

Visceral leishmaniasis - Kala-Azar

Distribution

Currently, 90% of all visceral leishmaniasis occurs in India, Bangladesh, Nepal, Ethiopia, Sudan and Brazil. Visceral leishmaniasis may be responsible for 500,000 new cases and > 50,000 deaths per year.

Clinical aspects

After an initial multiplication in the skin, causing a transient small lesion, the parasites can further multiply in bone marrow, liver and spleen, causing visceral leishmaniasis. The incubation period is usually 2 to 6 months. The pathogens are usually *Leishmania donovani* and *L. infantum*, but rarely *Leishmania tropica*. *L. chagasi* is now considered identical to *L. infantum* and was possibly introduced into the New World via infected dogs or rats at the time of the Spanish and Portuguese conquests, although there are doubts about this.

Visceral leishmaniasis in Southern Europe was initially considered a pediatric disease (hence the name *L. infantum*). However, it is clear that all age groups can be infected. The disease is characterized by a persistent inflammatory state with chronic fever, enlarged liver and spleen and a low blood count (pancytopenia = anemia + leukopenia + thrombocytopenia). This must be distinguished from an aplastic bone marrow. The patient becomes susceptible to other infections (pneumonia, tuberculosis, dysentery), which can sometimes prove fatal. Symptoms and signs of superimposed bacterial infections may confuse the clinical picture at the time of initial diagnosis. Low blood platelet counts result in a bleeding tendency (nosebleeds, bruising, etc.). Sometimes, there are other symptoms, such as swollen lymph nodes, which are more common in Sudan than in India. Weight loss and emaciation are frequent and substantial. The skin can turn a dark color: kala azar (Hindi) means "black fever" and refers to this hyperpigmentation. This was mainly described in Indian cases. The reason for this hyperpigmentation is not clear. The infection can proceed atypically in HIV patients (for example, without fever or splenomegaly or with negative serology). When

immunosuppression is induced by chemotherapy, latent visceral leishmaniasis can become clinically apparent.



Visceral leishmaniasis (kala azar) with hepatosplenomegaly. Copyright ITM

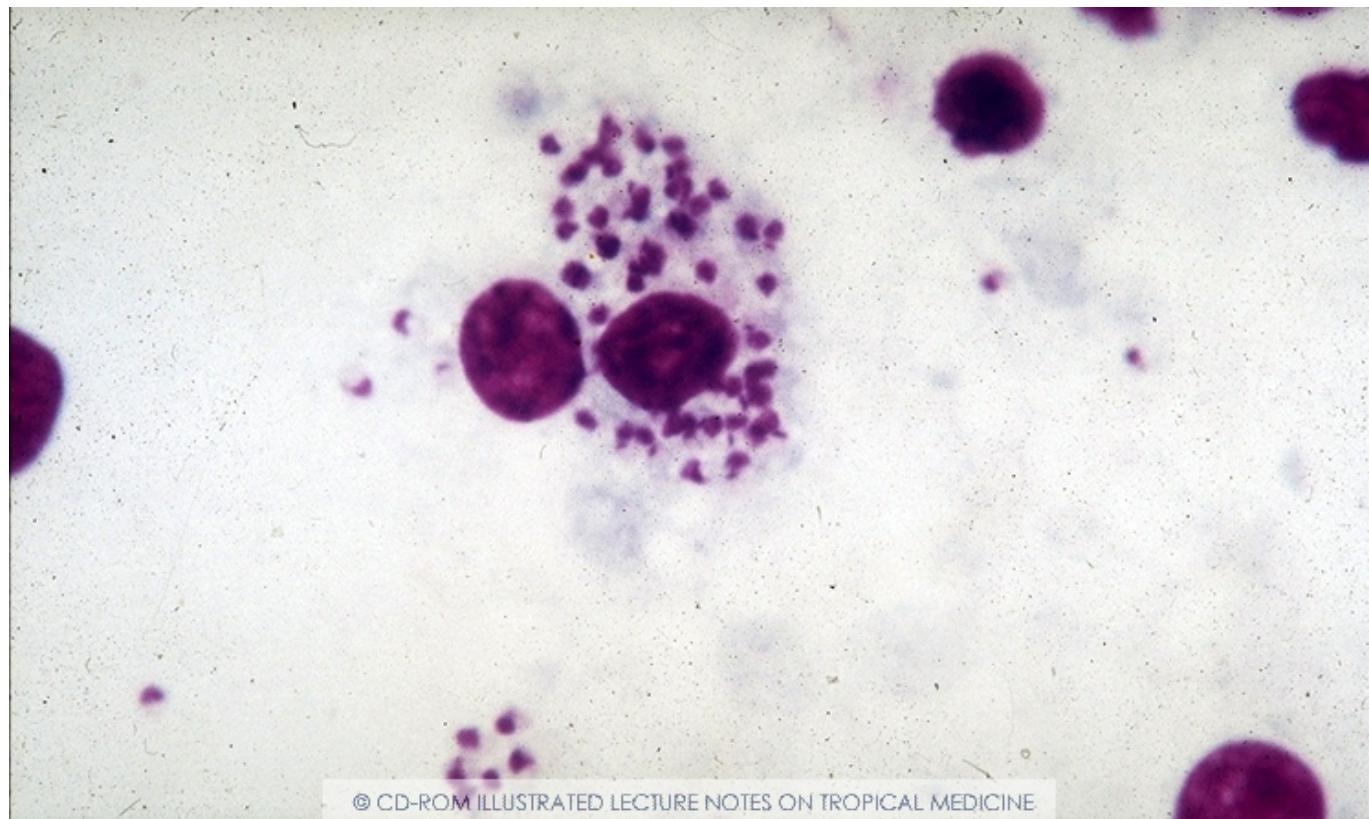
Post-Kala-azar dermal Leishmaniasis (PKDL)

