Leishmaniasis
<table>
<thead>
<tr>
<th>Leishmaniasis</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>3</td>
</tr>
<tr>
<td>Classification</td>
<td>6</td>
</tr>
<tr>
<td>Distribution</td>
<td>9</td>
</tr>
<tr>
<td>Vector</td>
<td>12</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>14</td>
</tr>
<tr>
<td>Life cycle, Leishmania sp.</td>
<td>15</td>
</tr>
<tr>
<td><strong>Visceral leishmaniasis - Kala Azar</strong></td>
<td>18</td>
</tr>
<tr>
<td>Distribution</td>
<td>18</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>19</td>
</tr>
<tr>
<td>Post- Kala azar Dermal Leishmaniasan (PKDL)</td>
<td>21</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>21</td>
</tr>
<tr>
<td>Treatment of VL</td>
<td>25</td>
</tr>
<tr>
<td><strong>Cutaneous leishmaniasis</strong></td>
<td>32</td>
</tr>
<tr>
<td>Distribution</td>
<td>32</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>32</td>
</tr>
<tr>
<td>Diagnosis of cutaneous leishmaniasan</td>
<td>37</td>
</tr>
<tr>
<td>Treatment</td>
<td>37</td>
</tr>
<tr>
<td><strong>Mucocutaneous leishmaniasis</strong></td>
<td>40</td>
</tr>
<tr>
<td>Distribution</td>
<td>40</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>40</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>42</td>
</tr>
</tbody>
</table>
Leishmaniasis

Summary

- In humans obligate intracellular parasite with replication in macrophages
- Cutaneous form: chronic painless ulcers or nodules, amastigotes in smear
- Visceral form: chronic fever, hepatosplenomegaly, pancytopenia, persistent inflammatory state. Lethal if not treated
- Diagnosis of kala azar: amastigotes in bone marrow and other sites, serology, antigen detection
- Mucocutaneous: chronic destructive lesions in mouth/nose, frequent clinical diagnosis
- Transmission via about 30 species of sandflies
- Zoonotic transmission: animal reservoir (especially dogs and rodents)
- Anthroponotic transmission: human reservoir, e.g. Indian kala azar and in cutaneous *L. tropica*
- Treatment with antimony derivatives, amphotericin B, miltefosin, pentamidine. Combination treatment increasingly in use.

General
Leishmania braziliensis ulcer on the wrist and spread via the lymphatics. Lesions occurred after a visit to rural Bolivia. Copyright ITM
There are several species of *Leishmania* parasites and these can cause various clinical conditions. They can be responsible for chronic ulcers and skin nodules. Sometimes both skin and mucosae are affected (mucocutaneous leishmaniasis). When deep organs are affected, the condition is called visceral leishmaniasis. The *Leishmania* species that cause these various clinical conditions always have the same morphology under the microscope. However, there are differences in parasite DNA, proteins, enzymes and mode of development in the insect vector, etc. *Leishmania* parasites can in turn be infected with a RNA virus (the “leishmania virus”) though the significance of this is not yet known.

The classification, distribution and pathogenicity of the various *Leishmania* species is quite complicated. New data are regularly becoming available (for example, *L. tropica* was shown to be able to cause visceral leishmaniasis in rare cases). The whole taxonomy will probably change as more and more genetic information becomes available. A distinction is made between zymodemes (iso-enzyme patterns), schizodemes (kDNA analyses with restriction enzymes), serodemes (via reactions with monoclonal antibodies) and rapdemes (using PCR with random primers). Some 30 different *Leishmania* species have been described (10 in the Old World and 20 in the New World). Many of these can infect humans. The genus *Leishmania* is frequently subdivided into the subgenera *Leishmania* and *Viannia*. There are substantial geographical genetic variations. Hence in the dry western part of Peru *L. peruviana* causes the disease “uta”, an ulcerative form without mucocutaneous lesions. This organism contains less DNA in some of the chromosomes than the virulent *L. braziliensis*, the pathogen causing Espundia, a disease which occurs in the forests on the other side of the Andes in Eastern Peru. One of the differences is the number of copies of the leishmanolysin gene, which codes for an important surface antigen (gp63). This zinc protease has a role in adhesion to macrophages and survival in the phagolysosome. It is regarded as an important virulence factor. *L. braziliensis* contains more leishmanolysin genes than *L. peruviana*. The protein is being studied as, among other things, the basis for an experimental vaccine.

**Classification**

There is still no generally accepted internationally agreed definitive taxonomy. The following is for orientation:

<p>| Leishmania species |</p>
<table>
<thead>
<tr>
<th>Leishmania species</th>
<th>L, D, V, CL, mucosal, visceral</th>
<th>Zoonotic</th>
<th>African, American, European, Indian, and Asian colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. (Viannia) braziliensis</td>
<td>LCL, mucosal</td>
<td>zoonotic</td>
<td>Latin America</td>
</tr>
<tr>
<td>L. (Viannia) panamensis</td>
<td>LCL, mucosal</td>
<td>zoonotic</td>
<td>Northern South America and southern Central America</td>
</tr>
<tr>
<td>L. (Viannia) peruviana</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Peru</td>
</tr>
<tr>
<td>L. (Viannia) guyanensis</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Viannia) lainsoni</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Viannia) columbiensis</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td>L. (Leishmania) amazonensis</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Leishmania) mexicana</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Central America, Mexico</td>
</tr>
<tr>
<td>L. (Leishmania) pifanoi</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Leishmania) venezuelensis</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td>L. (Leishmania) garnhami</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Leishmania) aethiopica</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Ethiopia, Kenya</td>
</tr>
<tr>
<td>L. (Leishmania) killicki</td>
<td>LCL</td>
<td>zoonotic</td>
<td>North Africa</td>
</tr>
<tr>
<td>L. (Leishmania) major</td>
<td>LCL</td>
<td>zoonotic</td>
<td>North and East Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td>L. (Leishmania) tropica</td>
<td>LCL</td>
<td>anthroponotic</td>
<td>North Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td>L. (Leishmania) donovani</td>
<td>LCL, visceral</td>
<td>anthroponotic</td>
<td>Central Asia, Africa</td>
</tr>
<tr>
<td>L. (Leishmania) infantum</td>
<td>LCL, visceral</td>
<td>zoonotic</td>
<td>South Europe, North Africa, Central and South America</td>
</tr>
</tbody>
</table>

