Recent advances in the diagnosis and treatment of kala-azar

SHYAM SUNDAR, JAYA CHAKRAVARTY

ABSTRACT
In India, about 100 000 cases of visceral leishmaniasis (VL) or kala-azar are estimated to occur annually, 90% of which occur in the state of Bihar. Currently, antibody-based tests such as the rK39-based immunochromatographic strip test and the direct agglutination test (DAT) are widely used for the diagnosis of VL. However, their major drawback is continued positivity both long after cure and in a high proportion of individuals living in endemic areas. Thus, antibody-based tests must always be used in combination with a standardized clinical case definition for VL. There have been many breakthroughs in the past decade in the treatment of kala-azar in India, such as approval of oral miltefosine and paromomycin, single-dose treatment with liposomal amphotericin B and multidrug treatment. Encouraged by these advances, an ambitious VL elimination programme was launched with the aim to eliminate VL as a public health problem in India, Nepal and Bangladesh by 2015. Early diagnosis, complete treatment of cases, integrated vector management, effective disease surveillance, and clinical and operational research should be the five key components of the strategy to achieve this goal.


THE ORGANISM
Leishmaniasis, a vector-borne disease, is caused by an obligate intracellular protozoan of the genus Leishmania. It broadly manifests as visceral leishmaniasis (VL); also known as kala-azar, cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). VL is typically caused by the Leishmania donovani complex: L. donovani, the causative organism of VL in the Indian subcontinent and Africa; L. infantum (L. chagasi) which causes VL in the Mediterranean basin, Central and South America.

TRANSMISSION
The natural transmission of leishmaniasis is carried out by female sandflies. In South Asia and the Horn of Africa, the predominant mode of transmission is anthroponotic and humans with kala-azar or post-kala-azar dermal leishmaniasis (PKDL) provide the major reservoir for ongoing transmission.1,2 In the Mediterranean, the Middle East and Brazil, the disease is zoonotic, with domestic dog as the most important reservoir host sustaining transmission.2

DISEASE BURDEN
An estimated 500 000 new cases of VL occur every year, 90% of these occur in the endemic areas of India, Nepal, Bangladesh, Sudan, Ethiopia and Brazil.3 In India, about 100 000 cases of VL are estimated to occur annually. Of these, the state of Bihar accounts for more than 90% of cases.4 However, environmental changes such as deforestation, urbanization, migration of non-immune people to endemic areas have led to an increase in the incidence of leishmaniasis. Migration from non-endemic to endemic areas is a major risk factor for the spread of VL as these people on their return can spread the disease in a non-immune population.

THE DISEASE
VL is the systemic and most severe form of leishmaniasis, characterized by prolonged fever, splenomegaly, hepatomegaly, pancytopenia, progressive anaemia and weight loss. If untreated, VL is uniformly fatal. Some patients with VL may develop a chronic form of dermal leishmaniasis characterized by indurated nodules or depigmented macules, which is called PKDL. In the Indian subcontinent, it occurs only in a small proportion of patients, 6 months to several years after an episode of VL.5 Treatment of PKDL is necessary as patients serve as a major reservoir of infection.

HIV–VL COINFECTION
The HIV/AIDS pandemic has modified the natural history of leishmaniasis.6 Both diseases exert a synergistic detrimental effect on the cellular immune response because they target similar immune cells. HIV infection increases the risk of developing VL in areas of endemicity, reduces the likelihood of a therapeutic response, and greatly increases the probability of relapse. HIV–VL coinfection has been reported from more than 35 countries. Initially, most of these cases were from south-western Europe but the number of cases are increasing in sub-Saharan Africa, Brazil and South Asia.6–8 The spread and overlap of both leishmaniasis and HIV infections in India is still not a major problem as it occurs in <2% of VL patients (unpublished data).

DIAGNOSIS
Clinical features of VL are non-specific and can be easily mistaken for any other common febrile illness such as malaria, enteric fever, tuberculosis, etc. VL is fatal if untreated. Therefore, highly sensitive diagnostic tests are needed which should also be highly specific as most of the current antileishmanial drugs are toxic. Besides being highly sensitive and specific, an ideal test should be simple and affordable.
Parasite detection

The visualization of the amastigote form of the parasite by microscopic examination of aspirates from bone marrow or spleen is the gold standard for the diagnosis of VL. Although the specificity is high, the sensitivity of microscopy varies, being higher for spleen (93%–99%) than for bone marrow (53%–86%). However, splenic aspiration can be complicated by life-threatening haemorrhage and therefore requires considerable technical expertise.9

Serological tests

Antibody-based tests, though widely used, have two major drawbacks. First, serum antibody levels remain detectable up to several years after cure;10-12 therefore, VL relapse cannot be diagnosed by antibody detection. Second, up to 32% of healthy individuals living in endemic areas with no history of VL are positive for antileishmanial antibodies due to asymptomatic infections.13,14

A recent study from an endemic region showed that almost half the healthy population were positive for signatures of infection, either serology or PCR-positive (unpublished data). This makes the diagnosis of VL in an endemic region difficult. Thus, antibody-based tests must always be used in combination with a standardized clinical case definition for VL.

