 mg/kg). One dosing regimen can be 5 mg/kg on days 1 to 5, 10, 17, and 24. However, different dosing approaches are used. Although this has good safety profile, it is less effective in eastern Africa and Latin America, and it is associated with delayed relapses in India. In an Ethiopian study, miltefosine monotherapy was found safer but less effective than antimonials. A compassionate use of combination therapy of L-AmB (30 mg/kg) and miltefosine, and a recent clinical trial in Ethiopia with this combination have shown promising results, with greater than 80% initial cure rate both in primary and relapsed VL patients. A clinical trial evaluating this combination therapy or L-AmB at 40 mg/kg has been conducted, and findings are to be reported soon (https://clinicaltrials.gov/ct2/show/NCT02011958).

Antiretroviral treatment is a cornerstone and should be initiated as soon as antileishmanial drugs are tolerated. The widespread use of antiretroviral treatment has resulted in dramatic reductions in the incidence of VL/HIV coinfection in southern Europe. However, it appears to have only partially protective effect against relapses. Repeated relapses tend be become progressively less acute, more atypical, and less responsive to treatment. Despite the use of ART, 1-year relapse rates of 30% to 60% have been reported. Consequently, secondary prophylaxis is recommended after achieving parasitologic cure for patients with CD4 count level below 200 cells/µL. These guidelines mention the limitation of data regarding when to stop secondary prophylaxis and suggest considering discontinuation of maintenance therapy when a sustained (3-6 months) CD4 level above 350 cells/µL is achieved with ART. L-AmB, antimonials, and pentamidine every 2 to 4 weeks have been used for secondary prophylaxis. Use of pentamidine infusion at a dose of 4 mg/kg every month in Ethiopia has resulted in a relapse-free survival rate of 71% 1 year after VL treatment. Although the development of resistance is of major concern in choosing drugs for secondary prophylaxis, the mode of transmission in the region needs to be taken into consideration. In antroponothic transmission regions, HIV coinfected patients may become reservoirs of resistant strains. Thus, it is wise to preserve the first-line options from use in secondary prophylaxis.

**Other immunosuppressive conditions**

VL has been reported in non-HIV immunosuppressive clinical conditions such as organ transplant recipients, rheumatologic diseases, malignancies, and long-term steroid use. The available data are based on case reports and case series to draw strong conclusions regarding choice of treatment. However, liposomal amphotericin B is often recommended for its good safety profile. Modifications of the immunosuppressive medications may be important during VL treatment. The treatment failure and relapse rates are less prominent when compared to patients with HIV co-infection. Thus, secondary prophylaxis is not indicated unless it was a relapsed disease.
<table>
<thead>
<tr>
<th>Guidelines from North America</th>
<th>Recommendations for Visceral Leishmaniasis Treatment</th>
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<tbody>
<tr>
<td>Centers for Disease Control and Prevention$^{65}$</td>
<td>Immunocompetent patients: L-AmB 3 mg per kg daily, by intravenous infusion, on days 1–5, 14, and 21 (total dose of 21 mg/kg). Imunosuppressed patients: L-AmB 4 mg per kg daily on days 1–5, 10, 17, 24, 31, and 38 (total dose of 40 mg/kg). Alternatives: Amphotericin B deoxycholate 0.5-1 mg/kg either daily or every other day for a total of 15–20 mg/kg. Pentavalent antimonial (SbV) 20 mg/kg/d intravenously or intramuscularly for 28 d.</td>
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<tr>
<td>AIDSinfo Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents$^{60}$</td>
<td>Preferred Therapy: L-AmB 2–4 mg/kg intravenously daily (All), or interrupted schedule (eg, 4 mg/kg on days 1–5, 10, 17, 24, 31, 38) (All) a total dose of 20–60 mg/kg (All). Alternative therapy: Other amphotericin B lipid complex dosed previously described, or Amphotericin B deoxycholate 0.5–1.0 mg/kg intravenously daily for total dose of 1.5–2.0 g (BII). Pentavalent antimony (sodium stibogluconate) 20 mg/kg intravenously or intramuscularly daily for 28 d (BII). Miltefosine (CIII) for patients who weigh 30–44 kg: 50 mg orally bid for 28 d; for patients who weigh ≥45 kg: 50 mg orally 3 times daily for 28 d. Chronic maintenance therapy for visceral leishmaniasis Indication: for patients with visceral leishmaniasis and CD4 count &lt;200 cells/mm$^3$ (All) Preferred therapy: Liposomal amphotericin B 4 mg/kg every 2–4 wk (All) Amphotericin B lipid complex 3 mg/kg every 21 d (All) Alternative therapy: Pentavalent antimony (sodium stibogluconate) 20 mg/kg intravenously or intramuscularly every 4 weeks (BII). Discontinuation of chronic maintenance therapy Some investigators suggest that therapy can be discontinued after a sustained (&gt;3–6 months) increase in CD4 count to &gt;200–350 cells/mm$^3$ in response to ART, but others suggest that therapy should be continued indefinitely. Therefore, no recommendation can be made regarding discontinuation of chronic maintenance therapy.</td>
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Infectious Diseases Society of America (IDSA)
and American Society of Tropical Medicine
and Hygiene (ASTMH)

