275 *Leishmania* Species: Visceral (Kala-Azar), Cutaneous, and Mucosal Leishmaniasis

Naomi E. Aronson, Nathanial K. Copeland, and Alan J. Magill[†]

SHORT VIEW SUMMARY

OVERVIEW AND GENERAL PRINCIPLES

- Leishmaniasis is an infection by the protozoan parasites in the genus *Leishmania* and causes three primary clinical syndromes: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML).
- *Leishmania* parasites exist as flagellated promastigotes in sand flies and transform into oval amastigotes after tissue infection in mammalian hosts.
- *Leishmania* organisms are transmitted by the bites of female sand flies of the *Lutzomyia* and *Phlebotomus* genera. Sand fly saliva and inoculum size influence human infection.
- Leishmaniasis is an intracellular infection, and as such adequate control requires a robust cell-mediated immune response. Parasite and host factors influence the tropism of infection.

VISCERAL LEISHMANIASIS

- VL (or *kala-azar*) is endemic in 75 countries worldwide; global incidence has significantly declined since 2010. *Leishmania donovani* occurs primarily in South Asia and East Africa, and *Leishmania infantum* (synonym *Leishmania chagasi*) predominantly occurs in Brazil and the Mediterranean littoral region.
- Polymerase chain reaction testing, parasite culture, or microscopic visualization of amastigotes on bone marrow or liver biopsy confirms diagnosis. Serology (rK39) may be helpful, although it is less sensitive in East African VL.
- Most infected individuals become latently infected and do not manifest symptoms, although they remain infected and at risk for developing symptomatic disease. Classic kala-azar can begin acutely or with a subacute/chronic picture.
- Fever, progressive weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia are the classic pentad of symptoms. Symptoms can vary, and multiple organ systems can be affected.
- Persons with human immunodeficiency virus/acquired immunodeficiency syndrome or other immunosuppressing conditions can present atypically and are at higher risk of relapse or death.
- Liposomal amphotericin B is generally the treatment of choice where available. In East Africa, combinations of pentavalent antimony with paromomycin are often used as first-line treatment. Immunocompromised patients require higher doses and longer courses of therapy.

CUTANEOUS LEISHMANIASIS

- CL is a neglected tropical disease with an estimated 12 million people affected. Global prevalence has been greatly increasing in recent decades.
- Providers should think of CL after exposure in endemic regions when one or more chronic, generally painless skin lesions develop. There are a wide variety of clinical manifestations, but typically induration exists. Diagnosis requires sampling of a skin lesion and testing using several techniques including stained tissue smears, polymerase chain reaction, and parasite culture for species identification (requires a reference laboratory).
- Metastatic infection (e.g., ML) is a concerning complication, mostly associated with New World CL species such as *Leishmania (Viannia) braziliensis* and less commonly *Leishmania (Viannia) guyanensis* and *Leishmania (Viannia) panamensis*. In addition, cellular immunocompromised hosts are at risk for disseminated infection.
- Although healing may occur spontaneously after months, treatment is recommended for ML-associated species, immunocompromised hosts, and more extensive or severe infection (see Table 275.2). Treatment must be individualized; there is no treatment of choice. The objectives of treatment are to expedite healing of the skin lesion, to limit scarring, and to prevent metastatic infection. As CL ulcers are chronic wounds, wound care should be part of management.

PREVENTION AND CONTROL

- There is no chemoprophylaxis, immunoprophylaxis, or vaccine available for human use to prevent leishmaniasis. Standard personal protective measures against arthropod bites should be used.

OVERVIEW AND GENERAL PRINCIPLES

Leishmaniasis refers to a diverse spectrum of clinical syndromes caused by infection with protozoan parasites of the genus *Leishmania* transmitted by the bite of a phlebotomine sand fly. Leishmaniasis can be separated geographically into Old World and New World disease, referring to the Eastern and Western Hemispheres, respectively. The clinical manifestations of leishmaniasis vary widely but are often divided into three clinically distinct syndromes: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML). A single *Leishmania* species can produce more than one clinical syndrome, and each of the syndromes is caused by more than one species. The outcome in any one patient is a result of the interplay of parasite factors (e.g., invasiveness, tropism, pathogenicity) and host factors (e.g., genetic predisposition, immune status, medication exposure). It is useful to view infection as potentially leading to leishmaniasis, a heterogeneous collection of clinical diseases, each with its own relatively unique geographic distribution, biology, ecology, local mammalian reservoir, and sand fly insect vector. In this chapter, shared aspects will be discussed initially, followed by syndrome-specific information.

[†]Deceased.

Life Cycle and Morphology

Leishmania organisms are dimorphic protozoa existing in two distinct morphologies within sand fly vectors and mammalian hosts, respectively. Although no clear sexual form has been identified, there is evidence that sexual recombination does occur.[1] Additionally, whereas generally diploid, *Leishmania* organisms use aneuploidy at various points in their life cycle, likely as a mechanism of gene regulation.[2] Mammalian infection begins when a female sand fly, taking a blood meal, regurgitates promastigotes into the bite site. Promastigotes (1.5–3.5 μm × 15–200 μm) are elongate, flagellated, and motile forms found in the digestive tract and proboscis of the sand fly. Promastigotes avoid the innate immune response and are phagocytized by neutrophils, macrophages, and dendritic cells. Within 24 to 48 hours after infection, intracellular promastigotes transform into oval or round amastigotes (1.5–3 μm × 3–5 μm) that lack a visible flagellum on light microscopy. Amastigotes have a distinct, rod-shaped structure called a

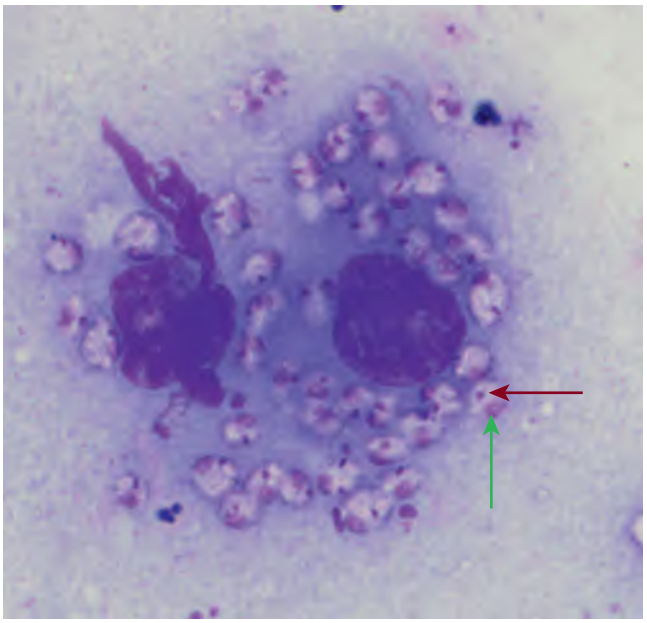

FIG. 275.1 Tissue smear demonstrating intracellular amastigotes from a *Leishmania major* infection. Note the rod-shaped kinetoplast *(red arrow)* seen next to the nucleus *(green arrow).* (Giemsa stain, magnification ×1000). *(Courtesy Mr. Juan Méndez, Silver Spring, MD.)*

