



Modern Views on Leishmaniasis Diagnosis, Preventive Measures and Treatment

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Abstract: The article presents the literature data on leishmaniasis. Leishmaniasis is a protozoan disease transmitted by sandflies that is most commonly seen in Europe, Africa, Asia, and Latin America. As travel patterns shift it is a disease being more frequently introduced into developed areas. The disease may either be cutaneous or systemic. Identification of the organism and knowledge of endemic species will guide interprofessional team members toward accurate diagnosis and targeted therapy if indicated. This activity outlines the epidemiology, clinical manifestations, diagnosis, and treatment of leishmaniasis. This review highlights the role of the interprofessional team in caring for affected patients and preparing for the potential for future cases of leishmaniasis in developed nations.

Key words: leishmania; cutaneous leishmaniasis; diagnostics; differential diagnosis; forecast; flow; treatment.

Leishmaniasis is a complex of diseases caused by the protozoa *Leishmania* and transmitted by the bite of infected phlebotomine sandflies.

Four major human diseases: (a) localized cutaneous leishmaniasis, (b) diffuse cutaneous leishmaniasis, (c) mucocutaneous leishmaniasis, and (d) visceral leishmaniasis. Which of the 4 diseases results depends mainly on the interaction between *Leishmania* species and the immunologic status of the host. Diagnosis is by organism isolation or serology, but species identification is only possible with isoenzyme analysis and new molecular techniques. Management ranges from observation to systemic therapy, primarily with antimonials, and vaccines in development.

Etiology

PARASITE AND LIFE CYCLE

The genus *Leishmania* consists of parasitic protozoa of the phylum *Sarcomastigophora*, order Kinetoplastida, and family Trypanosomatidae. Two subgenera, *Leishmania* and *Viannia*, exist. For clinical purposes, a nomenclature omitting the subgenus is often used. More than 20 species are

pathogenic for humans, which are transmitted by the bite of an infected female phlebotomine sand fly. Cases of venereal and vertical transmission, as well as transmission from infected blood transfusion or needles have, however, been reported.

Leishmania are dimorphic parasites. In the gut of the sand fly or in culture, they exist in a spindle-shaped motile (single anterior flagellum) promastigote form (10 to 20 μm). Upon transmission to the host, *Leishmania* parasites are ingested by macrophages and neutrophil granulocytes and—as a consequence of the shift toward a low pH in phagolysosomes—transform into the obligate intracellular, oval, nonmotile amastigote form (2 to 6 μm) that has a relatively large basophilic nucleus and a smaller rod-shaped kinetoplast at the base of the lost flagellum.

After ingestion during a blood meal of a sand fly, infected macrophages are ruptured and the amastigotes are released into the stomach of the insect where they immediately transform into the promastigote form. The promastigotes subsequently migrate to the alimentary tract of the fly, multiply extracellularly by binary fission, and, in a few days, reach the esophagus and the salivary glands of the fly, where they change into infective metacyclic promastigotes, which will be released into the skin at next bite. Promastigotes are then phagocytosed by resident host (skin) macrophages and neutrophils, where they transform into amastigotes that multiply by binary fission and get released following cellular burst to infect other cells of the host.

RESERVOIR HOST

Leishmaniasis is mostly zoonotic, being incidentally transmitted to humans from wild and domestic animals (primary reservoir hosts). The main reservoir hosts are the great gerbil, the fat sand rat, *Meriones* spp., and other rodents, as well as dogs, the opossum, sloths, and others. In several regions, however, the zoonotic host is not even fully characterized, leading to difficulties in reservoir and vector control strategies.

VL caused by *Leishmania donovani* and Old World CL caused by some *Leishmania tropica* strains are anthroponotic diseases (ie, humans are the primary reservoir hosts). Additional factors that may contribute to the increasing relevance of humans as reservoir hosts are alterations in the natural habitats (eg, human settlements in close proximity to forests, restrictions in the diversity and distribution of mammals available for sand fly feeding).