Leishmania species

Leishmaniasis | 7
<table>
<thead>
<tr>
<th>New World</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L. (Viannia) b</td>
<td>LCL, mucos</td>
<td>zoonotic</td>
<td>Latin America</td>
</tr>
<tr>
<td>L. (Viannia) p</td>
<td>LCL, mucos</td>
<td>zoonotic</td>
<td>Northern South America and southern Central America</td>
</tr>
<tr>
<td>L. (Viannia) p</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Peru</td>
</tr>
<tr>
<td>L. (Viannia) g</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Viannia) l</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Viannia) c</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Central America, Mexico</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>zoonotic</td>
<td>Northern South America</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>zoonotic</td>
<td>South America</td>
</tr>
<tr>
<td>Old World</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL, DCL</td>
<td>zoonotic</td>
<td>Ethiopia, Kenya</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>zoonotic</td>
<td>North Africa</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>zoonotic</td>
<td>North and East Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL</td>
<td>anthroponoti</td>
<td>North Africa, Middle East, Central Asia</td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL, visceral</td>
<td>anthroponoti</td>
<td>Central Asia, Africa</td>
</tr>
<tr>
<td>Old and New World</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. (Leishman)</td>
<td>LCL, visceral</td>
<td>zoonotic</td>
<td>South Europe, North Africa, Central and South America</td>
</tr>
</tbody>
</table>

Legend: LCL : localised cutaneous leishmaniasis ; DCL : diffuse cutaneous leishmaniasis

Visceral leishmaniasis is mainly caused by the *Leishmania donovani* complex. There are several species in this complex:
1. *Leishmania donovani* (India, Pakistan, sub-Saharan Africa, East Africa)
2. *Leishmania infantum* (Mediterranean Basin, Middle East)
3. *Leishmania chagasi* (South America = *Leishmania infantum*)
4. *Leishmania archibaldi* (Africa) – of unclear importance

In the Old World skin lesions are mainly due to:

1. *L. tropica* (Mediterranean basin, Middle East). Frequently dry lesions
2. *L. major* (Middle East, sub-Saharan Africa). Frequently moist lesions
3. *L. aethiopica* (Ethiopia, Kenya). Sometimes also affects mucosa
4. *L. killicki* (North Africa) – of lesser importance

In addition, *L. infantum* and *L. donovani* (more exceptionally) can also cause skin lesions.

In (mainly South and Central) America skin lesions are caused by the *L. mexicana* and *L. braziliensis* complex. These complexes are subdivided into species:

1. *L. mexicana* complex: *L. mexicana, L. venezuelensis, L. amazonensis*
2. *L. braziliensis* complex: *L. braziliensis, L. panamensis, L. guyanensis, L. peruviana*

Mucosal lesions are common in infections with *L. braziliensis*. One should always keep in mind that the clinical lesions of leishmaniasis are a consequence of the parasite species on the one hand and of the immunological resistance and reaction of the patient on the other.

Infections occur very rarely with other *Leishmania* species: *L.(Viannia) naiff, L. (Viannia) shawi.*

**Distribution**
Map Leishmania infantum, L. aethiopica, L. tropica, L. major. Adapted from Colour Atlas
Map Leishmania mexicana and L.braziliensis. Adapted from Colour Atlas.

Map of the areas endemic for Leishmania chagasi, L. infantum, L. donovani, pathogens leading to kala azar. Adapted from Colour Atlas
Mucocutaneous leishmaniasis occurs in Central and South America and occasionally in East Africa.

Visceral leishmaniasis occurs from western China to the Mediterranean Basin, East Africa and Central and South America. It is very rare in Africa south of the equator. The majority of cases occur in 6 countries: Bangladesh, Nepal, India, Ethiopia, Sudan and Brazil.

The cutaneous form is seen from India to the Mediterranean Basin, the northern half of the African continent and in Central and South America.

Leishmaniasis does not occur in Northern Europe, Canada, Uruguay, Chile, South Africa, Australia and Oceania. While Southeast Asia was thought to be leishmania free, an increasing number of visceral leishmaniasis cases have been reported from Thailand more recently.

For additional information and geographical risk in Europe; see www.leishrisk.net

Vector

The parasite is transmitted by the bite of infected female sandflies: Phlebotomus in the Old World and Lutzomyia in Central and South America. These genera, together with the blood-sucking genus Sergentomyia [little significance for man, as they suck blood from reptiles], belong to the Psychodidae family. Morphologically they very closely resemble each other. The name “sandfly” can be confusing as this name is sometimes used for other species as well. Sandflies are vectors of leishmaniasis, pappataci virus (an arbovirus) and Bartonella bacteria.
Sandfly. Lutzomyia and Phlebotomus species are vectors of leishmaniasis in the New, resp. Old World. Photo Cochabamba, Bolivia

Only some 10% of the approximately 600 known species of sandflies are vectors, and only 30 of these are important. A fly remains infected for life. In endemic areas, a minority of sandflies are infected usually below one per cent.