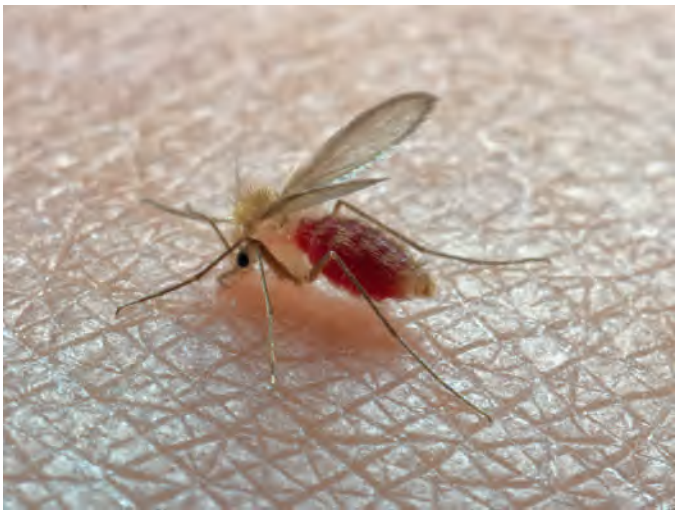

FIG. 275.2 *Phlebotomus papatasi,* the sand fly vector most commonly associated with transmission of zoonotic *Leishmania major* in the Old World. Note the feathered edges (fine hairs), characteristic "V" shape of wings at rest, and parallel venation on wings. *(Courtesy Dr. Ed Rowton, Silver Spring, MD.)*

kinetoplast, which is a specialized mitochondrial structure. Visualization of a kinetoplast, as seen in Fig. 275.1, confirms the diagnosis of leishmaniasis.

Amastigotes multiply by simple binary division, eventually rupturing the cell and invading other mononuclear phagocytes. Depending on the species and host factors, amastigotes may spread regionally or systemically from the bite site through lymphatics and/or the vascular system to infect mononuclear phagocytes elsewhere. This variable tropism is important in the clinical manifestations characteristic for a given species. The cycle is completed when female sand flies ingest parasitized cells. When in the digestive tract of the sand flies, *Leishmania* parasites develop through a series of flagellated intermediate stages to become infectious metacyclic promastigotes over the course of approximately 1 week.

Taxonomy and Classification

The taxonomy of *Leishmania* parasites can be confusing and is evolving. Traditional classification schemes using geographic, clinical, isozyme, or vectorial criteria are useful and widespread in the literature.[3] However, molecular techniques such as sequencing and multilocus sequence typing are influencing taxonomy, as exemplified by the recognition of the synonymous nature of *Leishmania donovani* and *Leishmania archibaldi*. The division of the genus *Leishmania* into the subgenera *Leishmania* and *Viannia* was originally based on the location of promastigote development within sand flies, and this has generally been confirmed by molecular techniques.[4] The advancement of molecular techniques, including rapid whole-genome sequencing, transcriptomics, and proteomics, will continue to refine the taxonomy of *Leishmania*.[5,6,7]

From the clinician's perspective, a useful classification accurately predicts the natural history of infection and response to treatment. However, different definitions, standards, and technical methods have been used over the past several decades to determine the species-level characterization of *Leishmania* parasites, making the correlation of species with treatment outcomes and prognosis challenging. The *Leishmania* species that infect humans and the clinical syndromes they produce are summarized in Table 275.1.

Transmission Principles and Sand Fly Biology

Transmission depends on the sand fly vector, the presence of a suitable reservoir, and susceptible humans. Female sand flies of the genera *Lutzomyia* and *Phlebotomus* transmit *Leishmania* species in the Americas and elsewhere, respectively.[5] As seen in Fig. 275.2, phlebotomine sand flies are small, delicate insects ranging from about 1.5 to 3.5 mm in length. Sand flies breed in the cracks of dwelling walls, rubbish, rubble, or rodent burrows. They are weak fliers and tend to remain close to the ground near their breeding sites and feed on nearby hosts. Sand flies make small cuts in host skin and feed on pooled blood. Saliva from the sand fly enhances the infectivity of promastigotes through its antihemostatic and immunomodulatory properties. Factors in the saliva such as adenosine or maxadilan influence the host immune response, favoring a Th2-type response along with decreased macrophage activation and diminished nitric oxide (NO) production.[8]

Transmission back to the sand fly occurs when a susceptible sand fly feeds on an infected reservoir host. It is unclear whether host parasitemia or parasites within the skin are responsible for this transmission. Sand fly saliva has been shown to increase host parasitemia after repeated feedings,[9] but the variability of sand fly infection seen in xenodiagnostic studies may be better accounted for by patchy skin infection.[10]

Depending on the *Leishmania* species, the sand fly genus, and the geographic location, the major reservoirs for leishmaniasis may be canines, rodents, or humans.

Pathogenesis and Immunology

Leishmaniasis can be thought of as a polar disorder similar to other intracellular infections such as leprosy (Fig. 275.3). On one end of the spectrum are polyparasitic infections (e.g., diffuse CL or VL) characterized by a predominantly Th2-type immune response with relative anergy, akin to lepromatous leprosy. Heavily parasitized macrophages are abundant, and diagnosis is readily made by smear. The oligoparasitic end of the spectrum includes ML and latent VL infection. Amastigotes are sparse, and a mononuclear cell infiltrate predominates in a Th1-type immune response, analogous to tuberculoid leprosy. In reality, the interaction between Th1 and Th2 is much more complex and includes variable modulation by Th17-type $CD4^+$ T cells among others.[11,12]

Why some species spread systemically causing visceral disease and others remain relatively confined to the skin is not fully understood. The A2 gene family, found as a functional gene among species that cause VL, is likely important in visceralization, protecting the parasite from heat shock and oxidative stress.[13] This is further supported by the observation that subspecies of *L. donovani* isolated from patients with CL have much less A2 production, but when A2 production is increased, the capacity to visceralize is restored.[14] Comparing the sequences of different CL and VL species shows that only 19 of >8000 total genes are *L. donovani*–specific genes.[15] Insertion of some of these *L. donovani*–specific genes into *Leishmania major* promotes the survival of *L. major* in the viscera of mice in murine models.[13]

TABLE 275.1 Major Clinical Syndromes, *Leishmania* Parasites, Key Details, and Treatment Overview