Serological methods can be grouped into non-specific and specific tests.

Non-specific tests. Tests such as formal gel test have been used in the past but should be abandoned because of their poor specificity and sensitivity.

Specific tests. Serological tests based on indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA) or western blot have shown high diagnostic accuracy in most studies but are poorly adapted to field settings. The direct agglutination test (DAT) and the rK39-based immunochromatographic test (ICT) are two serological tests that have been specifically developed for field use.

In DAT, whole promastigotes stained with coomassie brilliant blue are incubated overnight with sera of patients and agglutination observed. Initially, aqueous antigen was used but it had the drawback of requiring a cold chain and having a short life. Now, freeze-dried antigen has been developed which can be transported at ambient temperature. In a meta-analysis of studies using DAT, the test had sensitivity and specificity of 94.8% (95% CI 92.7%–96.4%) and 85.9% (95% CI 72.3%–93.4%), respectively.31 However, the major disadvantages of DAT are the need for multiple pipetting, long incubation time, high cost of antigen and limited production of quality controlled antigen.16,17

rK39 is a 39-amino acid repeat that is part of a kinesin-related protein in L. chagasi and is conserved within the L. donovani complex.13 Immunochromatographic strip tests (ICTs) based on rK39 are easy to perform, rapid, cheap and yield reproducible results. A meta-analysis that included 13 validation studies of the rK39 ICT showed sensitivity and specificity estimates of 93.9% (95% CI 87.7%–97.1%) and 95.3% (95% CI 88.8%–98.1%), respectively.18 However, this test shows a regional variation and has been shown to be less accurate in East Africa.19-21 Another format of rK39 ICT has been reported with higher sensitivity and specificity in Africa.22

Recently, the rK28 antigen has been introduced as a candidate for serological diagnosis of VL. In a micro-ELISA format it was compared to the rK39 ELISA, rK28 had 99.6% sensitivity (95% CI 97%–99%), which was similar to the sensitivity of rK39 ELISA (99.6%; 95% CI 97%–99%). Specificity of the rK28 antigen in non-endemic healthy, endemic healthy and different disease controls was 100% (95% CI 96%–100%), 94.2% (95% CI 88%–97%) and 95.5% (95% CI 84%–96%), respectively, which was similar to that of rK39 ELISA.23 A rapid format of rK28, though available, is yet to be commercialized and tested in the Indian subcontinent.24

Recently, a novel L. donovani antigen (BHUP2) was identified which when tested with sera of VL patients and controls in ELISA had a sensitivity of 94% whereas specificity in endemic healthy, non-endemic healthy and disease controls was 98%, 100% and 97%, respectively. Although preliminary, this 37kD protein has a strong potential for further development as a diagnostic tool.25

Antigen detection

This is an excellent method of diagnosing an infection. It is more specific than antibody-based immunodiagnostic tests and is expected to broadly correlate with the parasite load. This method is a better alternative to antibody detection. A latex agglutination test detecting a heat-stable, low molecular weight carbohydrate antigen in the urine of VL patients has shown promising initial results. Several studies conducted in East Africa and the Indian subcontinent showed good specificity but only low-to-moderate (48%–87%) sensitivity.21,26,27 The latex agglutination test correlated well with cure in a high proportion (97%–100%) of patients during antileishmanial treatment.27,28 Efforts are being made to improve the performance of this technique, as it holds promise as a test of cure, for which none of the current serological tests can be used.

Molecular diagnosis

The detection of parasite DNA by PCR in blood or bone marrow aspirates is more sensitive than microscopic examination. Because of its high sensitivity, PCR detects more asymptomatic infections than microscopic examination. Newer techniques such as quantitative nucleic acid sequence-based amplification (QT-NASBA) detects RNA in a background of DNA and may thus serve to measure viable parasites which significantly increase assay sensitivity. Recently, an easily readable format of PCR has been developed. This OligoC-TesT kit incorporates standardized PCR reagents with rapid oligochromatographic dipstick detection of PCR products. In a recent report from Kenya, the Leishmania OligoC-TesT had a sensitivity of 96.4% and a specificity of 88.8%, while the sensitivity and specificity of the NASBA-OC were 79.8% and 100%, respectively. These findings indicate high sensitivity of the Leishmania OligoC-TesT on blood while the NASBA-OC is a better marker for active disease.29 Despite advances, molecular diagnosis is currently restricted to only referral hospitals and research centres.