The female insects need blood in order to lay their eggs. Most species bite at night and at dusk. There are exceptions to this, such as Lutzomyia wellcomei, the main vector of L. braziliensis, which bites mainly during daytime. They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards, etc.) and man. They are small, soundlessly flying insects (approximately 2 mm in length). Because of these small dimensions they can get through standard mosquito nets. Impregnation with permethrine (cf. malaria) can help. Because of the very short mouthparts of the insects, they cannot bite through clothing. They are poor flyers. They will usually fly quite low and will remain in the vicinity of their breeding ground. They will also not fly when there is any wind. This knowledge can be exploited by having a fan or ventilator on at night in the bedroom to prevent
sandflies from flying. They require high humidity and temperature for breeding, although they can be observed in dry regions provided there are sites with a favourable local microclimate (crevices, termite mounds, caves, hollows and holes in tree roots, etc) where 15 to 80 tiny eggs can be laid. The larvae cannot survive drying out. They will feed on organic waste and then pupate. Sandflies reproduce optimally at 23-28°C and at a relative humidity of 70-100%. Temperatures below 10°C or above 40°C are unfavourable for their survival. Measures used to control adult sandflies include the use of insecticides for residual spraying of dwellings and animal shelters, space-spraying, insecticide-treated nets, impregnated dog-collars and personal protection through application of repellents/insecticides to skin or fabrics. Bednets will be most useful in areas with peridomestic vectors (e.g. P. argentipes in India) whereas in areas where the vector bites in the field (e.g. P. martini in Kenya and Uganda) this can be expected to be less effective. Because the breeding-sites of sandflies are generally unknown, control measures that act specifically against immature are not feasible. Reports of insecticide-resistance refer to only three sandfly species (P. papatasi, P. argentipes and S. shorttii) against DDT in one country (India), although there are reports of DDT-tolerance in several countries.

Pathophysiology

An important aspect of the immune system is the balance between two arms of the T-helper response. Broadly speaking, the T-helper1 (Th1) response is tailored to intracellular pathogens, such as viruses and some bacteria and parasites. Because these organisms live inside cells, they are not accessible to antibodies. The Th1 response therefore stimulates other defence mechanisms such as macrophages. The T-helper2 (Th2) system, by contrast, promotes a vigorous antibody response. The two arms are antagonistic, so a strong Th1 response means a weak Th2 response and vice versa. In leishmaniasis, where the parasites are intracellular, a strong Th1 response will kill the parasite and a strong Th2 response will lead to uncontrolled disease.

A gel produced by the Leishmania parasite in the gut of the sandfly prevents the insect from feeding properly. This causes more effort to feed, providing more chances for transmission of the parasite. The gel is injected into the human with the parasite and increases the severity of the infection. The crucial molecule in the gel, called filamentous proteophosphoglycan, interferes with the human immune system. The gel pushes the immune response to the non-protective T-h2 arm. The parasite thus manipulates the sandfly to make it feed more and then manipulates the host’s immune system so that it can spread unchecked. Sandfly saliva is important for the establishment of infection and disease pathogenesis. The sandfly saliva contains the vasodilator maxadilan. Saliva proteins seem to influence the immune response, resulting in a shift from Th1 to Th2 response. It is possible that the age-related decrease of susceptibility to leishmaniasis is due to anti-sandfly saliva antibodies.
Life cycle, *Leishmania sp.*

The parasite’s life cycle is quite simple. When an infected sandfly bites, the parasite (as a promastigote) is injected directly into the skin. This unicellular parasite then penetrates the cells of the reticuloendothelial system (macrophages), where it multiplies in the form of amastigotes (the non-flagellate form) (“a” = without; “mastix” = whip). It is this form that can be seen in a skin biopsy or bone marrow aspirate. Multiplication results in bursting of the host cell, whereupon other cells become infected.
Leishmania amastigotes. This is the form present in human tissue. Copyright ITM

Leishmania promastigotes. The parasite has this morphology when residing in the sandfly vector. Copyright ITM

When another sandfly later bites, these infected cells can be ingested. The parasite is then still located in infected macrophages. The blood meal in the stomach is completely surrounded by a peritrophic membrane. The parasite transforms into a different form (promastigote with flagellum) in the insect and then multiplies. After 2-3 days the peritrophic membrane is digested and the parasites are released into the lumen of the stomach and intestine. They then attach to the microvilli of the intestine by means of their flagellae. They produce an enzyme, chitinase which damages the chitin coating of oesophageal-gastric junction, so that the valve between stomach and oesophagus no longer functions adequately and leaks resulting in a backflow of parasites to the mouthparts. The parasites accumulate 7 to 10 days later in the insect’s proboscis and can be injected when the insect bites its next victim. The insect is infectious 7-10 days after an infected meal and has to survive for
this time in order to be transmitted. Haemoglobin degradation products inhibit the secretion of chitinase and/or inhibit the enzyme itself making backflow of parasites to the mouthparts more difficult. Certain plant sugars do not have this effect. The insects also feed on plant juices. A balance between plant and animal feeding is required for successful transmission. A botanical description of the vector’s environment (biotope) can be important in scientific studies.

Kala azar can be transmitted in other ways, but these are exceptional, namely shared use of needles among intravenous drug users or infected blood transfusion. Very rare cases of congenital kala azar infection have been reported.

**Historical note, discovery of the parasite**

The search for the origin of kala azar initially proceeded with great difficulty. Many hypotheses were investigated: for example, hookworm infection (ancylostomiasis) or malaria were thought to be responsible for the clinical condition. In 1900 an Irish soldier developed kala azar, after a stay in Dum Dum, near Calcutta, India. He died in England. The Scottish physician Dr. William Boog Leishman, later Director-General of the medical service of the British Army, carried out the autopsy. In spleen tissue he discovered small particles within the macrophages. He suspected that these were a sort of partly digested trypanosomes. A previously used name for visceral leishmaniasis was “Dum Dum fever” and refers to this historical event. The Irish physician Dr. Charles Donovan investigated splenic aspirates (needle biopsies of the spleen) from kala azar patients and confirmed Leishman’s discovery. The tiny particles were called Leishman-Donovan bodies.