INSECT VECTOR

The insect vector of leishmaniasis, the female phlebotomine sand fly, is grouped under the suborder *Nematocera* of the order *Diptera*.¹ Three genera (*Phlebotomus* in the Old World, and *Lutzomyia* and *Psychodopygus* in the New World), and approximately 70 species are implicated as vectors. They are widely distributed and have predilection to intertropical and warm temperate zones. Presumably as a consequence of globalization and global warming, Phlebotominae have been spreading into, for example, northern European regions in recent years.

Only the female sand fly is hematophagous. They live for approximately 40 days and are known as pool feeders, because they tear open the skin with their wide mouthparts and suck the blood as it collects. Phlebotomine sandflies are small (<3 mm), and do not fly far from their breeding site. Their activity is mostly crepuscular or nocturnal while the host is asleep. They rest during the day and lay their eggs in dark, cool, humid, and organic matter-rich places, such as rodent burrows, bird's nests, and house wall fissures. Being exophilic and exophagic, they prefer to rest and to have their meal outdoors, which limits their control through house spraying.

Epidemiology

Together with malaria and dengue, leishmaniasis is the third most frequent infectious disease transmitted by a vector. Annually, approximately 1.6 million new cases are reported; among these, it is estimated that approximately 200,000 to 400,000 cases represent visceral leishmaniasis, and 700,000 to 1.2 million cases represent cutaneous leishmaniasis. Around 12 million individuals are currently infected worldwide.

The World Health Organization (WHO) considers leishmaniasis to belong among the so-called neglected tropical diseases. Neglected tropical diseases are poverty-associated infectious diseases that are primarily prevalent in subtropical and tropical regions, and for which there is little to no public interest, little research activity, high morbidity and mortality, and no safe and long-lasting therapies (as of this writing).

Leishmaniasis is endemic in 90 countries around the world, mainly in tropical and subtropical regions (excluding Australia and Southeast Asia). The different clinical presentations, cutaneous, mucocutaneous and visceral disease, have a distinct distribution (cutaneous leishmaniasis) and (visceral leishmaniasis):

Cutaneous leishmaniasis (CL) is widely distributed, with approximately one-third of cases occurring in each of 3 epidemiologic regions: the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia. The 10 countries with the highest case counts are Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Peru, Sudan, and Syria. Together these 10 countries account for 70% to 75% of all CL cases. More than 90% of global visceral leishmaniasis (VL) cases occur in 6 countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan. Close to 90% of mucocutaneous leishmaniasis (MCL) cases occur in Bolivia, Brazil, and Peru.

The distribution of disease is strongly associated with the distribution of its vector, the sand fly. Outside of endemic regions, the disease is very often only recognized by travelers after their return home. In recent years, coinfections of *Leishmania* with HIV have become a major concern. Because HIV-infected individuals have longer periods of parasitemia, humans potentially become reservoirs for the parasite as well. This is one possible explanation for the increased rate of leishmaniasis cases in some regions that reportedly have high numbers of HIV/*Leishmania* coinfecting patients.

Pathophysiology

The resulting disease depends on the fate of the phagocytosed amastigotes. This, in turn, is a function of numerous parasite-related and host-related factors, as well as other factors that may account for geographical differences. In general, parasites interfere with the signaling pathways, intracellular kinases, transcription factors, and gene expression of macrophages, compromising their ability to generate leishmanicidal substances. In addition, they impair dendritic cell activation, migration, and the ability to secrete T-helper 1 (Th1) cytokines.

PARASITE-RELATED FACTORS

Sand fly saliva is increasingly recognized as an essential element in the pathogenesis of the disease. Besides containing vasodilators, anticoagulants, and immunomodulators, it may also increase the inoculum size and the diameter of the lesion in previously unexposed individuals. Intraspecific variations of sand fly saliva may even affect the overall clinical outcome of *Leishmania infantum*-induced disease by shifting the adaptive immunity from a Th1 to a Th2 immune response. The development of antisaliva antibodies after exposure may account for the decline with age of susceptibility to the infection in endemic areas.