A skin condition called post-kala azar dermal leishmaniasis (PKDL) can occur after a patient has suffered from kala azar. PKDL rarely occurs without being preceded by kala-azar. PKDL occurs on average 4-8 months after kala azar (range 0-3 years), though there are strong regional variations (In India 2-3 years after the disease, in Sudan typically within six months). This disease occurs mainly in India (up to 20% of visceral leishmaniasis patients) and to a much lesser extent in the Middle East. In Sudan, the disease occurs regularly (56% of visceral leishmaniasis patients were diagnosed in one study). It is virtually unknown in the Mediterranean Basin or South and Central America. It involves discolored patches and

painless nodules on the skin that usually contain high numbers of amastigotes. Most of the lesions occur on the face (98%) and to a lesser extent on the thorax (80%), arms (70%), legs (40%), tongue (40%) and genitals (6%). This disease has a chronic course (years) and is, therefore, important for transmission. Parasites do not affect internal organs in PKDL. There is sometimes a concomitant neuritis, which can further contribute to the clinical resemblance to leprosy. Contrary to India, in East Africa, this condition heals spontaneously in up to 80% of patients. Treatment with glucantime can be given for 2 months or longer (4 months in India, where resistance to antimony is higher). Amphotericin B is also effective. The therapeutic place of miltefosine for PKDL is not clear at present.

Diagnosis

In endemic areas, fever lasting more than 2 weeks and accompanied by splenomegaly not responding to antimalarial therapy strongly increases the suspicion of visceral leishmaniasis, but this clinical picture is insufficient to confirm the diagnosis.



Leishmania amastigotes. Copyright ITM

Diagnosis of visceral leishmaniasis is not easy, as none of the tests have 100% sensitivity and 100% specificity. Clinical syndromic diagnosis lacks specificity as malaria, hyperreactive malaria splenomegaly, trypanosomiasis, typhoid fever, disseminated tuberculosis, brucellosis, hematological disorders, splenic abscess or splenomegaly due to portal hypertension all can be accompanied by an enlarged spleen, fever, wasting, anemia and/or lymphadenopathy. Because of the high cost and toxicity of current therapeutic options, empirical treatment is not advised. Therefore, confirmatory diagnostic tests must be used. The leishmanin skin test indicates past infection or cutaneous leishmaniasis and is not used to diagnose visceral leishmaniasis.