CLINICAL SYNDROMES	*LEISHMANIA* SPECIES	KEY DETAILS	TREATMENT OVERVIEW[a]
VL	*Leishmania donovani*	Classic kala-azar in South Asia and East Africa	Regimen may vary by region in which infection is acquired, but for providers in North America the general preference is L-AmB (various regimens, but FDA-approved regimen is 3 mg/kg/d on days 1–5, 14, and 21, with higher doses for immunocompromised hosts)[1]; other options include SbV (20 mg/kg/d for 28 days) alone or in combination with PM (preferred in East Africa VL over L-AmB),[2,3] MIL (2.5 mg/kg/d for 28 days, FDA-approved regimen for *L. donovani* infection), PM, or combination therapy
	Leishmania infantum (syn *Leishmania chagasi*)	Major species in Americas; in Mediterranean causes infantile splenomegaly or, in adults, is often immunocompromise-related	
	Leishmania amazonensis, Leishmania martiniquensis, Leishmania tropica, and others	Rare causes of atypical, viscerotropic leishmaniasis	
Post–kala-azar dermal leishmaniasis	*L. donovani*	Generally macular in South Asia and nodular in East Africa	MIL for at least 12 weeks (India)[4] or SbV for 8–12 weeks (East Africa; treatment reserved for severe or persistent disease),[5] potentially amphotericin B formulations or adjunctive immunotherapy[6]
	L. infantum (syn *L. chagasi*)	Rare and often immunocompromise-related	Not well established[7]
CL	Various species	Chronic, often ulcerating, skin lesions with some characteristic features by region and species	See Table 275.2; for simple CL, options include watchful waiting, local therapy, and azoles; for complex CL, systemic therapy with amphotericin B formulations, SbV, MIL, or pentamidine is generally indicated
Old World CL	*Leishmania major*	So-called wet, rural oriental sore; widespread including Africa, Mediterranean littoral region, and Southwest and Central Asia	See Table 275.2
	L. tropica	So-called dry, urban oriental sore with predilection for the face	See Table 275.2
	Leishmania aethiopica	Major cause of CL in Ethiopia	Limited data; cryotherapy, SbV, and pentamidine reported[8]
	L. donovani and *L. infantum* (syn *L. chagasi*)	Less common causes of CL; often genetically distinct from strains causing VL	Can often be treated as simple CL in immunocompetent hosts
New World CL	*Leishmania mexicana*	Involves exposed skin including ear (chiclero's ulcer); seen from Texas to South America	Ketoconazole[9] or local therapies are first-line options
	Leishmania (Viannia) braziliensis	Widespread in Central and South America; challenging to treat; associated with ML and disseminated leishmaniasis	Treat as complex CL, usually systemically; some believe that *L. (V.) braziliensis* north of Costa Rica has less risk of ML and can be treated as simple CL; see IDSA-ASTMH guidelines[1]
	Leishmania (Viannia) guyanensis	Bush yaws or pian bois in northern Amazon basin; may be associated with ML	May be role for systemic pentamidine; recently intravenous therapy reported better than intramuscular[10]; treat systemically due to risk of ML
	Leishmania (Viannia) panamensis	Causes high rates of CL in Panama, Costa Rica, and Colombia; may be associated with ML	See Table 275.2
	Leishmania (Viannia) peruviana	La uta in Peru	Typically treated with systemic SbV, but resistance noted (efficacy 75.5%)[11]
	L. infantum (syn *L. chagasi*)	Nodular CL in scattered areas of Central and South America	Can often be treated as simple CL in immunocompetent hosts
	L. amazonensis, Leishmania (Viannia) colombiensis, Leishmania venezuelensis, Leishmania naiffi Leishmania pifanoi, Leishmania garnhami, and others	Other agents of New World CL spread across Central and South America	See Table 275.2
Leishmaniasis recidivans	*L. tropica*	Relapsing, satellite lesions around scar of primary CL lesion	Challenging; amphotericin formulations may have effect[12,13]; many relapse, retreatment depends on what was used in initial therapy
Diffuse CL	*L. mexicana, L. amazonensis,* and *L. aethiopica*	Anergic CL with spreading nodular disease progressing out from primary lesion	Resistant to treatment; may respond to MIL, but relapse is expected; immunotherapy has also been used[14–18]; amphotericin formulations seem to have effect[19–22]; WHO recommends SbV and MIL; if due to *L. aethiopica,* SbV and allopurinol[6]
	Other *Leishmania* spp.	Less common and generally in immunocompromised hosts	
Disseminated leishmaniasis	*L. (V.) braziliensis* and *L. amazonensis*; less commonly other *Viannia* spp.	Widespread, noncontiguous, pleomorphic lesions in immunocompetent hosts; particularly noted in Bahia, Brazil; oligoparasitic	Not well established; options include L-AmB with total doses of 17–37 mg/kg,[23] combination of L-AmB and MIL,[24] or amphotericin B deoxycholate[25]

Continued

TABLE 275.1 Major Clinical Syndromes, *Leishmania* Parasites, Key Details, and Treatment Overview—cont'd

CLINICAL SYNDROMES	*LEISHMANIA* SPECIES	KEY DETAILS	TREATMENT OVERVIEW[a]
ML	*L. (V.) braziliensis*; less commonly *L. (V.) guyanensis and L. (V.) panamensis*	Metastatic involvement of mucous membranes of upper airways in 2%–10% of CL cases caused by these species; often seen months to years after primary lesion	Regimen is tailored to individual with few randomized trials to guide therapy; options include SbV (20 mg/kg/d for 28 days), amphotericin B formulations (total dosage 20–40 mg/kg), or MIL (2.5 mg/kg/d for 28 days); adjunctive pentoxifylline and/or plastic surgery may be indicated
	L. tropica, L. major, L. donovani, L. infantum (syn *L. chagasi*), and others	Rare mucosal involvement either as a primary focus or contiguous spread from CL; may be associated with immunocompromise	Not well established; L-AmB and MIL have been used with success in *L. infantum* (syn *L. chagasi*)[26]

[a]The treatment of leishmaniasis must be individualized. Please see the clinical practice guidelines for additional details.[1,27] In general, treatment studies are not robust, and many comments included here are based on data from small observational series or individual case reports. The nuances of *Leishmania* species, geographical variability in drug resistance, and host factors are often ignored.

CL, Cutaneous leishmaniasis; *FDA,* US Food and Drug Administration; *IDSA-ASTMH,* Infectious Diseases Society of America–American Society of Tropical Medicine and Hygiene; *L-AmB,* liposomal amphotericin B; *MIL,* miltefosine; *ML,* mucosal leishmaniasis; *PM,* paromomycin; *SbV,* pentavalent antimony; *syn,* synonym; *VL,* visceral leishmaniasis; *WHO,* World Health Organization.