<table>
<thead>
<tr>
<th>Immunocompetent</th>
<th>Immunocompromised host (HIV)</th>
<th>Other immunocompromised host (postorgan transplant)</th>
<th>Special population (too young, elderly, renal or hepatic failure, pregnant, lactating)</th>
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</thead>
<tbody>
<tr>
<td>Ambisome 3 mg/kg/d intravenously on days 1–5, 14, and 21 (total dose, 21 mg/kg); 40 mg/kg for eastern Africa.</td>
<td>AmBisome 4 mg/kg/d intravenously, on days 1–5, 10, 17, 24, 31, and 38 (10 doses over a 38-day period), for a total dose of 40 mg/kg.</td>
<td>AmBisome 4 mg/kg/d intravenously, on days 1–5, 10, 17, 24, 31, and 38 (10 doses over a 38-day period), for a total dose of 40 mg/kg.</td>
<td>All need treatment; otherwise VL is fatal</td>
</tr>
<tr>
<td>Miltefosine 2.5 mg/kg/d (max 150 mg) for 28 d for VL acquired in Indian subcontinent (for age ≥12, weight ≥30, nonpregnant or breast feeding)</td>
<td>AmBisome + miltefosine combination</td>
<td>Secondary prophylaxis for CD4 &lt;200</td>
<td>Care should be given on selection of drug, dosage, and monitoring approach</td>
</tr>
<tr>
<td>Alternatives:</td>
<td>Other</td>
<td>Other</td>
<td>Other</td>
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<tr>
<td>Pentavalent antimonial 20 mg/kg/d intravenously or intramuscularly for 28 d (where resistance is low)</td>
<td>Failure with L-AmB - higher dose L-AmB, prolonged L-AmB or alternatives</td>
<td>Failure with miltefosine or antimonials – L-AmB or alternative</td>
<td>All need treatment; otherwise VL is fatal</td>
</tr>
<tr>
<td>Amphotericine B (for persons with liposome-induced complement activation-related psuedoallergy)</td>
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<td>Care should be given on selection of drug, dosage, and monitoring approach</td>
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<td>Nonresponding or relapsing patients</td>
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<td>Failure with miltefosine or antimonials – L-AmB or alternative</td>
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<td><strong>WHO recommendations per region</strong>&lt;sup&gt;10&lt;/sup&gt;</td>
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</table>
| **L donovani - Indian subcontinent** | • Liposomal amphotericin B: 3–5 mg/kg/d intravenously over 3–5 d for total dose of 15 mg/kg or 10 mg/kg intravenously sd  
  • Combination regimens (sequential coadministration)  
  • Liposomal amphotericin B (5 mg/kg intravenously sd) + miltefosine for 7 d  
  • Liposomal amphotericin B (5 mg/kg intravenously sd) + paromomycin for 10 d  
  • Paromomycin + miltefosine for 10 d  
  • Amphotericin B deoxycholate 0.75–1 mg/kg/d intravenously, daily or on alternate days, for 15–20 doses  
  • Miltefosine: children 2–11 y: 2.5 mg/kg/d; ≥12 y and < 25 kg body weight: 50 mg/d; 25–50 kg: 100 mg/d; > 50 kg: 150 mg/d; orally for 28 d  
  • Paromomycin 15 mg (11 mg base)/kg/d intramuscularly for 21 d  
  • Pentavalent antimonials: 20 mg Sb<sub>5+</sub>/kg/d intramuscularly or intravenously for 30 d in areas where they remain effective (including Nepal, Bangladesh, and certain areas in India)  
  • Rescue treatment in case of nonresponse: conventional amphotericin B deoxycholate or liposomal amphotericin B at higher doses |
| **L donovani - eastern Africa** | • Combination therapy: pentavalent antimonials + paromomycin for 17 d  
  • Pentavalent antimonials monotherapy  
  • Liposomal amphotericin B 3–5 mg/kg/d intravenously over 6–10 d for total dose of 30 mg/kg  
  • Amphotericin B deoxycholate  
  • Miltefosine |
| **L infantum regions** | • Liposomal amphotericin B 3–5 mg/kg/d intravenously in 3–6 doses for a total dose of 18–21 mg/kg  
  • Pentavalent antimonials 20 mg/kg Sb<sub>5+</sub>/kg/d intramuscularly or intravenously for 28 d  
  • Amphotericin B deoxycholate 0.75–1 mg/kg/d intravenously, daily or on alternate days for 20–30 doses, total dose of 2–3 g |