Other parasite-related factors include infectivity, pathogenicity, virulence, and tissue tropism. These differ from one species to the other. For example, lipophosphoglycan and gp63 are 2 important promastigote virulence factors that impair the overall functions of infected cells, with lipophosphoglycan being clearly involved in *Leishmania major* disease, but absent in *Leishmania mexicana*. Although viscerotropic species tend to spread to the reticuloendothelial system, they may become dermatropic as a consequence of treatment such as seen in post- kala-azar dermal leishmaniasis (PKDL). Similarly, *L. tropica*, classically dermatropic, may cause visceral disease.

HOST-RELATED FACTORS

Malnutrition, immunosuppression, and the host genetic background influence host susceptibility as well as resistance to disease.

The output of acquired T-cell immunity, which depends on the net effect of the opposing Th1 and Th2 responses, largely determines the course and the therapeutic response of the infection. A dominant Th1/cytotoxic T-cell Type 1 response resulting in the production of interferon- γ and nitric oxide leads to a leishmanicidal state of macrophages, and accounts for subclinical infection or self-healing LCL and the positive Montenegro skin test (that assesses delayed-type hypersensitivity to leishmanial antigen). A dominant Th2 response accounts for progressive disease such as diffuse cutaneous leishmaniasis (DCL), and is characterized by anergy to leishmanial antigen (negative Montenegro skin test). In addition, regulatory T cells, as well as the so-called Th17 cells producing interleukin-17A, contribute to disease susceptibility. The majority of parasite-specific T cells home to the site of infection, thus, cytokine profiles of T cells in peripheral blood may not reflect the actual immune status well. Mucosal leishmaniasis patients will have both Th1 and Th2 responses, with slight predominance of Th2 immunity, explaining persistence of the disease. To summarize, T-cell immunity would be intact in localized cutaneous leishmaniasis (LCL), defective in DCL, and pathologically exuberant in MCL. Humoral immunity seems to play a role during parasite opsonization, but little or no role in determining the course of the infection. High titers of antileishmanial immunoglobulin (Ig) G correlate more with chronic, nonhealing, and visceral disease. HIV coinfection of individuals both leads to a more severe course of infection with *Leishmania*, but also worsens disease outcome of the HIV infection. Other coinfections prevalent in endemic countries, such as malaria, also are known to have an effect on the course of disease.

Histology

The histopathologic examination of early lesions of LCL reveals a dense and diffuse mixed inflammatory cell infiltrate composed predominantly of histiocytes, and scattered multinucleated giant cells, lymphocytes, and plasma cells (sometimes with intracytoplasmic homogenous eosinophilic immunoglobulin material called *Russell bodies*). The hallmark of the disease (in approximately 70% of the cases) is the presence of numerous extracellular and intracellular (within histiocytes) amastigotes (also known as *Leishman-Donovan bodies*). Giemsa stain stains the parasite nonmetachromatically and the kinetoplast bright red. The organisms also may be highlighted by the Wright and Feulgen stains. Monoclonal antibodies can identify amastigotes and promastigotes in smear, biopsy, or culture specimens, constituting a rapid screening test for leishmaniasis; however, they are not commercially available.

The histologic differential diagnosis includes diseases characterized by parasitized macrophages. The presence of halo surrounding the yeasts in histoplasmosis, safety pin-like encapsulated Donovan bodies in granuloma inguinale, and Mikulicz cells in rhinoscleroma distinguishes these conditions from leishmaniasis. Other considerations are blastomycosis, paracoccidiomycosis, toxoplasmosis, and trypanosomiasis. As the lesion evolves, the number of amastigotes per section decreases and the histology approaches that of a chronic LCL, where the predominant histologic pattern is nodular

diffuse noncaseating tuberculoid granulomatous dermatitis. Epidermal hyperplasia and ulceration are variable. Scarring with marked loss of elastic fibers may be seen.