References

1. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clin Infect Dis.* 2016;63:e202–e264.
2. Kimutai R, Musa AM, Njoroge S, et al. Safety and effectiveness of sodium stibogluconate and paromomycin combination for the treatment of visceral leishmaniasis in Eastern Africa: results from a pharmacovigilance programme. *Clin Drug Investig.* 2017;37:259–272.
3. Musa A, Khalil E, Hailu A, et al. Sodium stibogluconate (SSG) and paromomycin combination compared to SSG for visceral leishmaniasis in East Africa: a randomised controlled trial. *PLoS Negl Trop Dis.* 2012;6:e1674.
4. Ramesh V, Singh R, Avishek K, et al. Decline in clinical efficacy of oral miltefosine in treatment of post kala-azar dermal leishmaniasis (PKDL) in India. *PLoS Negl Trop Dis.* 2015;9:e0004093.
5. el Hassan AM, Ghalib HW, Zijlstra EE, et al. Post kala-azar dermal leishmaniasis in the Sudan: clinical features, pathology and treatment. *Trans R Soc Trop Med Hyg.* 1992;86:245–248.
6. World Health Organization. *Control of the Leishmaniases: Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases.* Geneva: WHO; 2010. WHO TRS No. 949.
7. Celesia BM, Cacopardo B, Massimino D, et al. Atypical presentation of PKDL due to *Leishmania infantum* in an HIV-infected patient with relapsing visceral leishmaniasis. *Case Rep Infect Dis.* 2014;2014:370286.
8. van Griensven J, Gadisa E, Aseffa A, Hailu A, Beshah AM, Diro E. Treatment of cutaneous leishmaniasis caused by *Leishmania aethiopica*: a systematic review. *PLoS Negl Trop Dis.* 2016;10:e0004495.
9. Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF. Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. *J Infect Dis.* 1992;165:528–534.
10. Christen JR, Bourreau E, Demar M, et al. Use of the intramuscular route to administer pentamidine isethionate in *Leishmania guyanensis* cutaneous leishmaniasis increases the risk of treatment failure. *Travel Med Infect Dis.* 2018;24:31–36.
11. Arevalo J, Ramirez L, Adaui V, et al. Influence of *Leishmania (Viannia)* species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. *J Infect Dis.* 2007;195:1846–1851.
12. Dassoni F, Daba F, Naafs B, Morrone A. Leishmaniasis recidivans in Ethiopia: cutaneous and mucocutaneous features. *J Infect Dev Ctries.* 2017;11:106–110.
13. Gunduz K, Afsar S, Ayhan S, et al. Recidivans cutaneous leishmaniasis unresponsive to liposomal amphotericin B (AmBisome). *J Eur Acad Dermatol Venereol.* 2000;14:11–13.
14. Calvopina M, Gomez EA, Sindermann H, Cooper PJ, Hashiguchi Y. Relapse of New World diffuse cutaneous leishmaniasis caused by *Leishmania (Leishmania) mexicana* after miltefosine treatment. *Am J Trop Med Hyg.* 2006;75:1074–1077.
15. Convit J, Ulrich M, Polegre MA, et al. Therapy of Venezuelan patients with severe mucocutaneous or early lesions of diffuse cutaneous leishmaniasis with a vaccine containing pasteurized *Leishmania* promastigotes and bacillus Calmette-Guerin: preliminary report. *Mem Inst Oswaldo Cruz.* 2004;99:57–62.
16. Ordaz-Farias A, Munoz-Garza FZ, Sevilla-Gonzalez FK, et al. Case report: transient success using prolonged treatment with miltefosine for a patient with diffuse cutaneous leishmaniasis infected with *Leishmania mexicana mexicana*. *Am J Trop Med Hyg.* 2013;88:153–156.
17. Schraner C, Hasse B, Hasse U, et al. Successful treatment with miltefosine of disseminated cutaneous leishmaniasis in a severely immunocompromised patient infected with HIV-1. *Clin Infect Dis.* 2005;40:e120–124.
18. Zerpa O, Ulrich M, Blanco B, et al. Diffuse cutaneous leishmaniasis responds to miltefosine but then relapses. *Br J Dermatol.* 2007;156:1328–1335.
19. Alcover MM, Rocamora V, Guillen MC, et al. Case report: diffuse cutaneous leishmaniasis by *Leishmania infantum* in a patient undergoing immunosuppressive therapy: risk status in an endemic Mediterranean area. *Am J Trop Med Hyg.* 2018;98:1313–1316.
20. Hashemi SA, Badirzadeh A, Sabzevari S, Nouri A, Seyyedin M. First case report of atypical disseminated cutaneous leishmaniasis in an opium abuser in Iran. *Rev Inst Med Trop Sao Paulo.* 2018;60:e5.
21. Morrison B, Mendoza I, Delgado D, Reyes Jaimes O, Aranzazu N, Paniz Mondolfi AE. Diffuse (anergic) cutaneous leishmaniasis responding to amphotericin B. *Clin Exp Dermatol.* 2010;35:e116–119.
22. Supsrisunjai C, Kootiratrakarn T, Puangpet P, Bunnag T, Chaowalit P, Wessagowit V. Disseminated autochthonous dermal leishmaniasis caused by *Leishmania siamensis* (PCM2 Trang) in a patient from central Thailand infected with human immunodeficiency virus. *Am J Trop Med Hyg.* 2017;96:1160–1163.
23. Machado PR, Rosa ME, Guimaraes LH, et al. Treatment of disseminated leishmaniasis with liposomal amphotericin B. *Clin Infect Dis.* 2015;61:945–949.
24. Hamzavi SS, Sanaei Dashti A, Kadivar MR, Pouladfar G, Pourabbas B. Successful treatment of disseminated cutaneous leishmaniasis with liposomal amphotericin B and miltefosine in an eight-year-old girl. *Pediatr Infect Dis J.* 2018;37:275–277.
25. Noppakun N, Kraivichian K, Siriyasatien P. Disseminated dermal leishmaniasis caused by *Leishmania siamensis* in a systemic steroid therapy patient. *Am J Trop Med Hyg.* 2014;91:869–870.
26. Mosimann V, Neumayr A, Paris DH, Blum J. Liposomal amphotericin B treatment of Old World cutaneous and mucosal leishmaniasis: a literature review. *Acta Trop.* 2018;182:246–250.
27. Copeland NK, Aronson NE. Leishmaniasis: treatment updates and clinical practice guidelines review. *Curr Opin Infect Dis.* 2015;28:426–437.

Regardless of tropism, intracellular survival is essential to the pathogenesis of all forms of leishmaniasis. Multiple virulence factors enable *Leishmania* organisms to survive intracellularly, including lipophosphoglycan and leishmanolysin. The former aids in the transformation of phagosomes into parasitophorous vacuoles by promastigotes. Leishmanolysin, or GP63, is expressed on the parasite cell surface and secreted in exosomes; it facilitates complement and natural killer cell inactivation, prevents antigen cross-presentation to T cells, and modulates macrophage signaling and transcription pathways.[16,17]

Diagnostic Principles

There are three ways to approach the diagnosis of all suspected *Leishmania* infections: clinical, parasitologic, and immunologic. Clinical diagnosis combines epidemiology with clinical manifestations and serves as a useful guide but is rarely adequate in and of itself due to the cost and toxicity of available therapies as well as the prognostic importance of species identification, particularly in CL.

A parasitologic diagnosis is confirmed by visualizing amastigotes in a tissue biopsy specimen or smear, visualizing promastigotes in culture,

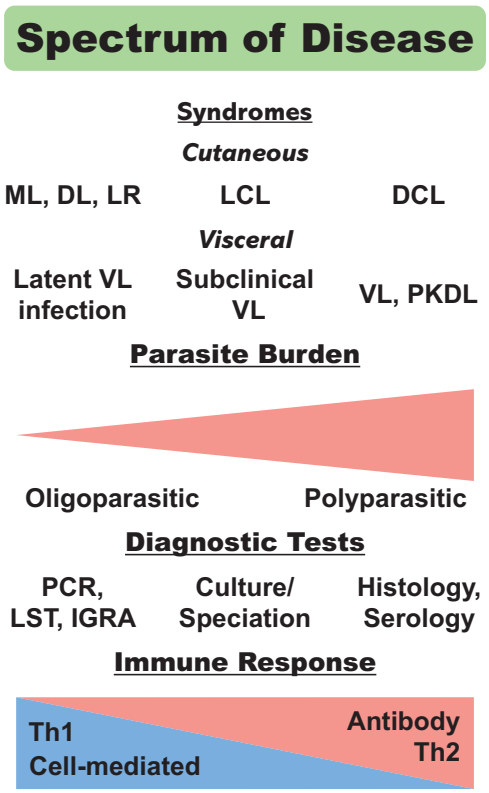

FIG. 275.3 Spectrum of *Leishmania* infection and disease. *DCL,* Diffuse cutaneous leishmaniasis; *DL,* disseminated leishmaniasis; *IGRA,* interferon-γ release assay; *LCL,* localized cutaneous leishmaniasis; *LR,* leishmaniasis recidivans; *LST,* leishmanin skin test; *ML,* mucosal leishmaniasis; *PCR,* polymerase chain reaction; *PKDL,* post–kala-azar dermal leishmaniasis; *Th1,* Th1-predominant response; *Th2,* Th2-predominant response; *VL,* visceral leishmaniasis.