**Visceral leishmaniasis - Kala Azar**

**Distribution**

At present 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. This causes visceral leishmaniasis. The incubation period is usually 2 to 6 months. The pathogens are usually Leishmania donovani and L. infantum. 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 to be a paediatric disease (hence the name L. infantum). However it is clear that all age groups can be infected. The disease is characterised by a persistent inflammatory state with chronic fever, enlarged liver and spleen and a low blood count (pancytopaenia = anaemia + leukopaenia + thrombocytopaenia). This must be distinguished from an aplastic bone marrow. The patient becomes very 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 also other symptoms, such as swollen lymph nodes, more common in Sudan than in India. Weight loss and emaciation are frequent and substantial. The skin can turn a dark colour: kala azar (Hindi) means “black fever” and refers to this hyperpigmentation. This was mainly described from Indian cases. The reason of 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 kala azar 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 kala azar patients) and to a much lesser extent in the Middle East. In Sudan the disease occurs regularly (56% of kala azar patients in one study). It is virtually unknown in the Mediterranean Basin or in South and Central America. It involves discoloured patches and painless nodules on the skin that usually contain few, but sometimes moderate 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 very 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. 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 an alternative. 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 not sufficient for diagnosis.
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, haematological disorders, splenic abscess or splenomegaly due to portal hypertension all can be accompanied by enlarged spleen, fever, wasting, anaemia 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 is an indicator of past infection and is not used to diagnose visceral leishmaniasis.

Direct diagnosis

Direct diagnosis is made by demonstrating the presence of amastigotes (in 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 very nearly 100%, though in reality slightly lower) than bone marrow aspiration but can be risky (spleen rupture, haemorrhage). The platelet count should be above $40 \times 10^9$/litre. Active bleeding, severe anaemia, 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 haemorrhage 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, 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 immunoelectrophoresis, complement fixation test, indirect haemagglutination, Western Blot and countercurrent immunoelectrophoresis 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 antigen during storage and transport). Note that freeze-dried antigen does 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 of 1/3200 is often used but should be evaluated in each setting. An alternative is to consider titres $< 1/1600$ to be 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 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 with 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 to punch out a standard size disk from the blood spot. In 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 not practical in many field conditions alternatives are being studied. ELISA is highly sensitive, but specificity depends upon the antigen used (amastigotes or promastigotes). Recombinant K39 antigen-based dipsticks using immunochromatography (ICT) have been an important step forward and have replaced DAT as 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 µl of serum are added on 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 the diagnosis of visceral leishmaniasis in peripheral centres. The specific format (brand) of dipstick may play an important role (e.g. Opti-Leish™, DiaMed IT Leish™, DiaMed DUAL IT L/M™ versus Kalazar Detect™).

**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, but this impractical in field conditions. The proteins can be precipitated as a gel by formalin. Twenty µl of 40% formaldehyde are added to 200 µl 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 µl 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 a temperature of 4°C, but for only 1 day at room temperature, in Locke transport medium (a buffered glucose-salt solution with antibiotics).

**Genome assays**
Lack of standardisation 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 in order to amplify the kinetoplast minicircle of *Leishmania* species (it can be 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 pick up also 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 kala azar, 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. To perform the test 0.1 ml is injected intradermally and the local reaction 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**

Pentavalent antimonial compounds.

One of the treatment options for visceral leishmaniasis are 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). The drugs can be administered IM (intramuscularly, painful) or by slow IV (intravenous) injection or infusion (diluted with 5% glucose solution, otherwise local thrombophlebitis occurs). The dose is always expressed as mg Sb: 2 x 10 mg/kg IM or slow IV infusion per day for at least 30 days. On an ampoule might be written 1500 mg/5 ml, which is 1500 mg calculated as the salt, not as stibium itself. This can lead to underdosing if one is not aware of this detail. As a dose is practically totally excreted and eliminated via the urine within 6 hours after administration, a twice daily administration would pharmacokinetically be more logical than an injection once daily. However, a single administration per day appears to suffice in practice. The dose should be reduced in patients with kidney failure. A maximum of 850 mg/day [10 ml Glucantime®] has been previously set due to
the risk of cardiotoxicity with higher doses. This limit has been contested and has been abandoned in the latest WHO guidelines. 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.

### Antimony

Antimony is just below arsenic in the periodic table. It mimics the toxic effects of arsenic, which result from binding to adjacent thiol groups on enzymes, thereby impairing their function. Antimony is found in trivalent and pentavalent forms. Inhalation of stibine gas (SbH₃) causes massive haemolysis. Pentavalent antimonials (e.g. meglumine antimoniate, sodium stibogluconate) are used for treatment of leishmaniasis. One of their actions is to inhibit phosphofructokinase, the rate-limiting step in the parasites’ glycolytic pathway.

**Follow-up and response in the event of recurrence**

Follow-up is necessary as a number of patients will relapse. This usually happens in the first 6 months after treatment. Upon recurrence of visceral leishmaniasis (relapse), higher doses of Glucantime® can be used for a longer time (2-3 months). Alternatively and preferably another drug or combination therapy can be used to treat relapses.

Cases of complete treatment unresponsiveness can occur. Splenectomy sometimes has to be carried out in cases of life-threatening anaemia or thrombocytopenia. If possible pneumococcal vaccination should be given before the operation, and lifelong antimalarial prophylaxis is indicated thereafter if the patient stays in an endemic area.