Thus, to conclude, at present, rK39-based rapid tests are widely used for the diagnosis of VL. However, their continued positivity long after cure and in a high proportion of individuals living in endemic areas is a major drawback.

TREATMENT

The treatment of VL is far from satisfactory. All antileishmanials with the exception of miltefosine have to be administered by the parenteral route. The duration of treatment is long, drugs are toxic and hospitalization is required for monitoring. However, during the past decade several breakthroughs in the treatment of kala-azar have been reported from India, which will most certainly change the face of therapy of VL in India and ultimately in other countries. These include the development and approval of oral
mitelofosine and paromomycin, single-dose treatment with liposomal amphotericin B (LAmB) and multidrug treatment.

**Pentavalent antimonials (Sh**$^{5+}$**)**

Sodium stibogluconate in doses of 20 mg/kg body weight for 28–30 days has been the standard first-line therapy in India. Large-scale misuse of this drug in Bihar, which included improper dosing, splitting of daily dose, and substandard batches of drugs caused the emergence of widespread antimony resistance in the region.\(^{40}\)

The level of unresponsiveness reached as high as 60% in India and adjoining Nepal.\(^{31,32}\) Due to the emergence of high level of resistance, sodium stibogluconate lost its utility in this region.

**Amphotericin B deoxycholate**

Amphotericin B deoxycholate is the drug most commonly used for the treatment of refractory VL in India.\(^{33,34}\) Amphotericin B has a high cure rate (\(>100\%\)) at a dose of 0.75–1 mg/kg for 15–20 intravenous infusions. However, it has many adverse effects, which necessitate close monitoring and hospitalization for 4–5 weeks, which ultimately increases the cost of therapy.

Lipid formulations of amphotericin B have lower toxicity and shorter duration of therapy. The total dose requirement for treatment of VL varies by geographical region. In India (\(L.\) donovani), a total dose of 20 mg/kg results in a cure rate of \(>95\%\) while a total dose of 18–21 mg/kg, has 90%–100% efficacy in southern Europe. Thus far, the prohibitively high cost of these lipid formulations has been a limiting factor in their use in endemic countries including India. Of all the lipid formulations, LAmB (Ambisome, Gilead Sciences, USA) has been tested most widely in all the leishmaniasis-affected regions including India, and is the only antileishmanial drug approved by the Food and Drug Administration, USA.\(^{35,36}\)

A preferential pricing agreement with WHO (agreement between Gilead and WHO of 14 March 2007) has reduced the price of LAmB (Ambisome®) for endemic regions initially to US$ 20 and now to US$ 18 per 50 mg vial.\(^{37}\)

After a series of studies with multiple and single doses, it was established that LAmB is highly effective in Indian VL.\(^{38–40}\)

Recently, a landmark study from India included 412 patients randomly assigned in a 3:1 ratio to receive either LAmB (at a dose of 10 mg/kg body weight) as a single dose or the conventional amphotericin B deoxycholate administered in 15 infusions of 1 mg/kg, given every other day during a 29-day hospitalization. Cure rates at 6 months were similar in the two groups: 95.7% (95% CI 92.6%–99.9%) in the conventional therapy group.\(^{41}\)

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**Paromomycin (aminosidine)**

This aminoglycoside-aminomycyclitol antibiotic has been used for the treatment of VL in a parenteral formulation and CL in both topical and parenteral formulations. In a phase 3 trial in the Indian subcontinent, a dose of 15 mg/kg of paromomycin sulphate (11 mg base) for 21 days gave a cure rate of 95%. It was shown to be non-inferior to amphotericin B and was approved by the Indian government in August 2006 for the treatment of patients with VL.\(^{46}\) The main advantage of this agent is its cost, approximately US$10 per patient.\(^{47}\) The disadvantages include the need for administering intramuscular injections, monitoring of serum transaminases and inadequate data regarding its use in pregnancy.

**Combination therapy**

The growing resistance of the parasite to antileishmanial drugs suggests that the current practice of monotherapy needs to be reviewed. Multidrug combination therapy has been used successfully in tuberculosis, leprosy and malaria. The rationale behind combination therapy is increased activity through the use of compounds with synergistic or additive activity, prevention of drug resistance, requirement of lower dose, thereby reducing the chances of toxic side-effects and cost, and increased spectrum of activity. Multidrug therapy is expected to reduce the probability of developing resistance in parasites, thereby prolonging the useful therapeutic life span of the existing drugs.