Direct diagnosis

Direct diagnosis is made by demonstrating the presence of amastigotes in a bone marrow, spleen or lymph node aspirate. The parasite is egg-shaped and measures 2-3 x 5 μm . With Giemsa staining, there is a pale blue cytoplasm, a well-defined nucleus and a smaller kinetoplast. Microscopy requires considerable expertise and training. Usually, bone marrow is obtained by sternum aspiration. The technique of spleen aspiration is more sensitive (in some studies approaching 100%, though in reality slightly lower) than bone marrow aspiration but can be risky (spleen rupture, hemorrhage). The platelet count should be above $40 \times 10^9/\text{litre}$. Active bleeding, severe anemia, jaundice, moribund state, pregnancy and lack of cooperation are contra-indications. Patients must lie in bed for several hours after the procedure. Vital signs must be checked frequently to allow early recognition of hemorrhage, and blood transfusion facilities must be available. To perform the procedure a 21-gauge needle and a 5 ml syringe is required. After penetration of the skin, the plunger is withdrawn, and the needle is quickly inserted into the spleen while maintaining suction and withdrawn immediately (i.e., less than 1 second). Lymph node aspiration and/or liver biopsy are sometimes necessary. The parasites can rarely be detected in peripheral blood monocytes.

Serology

Serology is positive in most cases of visceral leishmaniasis. Gel diffusions immunolectrophoresis, complement fixation test, indirect hemagglutination, Western Blot and countercurrent immunolectrophoresis have limited diagnostic accuracy and/or feasibility in the field. Indirect fluorescence tests (IFA) are an alternative but require a

fluorescent microscope. The direct agglutination test (DAT) is often used as this has a high sensitivity and specificity. Both liquid and freeze-dried antigens can be used, although liquid antigen is associated with poor reproducibility in East Africa (most likely due to decay of liquid antigens during storage and transport). Note that freeze-dried antigens do not require refrigeration. The DAT is simpler than many other tests but requires equipment, such as microplates and micropipettes, training and regular quality control. A suggested cut-off value 1/3200 is often used but should be evaluated in each setting. An alternative is to consider titers < 1/1600 negative, borderline between 1/1600 – 1/12800, and positive > 1/12800. It can be defended that in a rural endemic area, a patient with more than two weeks of fever and splenomegaly with strongly positive DAT values and no response to antimalarials doesn't necessitate formal demonstration of parasites. With borderline serological values, tissue aspiration and a search for amastigotes will be needed. A possibility in a small regional clinic is to absorb a drop of blood from a patient suspected to have kala-azar on a small filter paper and then punch out a standard-size disk from the blood spot. This way, one obtains a well-defined, accurate aliquot of absorbed blood. This can be transported and used for DAT in a well-equipped laboratory. Serology remains positive after cure. The fast agglutination screening test (FAST) is a simplified (single serum dilution) and more rapid version of the DAT (2-3 hours versus 18h). Because DAT is impractical in many field conditions, alternatives are being studied. ELISA is highly sensitive, but specificity depends on the antigen (amastigotes or promastigotes). Recombinant K39 antigen-based dipsticks using immunochromatography (ICT) have been an important step forward and have replaced DAT as a first-line test. K39 is a 39-amino acid repeat that is part of a kinesin-related protein of *L. chagasi*. This repeat is conserved within the *L. donovani* complex. The ICT tests are easy to perform, rapid and cheap. Twenty μ l of serum is added to the dipstick, which is then placed vertically in a test tube. Two drops of chase buffer solution provided with the dipstick are then added. The results are read after 5 to 10 minutes. Even a weak band in the test region is considered positive. A control line has to be visible. It is the most promising tool for diagnosing visceral leishmaniasis in peripheral centers.