**Alternative treatments:**

While antimonials have been the mainstay for treatment of visceral leishmaniasis for many decades, alternative options have been explored, mainly driven by the emergence of antimonial resistance in India, but also by their toxicity. Currently first line drugs entail antimonials, conventional amphotericin B and the lipid-containing formulations, paromomycin and miltefosine.

**Amphotericin B** is a polyene and has a fairly complex structure with a hydrophilic and a lipophilic component. The recommended dose of amphotericin B [Fungizone®] is 0.5-1 mg/kg/day IV, to be 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, anaemia, phlebitis at the site of the infusion, cardiotoxicity, kidney failure, hypokalaemia and hypomagnesaemia are frequent side effects. Side effects occurring shortly after administration can be reduced by cortisone IV or meperidine (pethidine), a morphine analogue. 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 prior to the administration. The drugs are then concentrated in the reticuloendothelial system and not in the kidneys so that a higher daily dose per kg of bodyweight 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**

2. Emulsification of Fungizone® in Intralipid 20%: little reduction of toxicity
4. Abelcet®: ABLC or Amphotericin B Lipid Complex. Microscopically small ribbon-like membranes formed by complexing with phospholipids.

**Injectable aminosidine** (paromomycin) is now also a first line drug. 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 noninferior 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 found 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** (Miltex®) was approved for use in India in 1992. It became more widely became available in subsequent years. Miltefosine or hexadecylphosphocholine is a lecithin analogue (=phosphatidyl-choline analogue). In the molecule phosphatidylcholine is bound to a carbohydrate
component via an ether bridge instead of an ester. Miltefosine interferes with certain cellular signal cascades and with membrane synthesis, though its precise mode of action is still unknown. It was initially developed as an antineoplastic agent. In the 1990s it was also discovered that in vitro and in animal models it was active against *Leishmania* parasites. These organisms contain 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 haemolysis. The molecule is fairly easy to produce and this 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 in 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 the use of liposomal amphotericin B (AmBisome) as first line treatment in the Indian subcontinent.

**Combination therapy** This 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 is now first line treatment in East-Africa. Other combinations including liposomal amphotericin B, paromomycin and miltefosine were found effective in India in phase III trials. Phase IV studies are ongoing.

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

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Regimen</th>
<th>Marketing</th>
<th>Clinical efficacy</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>20 mg/kg iv or im daily for 28-30 days</td>
<td>Albert David (SSG); GSK (Pentostam®) Sanofi Aventis (Glucantime®)</td>
<td>35-95% (depending on geographic area)</td>
<td>As high as 60% (Bihar, India)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.75-1 mg/kg iv for 15-20 doses (daily or alternate days)</td>
<td>Bristol Meyers Squibb (Fungizone®) Generic companies</td>
<td>&gt; 97% all regions</td>
<td>Not documented</td>
</tr>
<tr>
<td>Drugs</td>
<td>Toxicity</td>
<td>Cost/course</td>
<td>Issues</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Liposomal Amphotericin B</strong></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 (AmBisome®)</td>
<td>Europe and Asia: &gt; 95%; Africa: not fully established (higher dose required?)</td>
<td>Not documented</td>
</tr>
<tr>
<td><strong>Miltefosine</strong></td>
<td>2-2.5 mg/kg/d orally daily over 28 days (India only)</td>
<td>Paladin (Impavido®)</td>
<td>Asia: 94% (India) Africa: single field study (93% in HIV(-))</td>
<td>Readily obtained in lab isolates</td>
</tr>
<tr>
<td><strong>Paromomycin sulphate</strong></td>
<td>15 mg/kg im daily for 21 days (India only)</td>
<td>IOWH/Gland Pharma</td>
<td>Asia: 95% (India) Africa: 15 mg/kg: 64% (Sudan &lt;50%) 20 mg/kg: 80% (Sudan)</td>
<td>Readily obtained in lab isolates</td>
</tr>
</tbody>
</table>

* marketing authorization holder iv: intravenous; im: intramuscular; SSG: sodium stibugluconate

Table: The main drugs currently used for treatment of visceral leishmaniasis (continued).
Leishmaniasis

**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; gastrointestinal (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 (?)

---

**Table. Treatment recommendations for visceral leishmaniasis per geographical region, as recommended by the WHO (in order of preference)**

<table>
<thead>
<tr>
<th><strong>L Donovani - Indian subcontinent</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Liposomal amphotericin B: 3-5 mg/kg/d iv over 3-5 days for total dose of 15 mg/kg or 10 mg/kg iv sd</td>
</tr>
<tr>
<td>2. Combination regimens (sequential co-administration)</td>
</tr>
<tr>
<td>Liposomal amphotericin B (5 mg/kg iv sd) + miltefosine (dosage as below) for 7 days</td>
</tr>
<tr>
<td>Liposomal amphotericin B (5 mg/kg iv sd) + paromomycin (dosage as below) for 10 days</td>
</tr>
<tr>
<td>Paromomycin + miltefosine (dosages as below) for 10 days</td>
</tr>
<tr>
<td>3. Amphotericin B deoxycholate 0.75-1 mg/kg/d iv, daily or on alternate days, for 15-20 doses</td>
</tr>
<tr>
<td>4. Miltefosine: children 2-11 years: 2.5 mg/kg/d; ≥12 years and &lt; 25 kg body weight: 50 mg/day; 25 -50 kg: 100 mg/day; &gt; 50 kg: 150 mg/day; orally for 28 days</td>
</tr>
<tr>
<td>5. Paromomycin 15 mg (11 mg base)/kg/d im for 21 days</td>
</tr>
</tbody>
</table>
6. Pentavalent antimonials: 20 mg Sb\(^{5+}\)/kg/d im or iv for 30 days in areas where they remain effective (including Nepal, Bangladesh and certain areas in India)