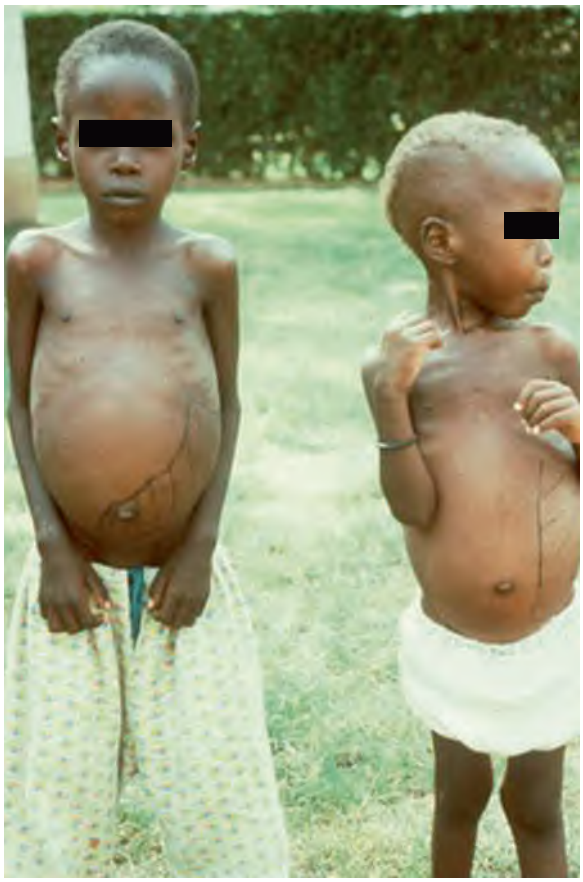

FIG. 275.4 Children with visceral leishmaniasis in Kenya. Note signs of malnourishment and protruding abdomen with massive splenomegaly. *(Courtesy Dr. Charles Oster, Washington, DC.)*

or amplifying *Leishmania*-specific nucleic acids by polymerase chain reaction (PCR). Optimally, parasites should be speciated using either isoenzyme techniques from cultured parasites or molecular methods such as multilocus sequence typing or PCR from a clinical sample, although molecular methods lack standardization. Specimens for culture can be inoculated into one of several media (Schneider's modified medium, Novy-MacNeal-Nicolle medium, and others) and maintained at 22°C to 26°C. In vitro, amastigotes transform into motile promastigotes and multiply, taking a few days to several weeks to reach detectable levels depending on the inoculum size. Parasite culture and *Leishmania* molecular assays are generally relegated to reference laboratories, and it is helpful to contact the laboratories in advance to ensure appropriate collection methods. In the United States, culture media and expert assistance are available from the Centers for Disease Control and Prevention (http://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html#dx). Protocols using matrix-assisted laser desorption/ionization may allow more widespread future availability of species identification.[18–20]

Immunologic diagnosis is an adjunct in most cases, with various antibody tests, cytokine release assays, and the leishmanin (Montenegro) skin test. The leishmanin skin test and cytokine release assays such as the interferon (IFN)-γ release assay (IGRA), evaluate for cell-mediated immune responses, but neither the skin test nor cytokine release assays are standardized or commercially available.

The choice of the optimal diagnostic test or procedure depends on the parasite burden of the leishmaniasis syndrome (see Fig. 275.3). The guidelines from the Infectious Disease Society of America (IDSA) and the American Society for Tropical Medicine and Hygiene (ASTMH) recommend a multipronged approach in most cases, with the use of molecular methods whenever possible.[21]

Treatment Principles

The diversity of *Leishmania* infections makes standard treatment recommendations impossible. Each region has different species complexes with a greater or lesser degree of genetic heterogeneity within the complex.[22,23] These genetic differences may be reflected in a variable natural history of infection and response to treatment. In addition, each geographic region has its own unique combination of sand fly vectors, mammalian reservoirs, and human hosts with different genetic backgrounds in varying zoonotic or anthroponotic cycles, leading to different outcomes and treatment responses. Optimal drug treatment regimens for each geographic region and major syndrome are best defined in consideration of demonstrated regional efficacy, available resources, and risk-benefit assessments. In resource-rich countries, the efficacy, safety, availability, and tolerability of drug regimens can be the primary factors regarding choice, whereas in low-resource endemic areas, cost and availability are crucial.

In addition to regional differences, variations in trial design, particularly treatment end points, make definitive recommendations difficult. The end points used in clinical trials include clinical, parasitologic, and immunologic cure. These refer to the resolution of clinical symptoms and signs within a defined time period; the absence of parasites by smear, culture, or PCR; and a falling antibody titer or conversion of a skin test from negative to positive. Although current chemotherapy options result in a clinical cure, they may seldom lead to true parasitologic cure, with persistence of *Leishmania* parasites in host tissue being the rule, not the exception.[24–27]

The IDSA-ASTMH guidelines summarize the treatment recommendations along with approved or recommended doses and side-effect profiles for providers practicing in North America.[21]