In DCL, a diffuse infiltrate composed of vacuolated macrophages with numerous intracellular and extracellular amastigotes is characteristic. The major differential diagnosis is lepromatous leprosy. In MCL, the histopathologic findings are similar to those of LCL but organisms are usually sparse. In PKDL, Pautrier-like epidermal microabscesses and a dense lymphoplasmacytic infiltrate with papillary dermal edema (in early lesions) are characteristic.

History and Physical

CUTANEOUS LEISHMANIASIS

Localized Cutaneous Leishmaniasis: LCL constitutes 50% to 75% of all incident cases. It is the mildest form of *Leishmania* diseases and the one that prevails in the Old World. It can be caused by all *Leishmania* species.

Old World LCL (Aleppo boil, Baghdad boil, Oriental sore, leishmaniasis tropica, Biskra button, Delhi boil, Bouton d'Orient, Lahore sore, Rose of Jericho, Kandahar sore, the little sister) is caused mainly by *L. major*, *L. tropica*, *Leishmania aethiopica*, and to a lesser extent *L. infantum*. The morphologic spectrum of Old World LCL is wide. Lesions start as erythematous papules that enlarge over a few weeks to form nodules/plaques and often ulcerate and become crusted. The "volcanic" noduloulcerative morphology is characteristic and consists of a painless crateriform ulcer with a rolled margin and a necrotic base that is often covered with an adherent crust. Two major types, (a) the moist type (mainly caused by *L. major*) and (b) the dry type (primarily caused by *L. tropica*), are identified. Both types may coexist in the same patient.

Other presentations include "iceberg nodules," and eczematoid, psoriasiform, erysipeloid, zosteriform, paronychia, chancreiform, annular, palmoplantar, verrucous, and keloidal lesions.

Depending on the causing subspecies, the following characteristics also may be noted:

- *L. major* tends to cause multiple, moist ulcerations resembling furuncles with lymphadenopathy.
- *L. tropica*—associated lesions are fewer, mainly in the face, without lymph node involvement.
- Recurrences are found in approximately 10% of patients close to the original lesions.
- *L. infantum*, the causative agent of Mediterranean VL in children, may cause a self-limited skin disease in adults with rare ulceration.
- *L. aethiopica* may cause a similar cutaneous disease to *L. tropica*, but carries a higher risk of evolving into DCL in up to 20% of affected individuals.

Species identification cannot be made clinically and requires biochemical/molecular techniques.

Satellitosis, regional lymphadenopathy, localized lymphadenitis, sporotrichoid lymphatic spread, subcutaneous lymphatic nodules, and localized hypoesthesia may occur. Mature lesions may be elongated and oriented parallel to skin creases.

New World LCL (Valley sickness, Andean sickness, white leprosy, chiclero ulcer, uta, pian bois, and bay sore) is caused by *L. mexicana* and *Leishmania (Viannia) braziliensis* complexes mainly. Pure cutaneous disease is very similar to Old World LCL and isolated ulcers in exposed areas are the most common presentations. The progression of the lesions gives rise to a characteristic scar consisting of thin pale skin at the ulcer site with a hyperpigmented border.

Infection with *L. mexicana* or *L. (V.) Leishmania (Viannia) guyanensis* causes only cutaneous disease in contrast to infection with *L. (V) braziliensis* and *Leishmania (Viannia) panamensis*, which may, in 40% to 80% of cases, progress to MCL. Approximately 50% of the lesions caused by *L. mexicana* heal within 3 months, whereas those caused by *L. braziliensis* persist much longer and are often associated with lymphadenopathy. Sporotrichoid lymphatic spread can be found more frequently, especially in children and immunosuppressed patients. Mature lesions may be elongated and oriented parallel to skin creases.