Formol-gel test

In kala-azar, there is a very high production of non-specific immunoglobulins (and a decrease in albumin), especially in advanced disease (i.e., more than 3 months). This can be demonstrated by serum protein electrophoresis, which is usually unavailable in field

conditions. The proteins can be precipitated as a gel by formalin. Twenty μ l of 40% formaldehyde are added to 200 μ l of serum in a glass tube. After twenty minutes, the gelification reaction is visually assessed as positive or negative. The test is simple and cheap. The test can also be positive in patients with hyperreactive malaria splenomegaly.

KAtex

A urinary antigen detection test using latex agglutination (KAtex) has been developed to circumvent the limitations of serological tests. It detects a heat-stable low molecular weight carbohydrate antigen. This will become negative upon successful treatment. It can, therefore, distinguish an active from a past infection. A very high specificity and moderate to high sensitivity were reported. The test requires the boiling of 1 ml of urine for 5 minutes. About 50 μ l of the treated urine sample is added onto a reaction zone on a glass slide, and a drop of latex is added. The liquids are stirred to a completely homogenous mixture. Any agglutination reaction discerned when compared with a negative control is considered positive. The sensitivity varies with the parasite load.

Culture

Culture can be done from peripheral blood, buffy coat or tissue aspirates. The microculture method improves sensitivity and decreases incubation periods. Cultures are expensive, time-consuming and require expertise. A *Leishmania* parasite can survive for 3 days at 4° C but for only 1 day at room temperature in a Locke transport medium (a buffered glucose-salt solution with antibiotics).

Genome assays

Lack of standardization and quality control is a major concern of PCR and related assays. A multitude of gene targets, protocols and applications have been described. A PCR assay was developed to amplify the kinetoplast minicircle of *Leishmania* species (it can also be used in vector studies). The kinetoplast minicircle is an ideal target because it is present in 10,000 copies per cell, and its sequence is known for most *Leishmania* species. The very high sensitivity of PCR-based assays may actually be a disadvantage by being a marker of infection (transient or permanent) instead of being a marker of disease, as it will also pick up

asymptomatic carriers. Detailed genomic analysis of *L. donovani* showed that parasites can have two, three, four or even five sets of chromosomes in one organism. Further study of this ploidy-variation will investigate the possible clinical implications of this unexpected finding.

Montenegro test

Leishmanin is a compound obtained via in vitro culture of promastigotes. A skin test with leishmanin (Montenegro test) is negative during active visceral leishmaniasis but later becomes positive (after 6 to 12 months). The Montenegro test reflects the suppressed cellular immunity during infection. There is a specific anergy for *Leishmania* parasites during active disease. This test is mainly of epidemiological value and may support the diagnosis of cutaneous leishmaniasis. To perform the test, 0.1 ml is injected intradermally, and the local reaction is read after 48 hours (>5 mm induration = positive). A positive test eliminates the existence of active kala azar. Cutaneous leishmaniasis produces a positive Montenegro test.

Treatment of VL

Without treatment, the case fatality rate for fully manifest clinical visceral leishmaniasis (VL), or kala-azar, exceeds 90%. Mortality is often due to hemorrhagic or infectious complications.

Treatment primarily involves antileishmanial therapy, with cost and availability being the main constraints on drug selection. Additionally, local drug resistance must be considered, especially for VL cases originating in South Asia, as treatment responses can vary significantly by region. Supportive therapy is also crucial, addressing nutritional status, concomitant anemia, hemorrhagic complications, and secondary infections to optimize treatment outcomes.

Patients with VL should be screened for human immunodeficiency virus (HIV) coinfection, and if HIV is present, it should be treated aggressively. Without effective immune reconstitution, treatment response in HIV-VL coinfect ed patients is generally poor.

Species identification usually is not critical to treatment decisions for VL (in contrast with cutaneous leishmaniasis). However, sensitivity to specific drugs varies substantially by region, and first-line treatment recommendations in major visceral leishmaniasis-endemic

areas have diverged.

Pentavalent antimonial compounds.