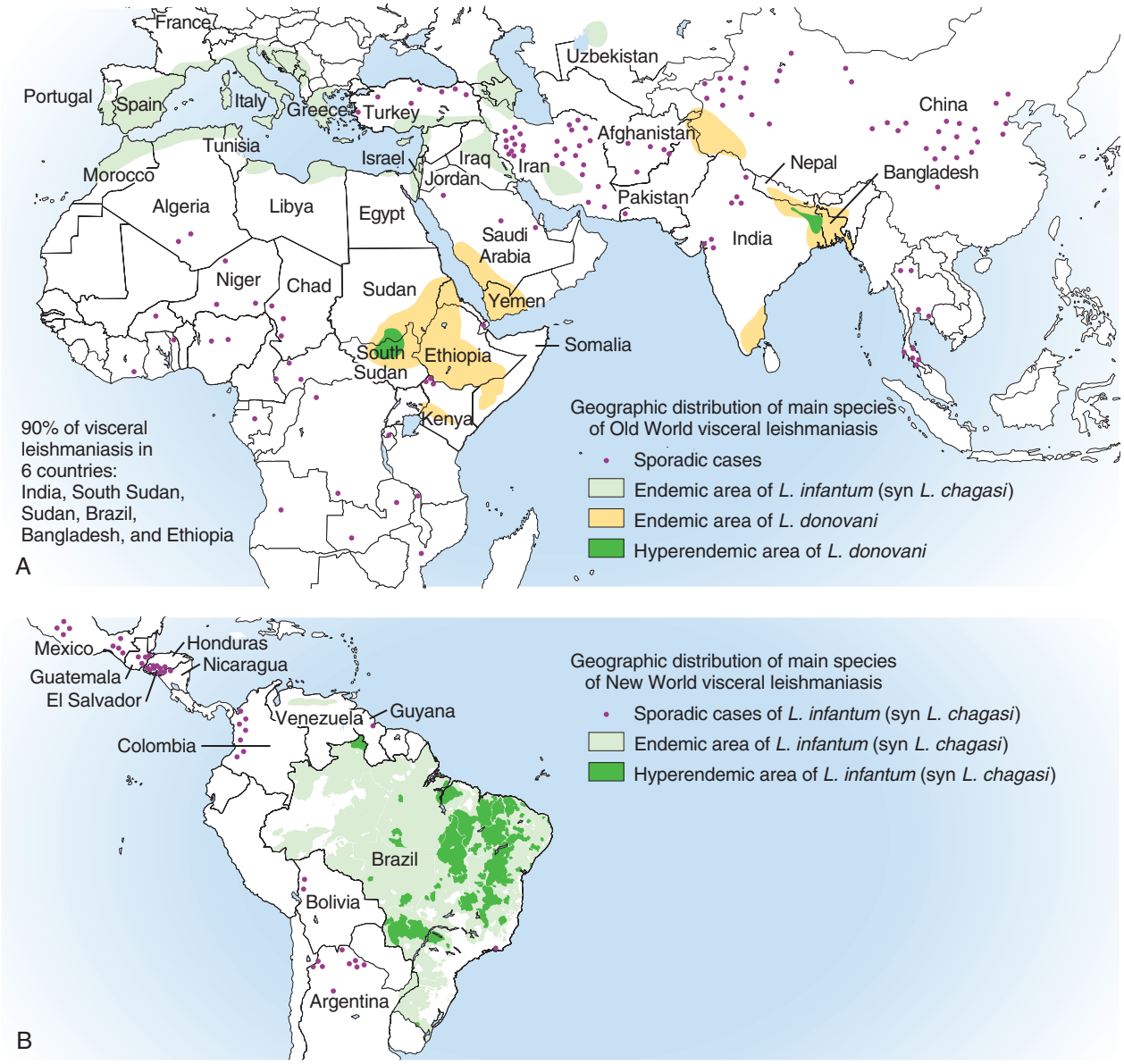

FIG. 275.5 Distribution of visceral leishmaniasis. (A) Old World (Eastern Hemisphere). (B) New World (Western Hemisphere).

VISCERAL LEISHMANIASIS

VL is a spectrum of symptoms and findings ranging from latent infection to classic VL, with the characteristic pentad of prolonged fever, weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia (Fig. 275.4). Parasites in the *L. donovani* complex are responsible for most cases of VL (see Table 275.1). The complex is made up of two principal species, *L. donovani* and *Leishmania infantum* (synonym [syn] *Leishmania chagasi*). The latter was previously considered two species, but it appears that early explorers from the Old World introduced it into the New World.[28] In the Indian subcontinent, VL is also known as *kala-azar* (Hindi for black or fatal sickness), *Dum Dum fever,* and *Assam fever,* among others. Mediterranean VL caused by *L. infantum* (syn *L. chagasi*) is also known as *infantile splenomegaly*.

Epidemiology

VL is endemic in 75 countries across the tropics and subtropics, predominantly in low-income or low-middle–income countries (Fig. 275.5). More than 90% of reported cases from the last 10 years were in Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan. The World Health Organization (WHO) estimated the incidence of VL to be 200,000 to 400,000 clinical cases/year in 2010[29]; as of 2015, the number of reported cases had fallen by nearly 60% corresponding to an estimated 82,000 to 164,000 cases/year globally (assuming similar rates of underreporting).[30] The global decline has largely been driven by declines seen in the Indian subcontinent. VL is often clustered locally and regionally and may undergo epidemic cycles in some regions.[31] VL is generally either an anthroponosis (sand fly–human–sand fly) or zoonosis depending on the region and parasite species. In anthroponotic disease, clinically ill patients with VL have the greatest potential of transmitting the parasite, although persons with post–kala-azar dermal leishmaniasis (PKDL) or latent infection also play a role in maintaining the disease.[32,33]

There is an increasing recognition of asymptomatic or latent infection occurring in endemic areas.[34] Depending on the region, testing modality, and definition of asymptomatic infection, the prevalence is 0.3% to 37%,[33,35–38] with the ratio of latent to active VL in the range of 4:1 to 50:1.[33,39]

Both *L. donovani* and *L. infantum* (syn *L. chagasi*) cause VL in the Old World (see Fig. 275.5A). In the Indian subcontinent, humans serve as the reservoir for *L. donovani,* and transmission is by *Phlebotomus argentipes* and other anthropophilic species. As a result of a multinational elimination effort in South Asia, reported cases and deaths in India have decreased by greater than 70% and 95%, respectively, from 2010 to 2015.[40]

In East Africa, VL caused by *L. donovani* is endemic in Eritrea, Ethiopia, Kenya, Somalia, South Sudan, Sudan, and Uganda and sporadic in other countries of the region. Conflict-related instability and resulting

displaced people have produced multiple waves of epidemic VL.[41] The exact reservoir in the region is unclear, but putative reservoirs include humans (especially during epidemics), rats, gerbils, other rodents, small carnivores, and potentially dogs.[42] Also, VL caused by *L. donovani* exists in southwestern Saudi Arabia, western Yemen, northeast Afghanistan, Pakistan, and western China, with humans and black rats serving as potential reservoirs, depending on the region.[43–46]

In Europe and the Mediterranean littoral region, VL caused by *L. infantum* (syn *L. chagasi*) is a zoonosis with dogs being the principal reservoir. Clinically apparent cases are typically encountered among infants, young children, and immunocompromised persons. In the 1990s VL emerged as an important opportunistic disease among patients with acquired immunodeficiency syndrome (AIDS) in southern Europe (Spain, France, and Italy).[47,48] The rates of coinfection with human immunodeficiency virus (HIV) and VL have declined significantly with the advent of antiretroviral therapy (ART). Highlighting the clustered, cyclic nature of VL, a large outbreak occurred near several parks in southwestern Madrid, Spain, in the years 2009–12.[49,50] VL caused by *L. infantum* (syn *L. chagasi*) also extends eastward out of the Mediterranean into Southwest and Central Asia as well as northwestern and central China, where dogs, other canines, cats, and hares are reservoirs.

In Latin America, *L. infantum* (syn *L. chagasi*) is endemic and broadly distributed (see Fig. 275.5B). Most areas have focal disease risk with a background of latent or subclinical infection with sporadic clinical cases in rural areas. The clustering of cases in households suggests that humans may also be reservoirs in these settings. Since the 1950s disease epidemiology has shifted from rural to predominantly urban, and major periurban outbreaks of VL have been reported from cities in northeastern Brazil.[51] Children are most frequently affected. *Lutzomyia longipalpis* is the major vector. Domestic dogs and wild foxes are reservoirs of infection.