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

### **L Donovani – East-Africa**

1. Combination therapy: pentavalent antimonials + paromomycin for 17 days (dosages as above)

2. Pentavalent antimonials monotherapy as above

3. Liposomal amphotericin B 3-5 mg/kg/d iv over 6-10 days for 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 iv in 3-6 doses for a total dose of 18-21 mg/kg

2. Pentavalent antimonials 20 mg/kg Sb\(^{5+}\)/kg/d im or iv for 28 days

3. Amphotericin B deoxycholate 0.75-1 mg/kg/d iv, daily or on alternate days for 20-30 doses, total dose of 2-3 g

---

iv: intravenous; im: intramuscular; sd: single dose

The nitroimidazole: **fexinidazole** has potential as a safe and effective oral drug therapy for treatment of visceral leishmaniasis (see also treatment of Human African Trypanosomiasis). Both metabolites of fexinidazole (sulfone and sulfoxine) were active against Leishmania donovani amastigotes. Reliance on a single enzyme for prodrug activation may leave fexinidazole vulnerable to the emergence of drug resistance. Clinical studies are ongoing. One option under exploration in East-Africa is the combination of miltefosine and fexinidazole (both given orally).

Several other drugs mentioned below have also been explored historically but have not made it to first line treatment.
Pentamidine isethionate (4 mg/kg every 48 hours IM for 4 months).

Combination therapy with gamma-interferon was explored, based on the importance of Th1 immunity in achieving control of visceral leishmaniasis, but efficacy was only modest.

High-dose allopurinol (Zyloric®), e.g. 3 x 7 mg/kg/day (that is, 20 mg/kg/day), for 4-12 weeks was also effective in clinical studies.

Terbinafine (Lamisil®) is an antimycotic drug with some clinical activity.

Sitamaquine. Due to relatively low efficacy rates and safety issues, this has largely been abandoned.

Pamidronate a bisphosphonate drug typically used in the treatment of osteoporosis is effective against experimental cutaneous leishmaniasis. Several bisphosphonates have significant activity against Leishmania donovani in vitro, and several are potent inhibitors of bone resorption and in clinical use for the treatment of osteoporosis and Paget’s disease. It is possible that currently approved clinical regimens of the drug are not high enough to cure human cutaneous leishmaniasis. Pamidronate could be a useful lead compound in the synthesis of new drugs against this disease.

LAST UPDATED BY ADMIN ON JULY 13TH, 2022

Cutaneous leishmaniasis

Distribution

Approximately 90% of all cases of cutaneous leishmaniasis now occur in Iran, Syria, Saudi Arabia, Afghanistan, Algeria, Peru and Brazil.

Clinical aspects

Various forms are clinically distinguished, the most important of which are:

1. Localised cutaneous leishmaniasis: skin ulcers that heal very slowly or nodular lesions, limited in extent and number. These chronic sores have regional names: clou de Biskra in Algeria and Aleppo boil in Syria.
2. Diffuse cutaneous leishmaniasis: cutaneous nodules and plaques that do not ulcerate but sometimes spread over the entire body.

3. Recurrent cutaneous leishmaniasis

“... After it is cicatrised, it leaves an ugly scar, which remains through life, and for many months has a livid colour. When they are not irritated, they seldom give much pain... It affects the natives when they are children and generally appears in the face, though they also have some on their extremities... In strangers, it commonly appears some months after their arrival. Very few escape having them, but they seldom affect the same person above more than once.”

Skin ulcer due to cutaneous leishmaniasis.
Diffuse cutaneous leishmaniasis. Infection with Leishmania aethiopica. Copyright ITM

**Localised cutaneous leishmaniasis**

After a bite by a sandfly infected with *L. tropica* (mainly urban infection), there is an incubation period of a few weeks or months, occasionally years. There is initially a small papule and usually only a single lesion, though sometimes there are several. This slowly spreads and can remain completely dry, become warty or nodular or develop into a painless, sharply delineated ulcer surrounded by a purplish raised border. Satellite lesions can occur. Spontaneous healing often occurs after 6 to 12 months, resulting in a depressed scar. Recurring cutaneous lesions – possibly with severe disfigurements – occasionally occur. There is usually immunity to any subsequent infection with the same organism. In infection with *L. major* (mainly rural infections, particularly from a rodent reservoir) the lesions are usually larger and develop more quickly, hence the name. There is a greater tendency to local spreading via the lymphatics and this has to be distinguished from sporotrichosis. The lesions will eventually spontaneously heal with scar formation. Clinical cure starts when macrophages become activated and start killing amastigotes. This is mediated via a T-helper cell type 1 (Th1) response. This immune reaction also prevents recrudescence of latent chronic infection. The Th1 response is accompanied by secretion of pro-inflammatory cytokines, such as interferon gamma and interleukin 12. If the immune response would be towards production of down-regulating cytokines (interleukin 4, 10, 13, TGF beta), macrophages will not be capable of eliminating the parasites, but tissue destruction will be limited.

In South America the lesions often have their own local names and clinical expressions. Hence in Peru they are called “uta” (a solitary ulcer or a few restricted lesions brought about by *L. peruviana*, frequently on the face). In Guyana they are known as “bush yaws” or (French) “pian bois” (*L. guyanensis*) with raspberry-like lesions that resemble yaws. In Yucatan, Mexico an ulcer on the ear (usually caused by *L. mexicana*) is known as “chiclero” ulcer.