For sodium stibogluconate contact the CDC Drug Service at 404-639-3670 or drugservice@cdc.gov; for emergencies, call 770-488-7100. For miltefosine available in the United States via www.Profounda.com.
Post kala-azar dermal leishmaniasis
PKDL is a chronic skin rash that starts as erythematous macules and papules around the perioral area and progresses with nodular rash spreading all over the body. It usually comes after effective treatment of VL in *L donovani* transmission regions. Geographic variation is observed in the nature of PKDL. Although it occurs in 50% to 60% of patients in Sudan within the first 6 months of VL treatment, it is less prevalent (5%–10%) and is occurring 1 to 2 years later in the Indian subcontinent. Spontaneous recovery occurs in the majority of cases in Sudan, whereas most of the patients with PKDL in India require pharmacologic treatment. PKDL may indicate an immune reconstitution syndrome. Although the circulating parasites are cleared from the reticuloendothelial system, the skin lesions are fully parasitized and may play role in disease transmission.4

Depending on the extent of body involvement, PKDL is classified into 3 grades. Grade 1 is rash on the face. This is usually self-limiting and needs simple observation. Grade 2 is when the trunk is involved, with the lesions spreading down from the face. Sometimes, there may be associated mucosal involvement. Grade 3 is when it involves the extremities in addition to the face and the trunk.

Patients who show grade 2 PKDL with mucosal involvement and grade 3 PKDL need pharmacotherapy.4 Limited data are available to guide treatment of PKDL. In eastern Africa, prolonged administration of antimonials (20 mg Sb5+/kg/d for 30–60 days) with or without paromomycin, or liposomal amphotericin B (2.5 mg/kg/d for 20 days) has been recommended.70 For the Indian subcontinent, conventional amphotericin B (1 mg/kg/d for 20 days, to be repeated up to 3–4 times at 20-day intervals) or miltefosine for 12 weeks is currently recommended by WHO.51 L-AmB, at a total dose of 15 mg/kg divided in 5 doses, was also found effective in this region.71 Promising findings were reported with therapeutic vaccination in Sudan.72

Complications and special situations
VL causes bone marrow suppression resulting in pancytopenia. VL patients are often prone to other infections because of leukopenia/neutropenia. Meticulous work-up for possible additional (predominantly bacterial) infections and timely management are critical for better outcome. They also have thrombocytopenia and anemia. Blood transfusion is another important aspect of the management.