Although sand fly bites account for most VL transmission, the parasite can be transmitted by blood transfusion, needle sharing, occupational exposure, and congenital exposure.[52–54] Clear evidence of sexual transmission exists in canines, but only a single case of human sexual transmission has been reported.[55,56]

Leishmania species that are typically associated with cutaneous syndromes—such as *Leishmania amazonensis* in Latin America[57,58] and *Leishmania tropica* in Kenya, India, Iran, and Saudi Arabia—are isolated in rare instances from patients with oligosymptomatic visceral syndromes.[59,60] For example, a small group of American military personnel who served in the Persian Gulf War acquired a viscerotropic form of *L. tropica* infection.[61] Additionally, *Leishmania martiniquensis* in the Caribbean[62] and related species in the *Leishmania enrietti* complex in Thailand[63] can cause visceral disease.

Pathogenesis and Immunology

After infection the parasites of the *L. donovani* complex are phagocytized and spread systemically. From a pathologic perspective, VL is characterized by inadequately controlled parasite replication leading to increasing numbers of amastigote-infected mononuclear phagocytes in the liver and spleen with progressive hypertrophy and clinically apparent hepatosplenomegaly. The splenic lymphoid follicles are replaced by parasitized mononuclear cells. There is a marked increase in the number and size of Kupffer cells in the liver, many of which contain amastigotes. Autopsy studies reveal wide dissemination of the parasite with infected mononuclear phagocytes observed in the bone marrow, lymph nodes, skin, and other organs.[64,65]

The immune response is complex and modulated by multiple factors.[13,66] A protective immune response involves the production and interaction of interleukin (IL)-12, IFN-γ, and tumor necrosis factor (TNF)-α, among others. IL-12 is produced by macrophages and dendritic cells in response to intracellular parasites and costimulation by T cells.[67,68] IL-12 not only promotes differentiation of Th1 $CD4^+$ T cells but also stimulates the production of IFN-γ by multiple cell types. IFN-γ stimulates the secretion of TNF-α, and together these cytokines activate macrophages to kill intracellular amastigotes through the production of NO and reactive oxygen species. In the liver, granuloma formation seems to be important to the control of VL, although perhaps not essential, and is influenced by TNF-α, various chemokines, and T-cell granzyme production.[69,70]

An ineffectual immune response is characterized by the production of IL-10 and transforming growth factor (TGF)-β. IL-10 inhibits IFN-γ–mediated macrophage activation, suppresses NO production, and inhibits the production of IL-12 and TNF-α. TGF-β interferes with macrophage activation and suppresses expression of major histocompatibility complex type II on monocytes. Although IFN-γ, TNF-α, and other Th1-type cytokines are often elevated in active VL, the influence of IL-10 and the Th2 environment results in a pathologic response.[71]

Multiple other cell types contribute to an ineffectual immune response including macrophages,[72–75] neutrophils,[76,77] and B cells.[78] B-cell proliferation and antibody production is generally regarded as nonspecific and ineffectual, but *N*-glycosylation patterns may be involved in the pathophysiology.[79]

The reason some people develop a protective response when others develop disease is not fully understood. The sequence of early cytokine responses and the manner in which *Leishmania* antigens are presented by macrophages and dendritic cells—influenced by vector, parasite, and host factors—may be important variables determining the response to an individual infection.[13]

Vector determinants associated with progressive disease include the vasodilatory effects of sand fly saliva, a smaller size of the parasite inoculum, and an absence of preexisting immune responses to sand fly salivary proteins. Parasite factors associated with visceralization were discussed previously. It is likely that there are subspecies differences among VL-causing *Leishmania* species that influence immune response and parasite virulence, but further genomic, transcriptomic, and proteomic comparisons are needed.[80]

Host determinants associated with the outcome of infection and the spectrum of clinical manifestations include genetically determined human immune responses, host nutritional status, and immunocompetence. Genetic risk factors include loci on chromosomes 22q12 and 2q22-23 in Sudan, the HLA-CDR-HLA-DQA1 locus in Brazil and India, and various cytokine pathways (including IL-12, IL-10, TNF-α, and mannose-binding lectin).[13,81–92] Additionally, risk for disease relapse has been seen in families from Sudan with mutations involving the alkylglycerol monooxygenase gene.[93] Malnutrition is a risk factor for progression of infection to disease and results in lower concentrations of leptin, which influences IFN-γ and IL-12 production.[94] As discussed subsequently, various forms of immunosuppression predispose infected individuals to develop symptomatic VL. Additionally, the extremes of age and in utero exposure to the parasite are associated with disease.

Natural History and Latent Visceral Leishmaniasis Infection

The natural history of leishmaniasis generally progresses along two pathways: latent VL infection or progression to disease. There is not a consensus in the literature regarding the definition of asymptomatic VL, with patients with latent infection being identified variably by skin test, IGRA, serology, or PCR.[33,39] When multiple tests are performed on the same asymptomatic population, they may not show agreement.[34] Latent infection progresses to active VL in 2% to 29% of individuals at up to 3 years.[33,95] Most individuals with latent infection remain asymptomatic, but such individuals may develop subclinical symptoms or splenomegaly before progressing to frank disease or resolving spontaneously. Activation of latent infection is more common in patients who are antibody positive or immunosuppressed and can occur many years after exposure.[96]

For patients progressing more directly to symptomatic disease, the incubation time has generally been regarded as 2 to 8 months but can range from 10 days to >1 year. However, incubation periods are often extrapolated from travelers with known exposure periods, and the latent infection paradigm adds further uncertainty. Individuals may have a subclinical course with spontaneous resolution or progression to overt disease, or they may immediately develop classic kala-azar. Although most patients with fully symptomatic kala-azar will die without intervention, there are reports of spontaneous disease resolution.[97] Various studies have shown that risk of death is increased with delayed diagnosis, incomplete treatment, lower social status, age <2 years or >40 to 45 years, HIV or tuberculosis coinfection, low body mass index, jaundice, ascites or edema, bleeding, severe anemia, thrombocytopenia, hypoalbuminemia, or hyponatremia.[98–101]

Persons with self-resolving infection and persons who have undergone successful chemotherapy develop protective immune responses. However, the parasite is not eradicated, and disease can redevelop years later if the infected person becomes immunocompromised.

Clinical Manifestations

The fully developed clinical manifestations of VL are similar in all endemic areas. Skin lesions at the site of inoculation are usually not apparent in persons with VL. In cases with a subacute or chronic course, there is an insidious onset of fever, weakness, loss of appetite, weight loss, failure to thrive, and abdominal enlargement caused by hepatosplenomegaly. In endemic areas, low-grade symptoms may persist for weeks to months but may not be sufficiently severe to warrant medical attention in low-resource areas, and the condition of such patients may be called subclinical when oligosymptomatic would be more appropriate. Fever may be intermittent, remittent with twice-daily temperature spikes (double quotidian), or, less commonly, continuous. Fever is relatively well tolerated, and older clinical references routinely noted that patients were not acutely ill or toxic in appearance.