One of the treatment options for visceral leishmaniasis is pentavalent antimony derivatives (antimony, chemical symbol Sb = Stibium). The derivative most frequently used is Glucantime® (meglumine antimonate, 85 mg Sb/ml) and rarely Pentostam® (sodium stibogluconate, 100 mg Sb/ml). One of their actions is to inhibit phosphofructokinase, the rate-limiting step in the parasites' glycolytic pathway. The standard dosing regimen consists of 20 mg/kg/day of antimony for 28 to 30 days. Monitoring for cardiotoxicity with ECG is advised: T-wave inversion and prolongation of the QT-time are indicative of threatening arrhythmia. The fever usually disappears after 1 week. The spleen begins to get smaller after 2 weeks but frequently requires 6 to 12 months to return to normal.

Alternative treatments:

Antimonials have been the mainstay for treating visceral leishmaniasis for decades, but the emergence of antimonial resistance in India and their toxicity caused a change in guidelines. Currently, the first-line drug in many settings is liposomal amphotericin B. Conventional amphotericin B, paromomycin, miltefosine and antimonials are alternatives. In HIV-infected patients, the combination of liposomal amphotericin B with a second drug is advised.

Amphotericin B is a polyene with a fairly complex structure and hydrophilic and lipophilic components. The recommended dose of amphotericin B [Fungizone®] is 0.5-1 mg/kg/day IV, given over 6 hours; total dose max. 1-3 g. This drug is mainly used for the treatment of deep mycoses, though it is also active against Leishmania. It is a rather toxic medication. Shivering, fever, nausea, vomiting, headache, anemia, phlebitis at the site of the infusion, cardiotoxicity, kidney failure, hypokalaemia and hypomagnesemia are frequent side effects. Side effects occurring shortly after administration can be reduced by cortisone IV or meperidine (pethidine), a morphine analog. Administration of 500-1,000 ml physiological isotonic saline solution before starting the IV drip reduces the risk of nephrotoxicity. The toxicity of the drug is reduced by pharmacological complexing with lipids before administration. The drugs are then concentrated in the reticuloendothelial system and not in the kidneys so that a higher daily dose per kg of body weight can be administered and

treatment time shortened (e.g., to 5 days). There are good indications that single-dose treatment (high dose; 10 mg/kg of the liposomal formulation) is useful, at least in the Indian subcontinent (India, Nepal, Bangladesh). In 1990, AmBisome® was developed as a first-choice drug. Several lipid formulations of amphotericin B are now available. They differ from each other in the type of phospholipid and the ratio of lipid to amphotericin B. Good results have been obtained with these lipid formulations. The price of these medications (AmBisome®, Amphotec®, Abelcet®) has come down but is still high for the average rural farmer in a developing country.

Formulations of Amphotericin B

1. Fungizone®: Amphotericin B deoxycholate. Contains no lipids.
2. Emulsification of Fungizone® in Intralipid 20%: little reduction of toxicity
3. AmBisome®: L-AmB: incorporation in liposomes (vesicles).
4. Abelcet®: ABLC or Amphotericin B Lipid Complex. Microscopically small ribbon-like membranes formed by complexing with phospholipids.
5. Amphotec®: ABCD (= Amphocil®) Amphotericin B Colloidal Dispersion: AmB-cholesteryl sulfate forms disc-shaped structures.

Injectable aminosidine (paromomycin) is now a valid alternative. It is an aminoglycoside antibiotic. In 2007, the results of an Indian study showed that paromomycin IM, at a dose of 11 mg/kg/day x 21 days, was non-inferior to amphotericin B at a dose of 1 mg/kg IV every other day x 30 days. The combination with antimonials for 17 days was also effective in East Africa. Pain at the injection site, liver toxicity and ototoxicity were reported as side effects. Paromomycin for IM administration is licensed in India and, since 2012, also in Nepal. Combined with antimonials, it is the first-line regimen in East Africa.