A “chiclero” is a man who collects chicle-latex in the forest. During their activity in the plantations the workers can get bitten by *Lutzomyia olmeca* and as such are exposed to a high risk of contracting leishmaniasis, hence the term “chiclero ulcer”.
Diffuse cutaneous leishmaniasis

Diffuse cutaneous leishmaniasis is a diffuse affliction of the skin with extensive non-ulcerative nodules and is a very chronic disease. It is sometimes followed by chronic lymphoedema of an affected part of the body. This disease is poorly understood but is probably caused by a diminished resistance to the parasite. This immunosuppression is possibly brought about by the parasite itself. One of the supposed mechanisms of escape of Leishmania parasites is downregulation of the expression of major histocompatibility complex (MHC) class II molecules on the macrophages they colonise. In East Africa diffuse cutaneous leishmaniasis is often caused by L. aethiopica and in the New World frequently by L. mexicana.

If there are generalised cutaneous lesions the condition must be differentiated from lepromatous leprosy, keloids, neurofibromatosis and post kala azar dermal leishmaniasis (PKDL). Due to the low
resistance of the patient very numerous amastigotes are present and most skin smears are positive. Treatment is difficult as the patient’s immune system itself is functioning poorly. DCL patients are anergic to leishmanial antigen. Patients with DCL have a predominantly Th2-type cytokine response. They have low concentrations of interferon gamma and interleukin 12. There is no tendency to self-cure. Differentiation from PKDL is important, as the latter can still be treated reasonably well. In Sudan 1 case of diffuse cutaneous leishmaniasis is found for every 100 cases of localised cutaneous leishmaniasis. The incidence varies greatly from district to district. It occurs frequently in South America, but in contrast to this it does not occur in India (or very exceptionally –eg in HIV patients).

Recurring cutaneous leishmaniasis

Recurring cutaneous leishmaniasis seldom occurs (Iraq, Iran). This disease, also known as leishmaniasis recidivans leads to significant tissue damage. Parasites are very difficult to detect in these very chronic lesions. Differentiation from cutaneous tuberculosis is important.

**Diagnosis of cutaneous leishmaniasis**

Attempts should be made to detect the parasite microscopically in a biopsy or smear from the edge of the wound. The biopsy should if possible, be divided up for pathology (seldom available, not very sensitive and is principally used more for exclusion of another cause) and cultures (bacteria, mycobacteria, fungi, *Leishmania*) and an impression preparation should also be made. Lesions on the face can be injected with 0.1 ml physiological saline and aspirated again while moving a small, thin needle back and forth in the skin. Serology is usually negative. Differential diagnosis includes ulcers due to mycobacteria, cutaneous diphtheria, tertiary syphilis, yaws, cutaneous carcinoma and deep or subcutaneous mycosis. Field sore (cutaneous diphtheria) and tropical ulcers are painful, particularly in the early phase.

Differential diagnosis of disseminated nodular and ulcerated lesion includes leishmaniasis, sporotrichosis, atypical mycobacteria and nocardiosis.

**Treatment**

The response to treatment varies according to the species. Drugs for systemic and topical treatment can be used. There is an urgent need for better and cheaper drugs.

**Indications for local treatment**
1. lack of risk of developing mucosal lesions
2. Old World cutaneous leishmaniasis
3. small, single lesion
4. absence of spread to lymph nodes

**Indications for systemic treatment**

1. presence of mucosal lesion or spread to lymph nodes
2. New World cutaneous leishmaniasis, except localised Leishmania mexicana infection
3. lesions unresponsive to local treatment

**Overview topical treatment of cutaneous leishmaniasis**

1. physical methods: cryotherapy (liquid nitrogen) for 15-20", repeated 2-3 times with an interval of e.g. 3 weeks. Blistering will occur.
2. application of local heat via a CO₂ laser or an infrared lamp (40°C to 42°C for 12 hours) has been used, but heat-induced skin bullae are common.
3. ointment with 15% paromomycin and 12% methylbenzethonium chloride in soft white paraffin (e.g. Leishcutan® ointment). Urea can be added as a keratolytic. Twice daily application is advised for a duration of 20-30 days.
4. skin infiltration with pentavalent antimony with a fine gauge needle. Blanching of the lesions should be obtained. Treatment is repeated every 5-7 days, in general 2-5 times, sometimes more.
5. imiquimod crème (Aldara®). This immunomodulator activates macrophage killing of Leishmania amastigotes, but is best used in combination with systemic meglumine antimonate. Experience with this drug is limited.
6. treatment with antimonium plus topical recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) has been described. GM-CSF (molgramostim = Leucomax®) was diluted for topical use to a concentration of 10 µg/ml. It was applied 3 times weekly for 3 weeks (1-2 µg/cm²/lesion). In vitro, GM-CSF has been shown to activate macrophages that kill Leishmania pathogens. Intralesional injection of GM-CSF (400 µg) has also been shown to reduce the healing time of leishmania ulcers.
7. Application of topical 5-aminolaevulinic acid (a porphyrin-precursor), followed by two laser irradiations, which photoactivates the compound. It is expected that very little scar tissue would form, so for aesthetically important places, this might become first choice treatment, if the clinical studies confirm this expectation.
Overview systemic treatment of cutaneous leishmaniasis

1. Pentavalent antimonials (meglumine antimoniate [85 mg Sb/ml, IM] or sodium stibogluconate [100 mg/ml, IM or filtered IV] can be given parenterally for extensive skin lesions. For unknown reasons, the incidence of herpes zoster is increased about 10 times during IV treatment with IV Pentostam relative to the incidence in the normal population. Cases of cutaneous leishmaniasis not treated with antimony do not have an increased incidence of herpes zoster.