Disease caused by certain subspecies has the following specific presentations:

- *L. mexicana* is the causative organism of chiclero ulcer, a chronic mutilating infection of the pinna of the ear of forest workers in Mexico and Central America. *Lutzomyia flaviscutellata* is the major vector.
- In Brazil, the lower limbs are commonly affected, causing the typical Bauru ulcers.

Diagnosis and treatment

Given the potential treatment toxicity, confirmation of the diagnosis is always mandatory. Parasite subspecies determination is important for treatment decisions.

Even when smear, histology, and culture results are combined, the parasite may not be detected in 10% to 20% of cases. The diagnostic challenge is often greater in New World disease and in chronic lesions. The sensitivity of both tissue smear and culture approaches 90% when specimens are taken during the first weeks of infection. The best approach is to use several diagnostic methods. Taken from the infiltrated margin, a skin biopsy may be divided into 3 parts: one for an impression smear, one for histologic examination, and another for culture.

IMPRESSION SMEAR

Several smear techniques may be used with a success rate ranging between 50% and 80%. They are obtainable from fine-needle aspirates or tissue scrapings, air-dried, fixed with methyl alcohol, stained with Giemsa stain, and viewed under oil-immersion microscopy. Impression smears, made by gently pressing the skin biopsy against a glass slide 2 to 5 times, provide better sensitivity than hematoxylinand-eosin examination.

Differential Diagnosis

Cutaneous ulcers in tropical regions include:

- Furuncular myiasis
- Staphylococcal infection
- Lepromatous leprosy (leonine facies)
- Tuberculoid leprosy (hypopigmented patches and plaques)
- Yaws (primary stage of ulcerative or nodular lesions on lower extremities)

Prognosis

Essential to prevention is the promotion of personal protective measures through the use of protective clothing, insect repellents containing 30% to 35% *N, N*-diethyl-3-methylbenzamide (DEET), and permethrin-treated bed nets; the avoidance of endemic areas; and staying on higher floors of buildings in the evening.

One method of reservoir control of *L. major* was achieved by destroying burrows made by the rodent host. In endemic areas where dogs could be hosts of parasites, a dog collar coated with deltamethrin could be a useful way to control transmission. Targeting anthroponotic foci that are at the origin of deadly epidemics, suspecting leishmaniasis in persons with skin lesions or febrile illnesses returning from endemic areas, and deferring prospective blood donors from donating blood for at least 1 year after their return are other measures. Control of HIV in southern Europe where leishmaniasis is closely associated with HIV has generated positive outcome.

New diagnostic tests, drugs, and vaccines are in development. Vaccine development for tropical diseases is often limited by the lack of financial return. "Leishmanization," the self-inoculation of the live parasite in inconspicuous areas of the body, has been practiced in endemic areas to prevent disfiguring facial lesions, but was later abandoned because of its complications. Killed promastigotes vaccines with and without adjuvant bacille Calmette-Guérin seem to be useful in some endemic areas. Adjuvants such as bacille Calmette-Guérin are important to prime a Th1 response. Attenuated live parasites-based and plasmid DNA-based vaccines using leishmanial antigens have shown efficacy in mouse models. Experimental vaccines containing a component of sand fly saliva proteins appear to be promising. Despite being experimentally successful, a safe nonliving prophylactic vaccine has not yet been able to confer significant protection in humans. A vaccine for dogs has been approved. The sequencing of the *Leishmania* genome in 2007 might lead the way to new ways for prevention, control, and treatment of the condition.

Conclusion

Thus, literature data indicate that, despite the obvious successes in the fight against cutaneous leishmaniasis, there remains a high likelihood of local outbreaks of this disease. Despite the ongoing complex work, not it is always possible to ensure early diagnosis and prevention of this disease, and therefore the problem treatment of patients with cutaneous leishmaniasis remains today one of the topical in dermatology.

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