Miltefosine (Impavido®, Miltex®) was approved for use in India in 1992. It became more widely became available in subsequent years. Miltefosine interferes with certain cellular signal cascades and membrane synthesis, though its precise mode of action is still unknown. *Leishmania* contains many ether lipids in the cell membrane. The main advantage of the compound is that it can be given orally, in contrast to the injectable antimony derivatives and amphotericin B. It cannot be given IV as this would lead to hemolysis. The molecule is fairly easy to produce and should eventually bring down the price, which is quite high in the West.

The daily dose for adults is 100-150 mg and for children, 2.5 mg/kg/day. It should be given for 4 weeks. The half-life is several weeks. The cure rate was high in studies in India, although lower efficacy was found in East Africa. Dose-dependent gastrointestinal discomfort often occurs, and reversible hepato- and nephrotoxicity sometimes occurs. It is teratogenic, and so cannot be given to pregnant women or women who want to conceive 6 months after treatment. How quickly resistance to miltefosine will develop when used as monotherapy in the field is not yet clear. It is relatively easy to induce resistance in vitro. In this regard, it is of concern that success rates have been declining over the last years in the Indian subcontinent, although it is not yet well defined whether this relates to true parasite resistance, underdosing or evolving parasite fitness are also considered as alternative explanations. This has led to using liposomal amphotericin B (AmBisome) as the first-line treatment in the Indian subcontinent.

Combination therapy is the suggested way forward to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration and possibly decrease cost. Pentavalent antimonials combined with paromomycin are now the first-line treatment in East Africa. Other combinations including liposomal amphotericin B, paromomycin and miltefosine were found effective in India in phase III trials.

Until now, the generic recommendation for treatment of a VL episode in an HIV co-infected patient was first to consider lipid formulations of amphotericin B, infused at a dose of 3-5 mg/kg daily or in 10 intermittent doses (on days 1-5, 10, 17, 24, 31 and 38) to a total dose of 40 mg/kg. Evidence from clinical trials in Ethiopia and India on the efficacy and safety of combination therapy (liposomal amphotericin B (L-AMB) plus miltefosine) to treat VL in HIV co-infected patients instead of monotherapy has offered new possibilities for case management. Some evidence has emerged for considering secondary prophylaxis after the first episode of VL, with pentamidine in Ethiopia and with amphotericin B or its lipid formulation in India.

Table: The main drugs currently used for the treatment of visceral leishmaniasis.

Drugs	Regimen	Marketing^a	Clinical efficacy	Resistance
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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)
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
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
Miltefosine	2-2.5 mg/kg/d orally daily over 28 days (India only)	Paladin (Impavido®)	Asia: 94% (India) Africa: single field study (93% in HIV(-))	Readily obtained in lab isolates
Paromomycin sulphate	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 lab isolates

^a marketing authorization holder iv: intravenous; im: intramuscular; SSG: sodium stibogluconate

Table: The main drugs currently used to treat visceral leishmaniasis (continued).

Drugs	Toxicity	Cost/course	Issues
Pentavalent antimonials	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	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	Uncommon and mild; Nephrotoxicity (limited)	Preferential price: \$280 (20mg/kg total dose) Commercial price: ~ 10x	Price; Need for slow iv infusion; Heat stability (stored <25° C)
Miltefosine	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 sulphate	Uncommon, Nephrotoxicity Ototoxicity Hepatotoxicity	~ \$15	Efficacy variable between and within regions; Potential for resistance (?)

Response to treatment

The response to treatment of visceral leishmaniasis is generally assessed clinically. Key indicators include the resolution of fever within one to two weeks, a decrease in spleen size

within a month of starting treatment, and weight gain. Additional parasitologic testing is usually not required, but patients should be monitored clinically for at least 12 months and instructed to return if symptoms recur. In immunocompetent patients, most relapses occur within 6 to 12 months after treatment completion, though relapses up to 18 months post-treatment have been described. Immunocompromised patients should be followed for a minimum of one year, ideally lifelong or until effective immune reconstitution, to monitor for post-treatment relapse symptoms.

For patients with an equivocal clinical response (e.g., no decrease in spleen size, continued fever) or those suspected of having a relapse, bone marrow or splenic aspirate should be performed to confirm relapse or to look for alternative diagnoses.

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