2. Pentamidine. First line against *L. guyanensis* (French Guyana). Several treatment schemes exist and the cure rate is dose-dependent. Some short-courses use 1200 mg as a total dose. In Guyana 3 mg/kg/day every other day is often used (4 injections).

3. Imidazoles, triazoles. Infections caused by *L. major* can be successfully treated with oral fluconazole 200 mg/day for 6 weeks (cure rate of 80%). Ketoconazole 600 mg per day x 28 days is moderately effective for *L. mexicana*, but much lower against *L. braziliensis*. Treatment with ketoconazole is sometimes complicated by hepatotoxicity, abdominal pain and nausea. Itraconazole (Sporanox®) gave good results in initial studies but this was not seen in the field.

4. Miltefosine. Not yet widely available, but allows oral therapy.

5. Amphotericin B and its liposomal formulations (IV).

6. Allopurinol. Not as monotherapy, but in combination with e.g. pentavalent antimony for *L. panamensis*.

Treatment of diffuse cutaneous leishmaniasis (*L. aethiopica*)

The treatment of diffuse cutaneous leishmaniasis caused by *L. aethiopica* is problematical, as this parasite is less sensitive to Glucantime®. Pentamidine can be used against *L. aethiopica*. A dose of 4 mg/kg/week which has to be continued for at least 4 months after disappearance of the parasites from the skin is an acceptable guideline here. Parenteral aminosidine sulphate is another therapeutic possibility. This is an antibiotic that is obtained from *Streptomyces chrestomyceticus*. It is an aminoglycoside and is thus potentially nephro- and ototoxic. It is chemically identical to paromomycin, which is obtained from a related *Streptomyces* strain. The compound is not resorbed from the intestine. Recurrences are frequently seen with aminosidine given as monotherapy. Aminosidine is however synergistic with stibogluconate and a permanent remission can be obtained with the combination of aminosidine with Glucantime® or Pentostam®. The dose is 14 mg/kg/day IM to be continued for up to 60 days after all parasites have been eliminated. The total treatment period takes 6 months or more. Good results were obtained with amphotericin B.
Mucocutaneous leishmaniasis

Distribution

At present 90% of all mucocutaneous leishmaniasis occurs in Bolivia, Peru and Brazil. Illustrations of skin lesions and disfigurements suggestive of leishmaniasis are encountered on pre-Inca earthenware. These indicate that the disease was already in existence in Peru and Ecuador in the 1st century AD. Texts dating from the 15-16th century Inca period and the Spanish conquest mention the risk of cutaneous ulcers in seasonal farmers. Espundia was also described as “white leprosy”.

Clinical aspects

When skin and mucosae are affected the disease is known as mucocutaneous leishmaniasis. This is very rare in East Africa but frequent in South America, where it is known as “espundia”. After an initial skin lesion, that slowly but spontaneously heals, chronic ulcers appear after months or years on the skin, mouth and nose, with destruction of underlying tissue (nasal cartilage, for example). Tissue destruction with disfigurement can be very severe. Parasites are usually rare in the lesions. A substantial part of the disfigurement is possibly due to immunological mechanisms. One hypothesis is a relationship between the occurrence of mucocutaneous lesions and the presence of certain alleles of polymorphic tumour necrosis factor a and b genes.
Espundia or mucocutaneous leishmaniasis often results from infection with Leishmania brasiliensis.

Photo Cochabamba, Bolivia
Espundia or mucocutaneous leishmaniasis often results from infection with *Leishmania brasiliensis*. Photo Cochabamba, Bolivia

**Diagnosis**

The lesions often contain few parasites. Diagnosis is sometimes made solely on a clinical basis. Culture of the parasites is possible, but not really feasible in primitive rural conditions. Serology in espundia can be positive or negative (the quality of the antigen is of crucial importance). A practical problem in South America is whether a certain skin lesion with *Leishmania* amastigotes is caused by *L. braziliensis* or not. The geographical origin of the lesion or PCR and/or zymodeme analyses may give an answer here, though these laboratory techniques are not available in rural areas.

**Mucocutaneous leishmaniasis, differential diagnosis:**
Differential diagnosis includes skin cancer, tertiary syphilis and yaws, leprosy, rhinoscleroma (a very chronic granulomatous infection with *Klebsiella rhinoscleromatis*), rhinosporidiosis, midline granuloma (a form of T-cell lymphoma), Wegener’s granulomatosis, sarcoidosis, skin tuberculosis, infection with the free-living amoeba *Balamuthia mandrillaris*, chronic nasal cocaine abuse, noma, and fungal infections such as cryptococcosis, histoplasmosis and South American blastomycosis (paracoccidioidomycosis). With this last disease, which is a very chronic infection, the lungs are frequently affected in a manner that can mimic tuberculosis. The yeast has typical oval cells with ectospores and can be detected in sputum.

**Overview: Differential diagnosis of nasal ulcers:**

1. Mucocutaneous leishmaniasis (espundia)
2. Fungal infections, such as paracoccidioidomycosis (syn. South American blastomycosis), histoplasmosis, cryptococcosis, coccidioidomycosis
3. Actinomycosis
4. Treponematoses (syphilis, yaws, bejel)
5. Leprosy
6. Tuberculosis
7. Rhinosporidiosis
8. Rhinoscleroma (chronic infection with *Klebsiella rhinoscleromatis*)
9. Balamuthiasis (infection with free-living amoeba)

**Non-infectious**

1. Granulomatosis with Polyangitis (formerly Wegener granulomatosis)
2. Midline granuloma (a form of T-cell lymphoma)
3. Other non-Hodgkin lymphoma
4. Squamous cell carcinoma
5. Sarcoidosis
6. Relapsing polychondritis
7. Cocaine abuse

LAST UPDATED BY ADMIN ON JULY 13TH, 2022