VL may occur in individuals with different kinds of condition such as pregnancy or lactating mothers, or individuals with different organ dysfunctions such as renal failure or hepatic failure. As untreated VL can rapidly lead to deterioration and has fatal outcome, treatment should not be delayed. L-AmB is a safe option to use in these conditions. The conventional amphotericine B can be used for people with liposome-induced complement activation-related pseudo-allergy. Drug dose adjustments should also be done depending on creatinine clearance, concomitant medications, and adverse events.

Treatment monitoring
VL treatment response typically starts after 5 to 7 days of treatment initiation, with resolution of fever and improvement in appetite. This is often followed by weight gain, regression of the splenomegaly, and improvement in hematologic profiles. By the end of treatment, the spleen will become nonpalpable or tipped, and the hematology profile normalizes. However, splenomegaly might need several months to disappear completely.

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%. However, treatment outcomes vary widely between different geographic regions, severity of disease, and the presence of coinfections and comorbidities. Up to 5% to 10% of immunocompetent individuals with apparent cure develop relapse, most commonly within 6 months after treatment. Patients with underlying immunosuppression, those with partial clinical response, and those with relapsed form of disease need parasitologic assessment at the end of treatment to decide on their next management. Close follow-up for possible relapse and PKDL is important.

SUMMARY

Significant progress has been made in VL diagnostics over the last decades. Remaining challenges include the development of RDTs performing well in all VL-endemic areas. Additionally, noninvasive tests of cure are highly needed, especially for immunosuppressed patients, as they are more likely to fail treatment. In that respect, quantitative PCR and antigen tests merit further research. The same applies for the diagnosis of VL relapse. Interestingly, an RDT testing for *L. donovani* antigen-specific IgG1 levels looks promising to diagnose VL relapse in immunocompetent Indian patients, with immunoglobulin 1 (IgG1) levels at very low levels in those disease-free at 6 months but high in those presenting with VL relapse.

Molecular tests are expected to be increasingly used globally. In currently available studies, different types of primers and protocols were used, with limited standardization across laboratories. Although available data generally suggest a consistently high sensitivity across these differences, standardization of these tests remains important. Moreover, given the relatively low number of studies on each primer, sample, species, or geographic area, additional studies are needed to get more reliable data and to discern clinically relevant differences in diagnostic performance across samples used or parasite strains. More data on the diagnostic accuracy of PCR on peripheral blood, especially in immunocompromised patients, are needed also. Immunologic markers are likely to be increasingly used to assess treatment response or to indicate safe discontinuation of secondary prophylaxis in immunocompromised patients. Whole blood assays similar to the interferon-γ release assays (IGRAs) used for TB screening have been developed and look promising.

Several safety and effectiveness studies were reported from major *Leishmania* endemic regions of the world, as the limited available antileishmanial medicines became more accessible. The effectiveness varies with geographic region where the infection is acquired and the presence of comorbidities, importantly HIV co-infection. L-AmB has been found to be safe and effective in most endemic regions of the world and is the preferred choice to treat VL. However, higher L-AmB doses were required to treat VL in eastern Africa, where antimonials still remain the most effective although highly toxic. Combination therapy is increasing being explored and recommended to maximize effectiveness and reduce resistance development. Although antimonial combination with paromomycin has reduced the hospital stay for the eastern Africa region, L-AmB combined with miltefosine has increased effectiveness of VL treatment in HIV coinfected patients.

ACKNOWLEDGMENTS

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