In a randomized, non-comparative, group-sequential, triangular design study, 181 subjects were assigned to treatment with 5 mg/kg of LAmB alone, 5 mg/kg of LAmB followed by miltefosine for 10 days or 14 days or 3.75 mg/kg of LAmB followed by miltefosine for 14 days. When it became apparent that all regimens were effective, 45 additional, non-randomized patients were assigned to receive 5 mg/kg of LAmB followed by miltefosine for 7 days. The final cure rates were high (\(>95\%\)) and similar in all the groups. These results suggest that a single infusion of LAmB (in most instances, administered in an outpatient setting) followed by a brief self-administered course of miltefosine could be an excellent option against kala-azar in India.\(^{48}\)

In a subsequent phase 3 trial in India, three-drug combinations (single injection of 5 mg/kg LAmB and 7-day 50 mg oral miltefosine or 10-day 11 mg/kg intramuscular paromomycin; or 10 days each of miltefosine and paromomycin) were used. All the combinations showed excellent cure rates (\(>97\%\) in all arms) and were non-inferior to the standard treatment.\(^{49}\)

As the efficacy and required dosage of the antileishmanial agents vary in different areas, the WHO recently published the treatment recommendations for VL based on these regional differences. The treatment recommendations for VL and PKDL caused by \(L.\) donovani are given in Tables I and II. According to WHO, the ideal treatment for VL should cure the patient, reduce the risk for relapse and for PKDL and reduce the transmission of resistant parasites. To ensure compliance and completion of the course of therapy, direct observation should be implemented, mainly for oral miltefosine.\(^{50}\)
TREATMENT OF HIV–VL COINFECTION

Patients with HIV–VL coinfection have a high parasite burden, a weak immune response, poor response to treatment and a high relapse rate. Pentavalent antimonials are more toxic to patients with HIV, who require close monitoring for pancreatitis and cardiotoxicity. The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity. The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8 The best option for these patients are LAmB at 4 mg/kg given on days 1–5, 10, 17, 24, 31 and 38. Secondary cardiotoxicity.8

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1. Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over 3–5 days period up to a total dose of 15 mg/kg (A) by infusion or 10 mg/kg as a single dose by infusion (A)

2. Combinations (co-administered) (A)
   - liposomal amphotericin B (5 mg/kg by infusion, single dose) plus miltefosine (daily for 7 days, as below)
   - liposomal amphotericin B (5 mg/kg by infusion, single dose) plus paromomycin (daily for 10 days, as below)
   - miltefosine plus paromomycin, both daily for 10 days, as below

3. Amphotericin B deoxycholate: 0.75–1.0 mg/kg per day by infusion, daily or on alternate days for 15–20 doses (A)

4. Miltefosine: for children aged 2–11 years, 2.5 mg/kg per day; for people aged ≥12 years and <25 kg body weight, 50 mg/day; 25–50 kg body weight, 100 mg/day; >50 kg body weight, 150 mg/day; orally for 28 days (A) or
   - Paromomycin: 15 mg (11 mg base) per body weight per day intramuscularly for 21 days (A)

5. Pentavalent antimonials: 20 mg Sb^5+/kg per day intramuscularly or intravenously for 30 days in areas where they remain effective: Bangladesh, Nepal and the Indian states of Jharkhand, West Bengal and Uttar Pradesh (A)

Rescue treatment in case of non-response: conventional amphotericin B deoxycholate infusions or liposomal amphotericin B at higher doses

VACCINE

Leishmaniasis is unique among parasitic diseases because a single vaccine has the potential to protect against more than one species and be successful at both treating and preventing the disease. Unfortunately, there is no vaccine approved for VL, though several vaccine development programmes are under way.

VL ELIMINATION PROGRAMME

India, Nepal and Bangladesh harbour an estimated 67% of the global VL disease burden,41 and the governments of these countries have launched a regional VL elimination programme. The target of this programme is to eliminate VL as a public health problem in these countries by 2015, by using a local approach to reduce the annual incidence of VL to less than 1 case per 10 000 individuals.

The rationale for VL elimination in the Indian subcontinent are as follows: the disease in this area is anthroponotic with humans as the only reservoir and Phlebotomus argentipes sandflies being the only known vector; new and more effective drugs and a rapid diagnostic test—the r39 ICT—are available that can be used in the field; there is strong political commitment and inter-country collaboration; and the disease is endemic in only a limited number of districts. Miltefosine is currently being used in the elimination initiative in the three countries.

CONCLUSION

The r39-based ICT remains one of the most sensitive, simple, rapid and affordable test for the diagnosis of VL. However, biomarkers of active disease and parasite resistance and test of cure are urgently needed.

Recently, advances have been made in the treatment of VL such as the use of single-dose LAmB, combination therapy and newer drugs such as paromomycin. However, the inventory of antileishmanial drugs is very small, and emergence of drug resistance is complicating the control of leishmaniasis. Combination therapy by reducing the duration of therapy and decreasing the chances of developing resistance should be encouraged. The development of prophylactic as well as therapeutic vaccines and immunomodulators would go a long way in controlling the disease.

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