Acute presentation in nonimmune persons comprises abrupt onset with high fever and chills, sometimes with a periodicity that suggests malaria.[102] Chills, but seldom rigors, accompany the temperature spikes. As time passes, the spleen can become massively enlarged. It is usually soft to firm and nontender. The presence of a hard spleen suggests a hematologic disorder or another diagnosis such as schistosomiasis. The liver also enlarges; it usually has a sharp edge, soft consistency, and a smooth surface. Lymphadenopathy is common in patients in Sudan[103] but uncommon in other geographic areas. Elevated liver enzymes and bilirubin may be observed. Peripheral edema may be seen late in disease, particularly in malnourished children. Hemorrhage can occur from one or more sites; epistaxis and gingival bleeding may be noted as well as petechiae and ecchymoses on the extremities in late-stage disease. Many patients with VL become cachectic, mediated in part by TNF-α and other cytokines known to have catabolic and anorectic effects.[104]

Secondary bacterial infections are common in persons with advanced VL. Patients can present with coinfection or acquire secondary bacterial infections during hospitalizations. It is important to recognize and treat clinically significant bacterial coinfections.[105–107] Death may result from pneumonia, septicemia, tuberculosis, dysentery, or measles, or it may be the consequence of malnutrition, severe anemia, or hemorrhage.

The laboratory findings include anemia, leukopenia, thrombocytopenia, and hypergammaglobulinemia. Anemia is almost always present and may be severe. It is usually normocytic and normochromic and appears to be due to a combination of factors including hemolysis, marrow replacement with *Leishmania*-infected macrophages, hemorrhage, splenic sequestration of erythrocytes, hemodilution, and marrow suppressive effects of cytokines such as TNF-α. Secondary hemophagocytic lymphohistiocytosis is increasingly reported, frequently in children.[108,109] Leukopenia is also prominent, with white blood cell counts occasionally as low as 1000/mm^3. Severe eosinopenia is frequently observed. Of note, anemia and neutropenia have not been prominent in patients with VL who have undergone splenectomy. Hypergammaglobulinemia, circulating immune complexes, and rheumatoid factors are present in the sera of most patients with VL.[110,111] The globulin level may be as high as 9 g/dL; the ratio of globulin to albumin is typically high. The erythrocyte sedimentation rate is usually elevated.[112]

Renal involvement in VL is common and includes proteinuria, acute renal failure, nephrotic syndrome, glomerulonephritis, acute interstitial nephritis, tubular necrosis, and tubulitis.[113–118] Elevated serum creatinine has been observed both retrospectively and prospectively in 26% to 28% of patients with VL in Brazil, but abnormalities in urinary concentration and acidification were seen in >60%.[119,120]

Viscerotropic disease caused by atypical species is characterized by chronic low-grade fever, malaise, fatigue, and in some cases diarrhea. Mild splenomegaly can occur, but generally patients do not develop classic kala-azar or progressive VL.[61]

Diagnosis

The positive predictive value of a clinical diagnosis of VL in patients with the pentad of prolonged fever, progressive weight loss, pronounced hepatosplenomegaly (especially splenomegaly), pancytopenia, and hypergammaglobulinemia from a known endemic area is very high. However, clinical diagnosis can be challenging in patients with atypical presentations such as oligosymptomatic or immunosuppressed patients or in returned travelers presenting to physicians in nonendemic regions months to years after an exposure.

Samples for parasitologic diagnosis can be obtained by splenic aspiration, bone marrow aspiration, liver biopsy, and lymph node aspiration. Splenic aspiration to obtain a few drops of fluid for Wright-Giemsa–stained smears, culture, or PCR assay is the most sensitive method for parasite identification.[121] Splenic aspirations have sensitivities of 93% to 99%, and the parasite burden can be quantified to stage patients and monitor response to treatment.[122] However, splenic aspiration carries the risk of life-threatening hemorrhage, particularly in advanced disease, and is not recommended in North America.[21] Bone marrow aspiration is safer and preferred in nonendemic settings but is less sensitive, ranging from 60% to 85%, but improving to as high as 95% when microscopists review smears for an hour.[123,124] Liver biopsy is even less likely to yield the diagnosis but occasionally is positive when bone marrow is negative.[125] Lymph node aspiration or biopsy may be diagnostic when enlarged nodes are present, as is often the case in East Africa. Rarely, amastigotes may also be seen on blood smears or cultured from the buffy coat or blood.[126] When the diagnosis of VL is suspected, parasitologic confirmation may require more than one technique and repeated procedures.[102] Whenever possible, aspirates should also be cultured.

Molecular approaches have the potential to replace traditional parasitologic methods due to their diagnostic accuracy even when using blood samples rather than more invasive splenic or bone marrow aspirates. A meta-analysis found a pooled sensitivity and specificity from whole blood of 93% and 96%, respectively.[127] Certain molecular assays are more sensitive than microscopic methods for the detection of parasites and can detect parasites in asymptomatic individuals with exposure risks. Whereas various techniques exist, quantitative PCR assay has the advantage of being able to assess parasite load in patients with VL, which is highly correlated to splenic score.[128] Also, quantitative PCR may provide a threshold for distinguishing latent infection from active disease and is useful in monitoring response to treatment.[129,130] There is a lack of standardization in methodology and target sequence, and larger studies are needed to compare different methods, both within and between VL regions. Assays targeting kinetoplast DNA minicircles have been shown to be the most sensitive target, likely as a result of each parasite containing approximately 10^4 minicircles, although this number is variable, making quantification less reliable.[131] Replacing traditional parasitologic methods with PCR has also been shown to be potentially cost saving.[132] However, most molecular methods require well-equipped laboratory facilities, minimizing their applicability to resource-limited settings. Techniques such as loop-mediated isothermal amplification or recombinase polymerase amplification may offer reliable alternatives for field application but require further standardization and development.[133–135]

Immunologic tests are widely used in the diagnosis of VL worldwide. High-titer antileishmanial antibodies are typically present in immunocompetent persons with symptomatic VL and are variably present in individuals with latent infection. A number of serologic tests using different antigens and assays are available; the most widely used include the recombinant K39 (rK39) (a kinesin-related protein) test and direct agglutination test (DAT). Both tests perform well with pooled sensitivities and specificities >90%, but sensitivity is diminished in some regions, particularly East Africa—potentially related to host differences or, for rK39, the extensive diversity of kinesin-related proteins found in East African isolates.[136,137] Performance of DAT may be improved by using locally derived antigens instead of commercial preparations.[138] Both rK39 and DAT are suited for use in resource-limited settings, with the former using rapid immunochromatographic test strips. Antibody tests are limited by remaining detectable for months to years after successful treatment and by the potential to cross-react with antibodies from past infections such as leprosy, Chagas disease, CL,[139] and others. Although antigen-based tests have the potential for overcoming many of these shortcomings, the commercially available KAtex antigen test has poor

sensitivity in clinical specimens, and other potential alternatives are still early in development.[137,140,141]

Other immunologic tests measuring cell-mediated immunity such as the IGRA or leishmanin skin test are often negative in patients with active VL, potentially becoming positive after spontaneous or treatment-related disease resolution.[142] Individuals with latent infection can be positive as well, making these tests epidemiologically useful. In transplant patients, cytokine release assays can be positive in asymptomatic patients but cannot distinguish between latent infection and successfully treated cases.[143]

A reasonable approach to the diagnosis of VL in immunocompetent hosts in North America consists of screening clinically suspect cases with serologic tests and confirming the diagnosis via bone marrow aspirate/biopsy for histopathology, parasite culture, and PCR.

The differential diagnosis of late-stage VL with the full complement of symptoms and signs is limited to hematologic and lymphatic malignancies and, occasionally, disseminated histoplasmosis and tropical splenomegaly syndrome. Acute VL has a much broader differential diagnosis including malaria, miliary tuberculosis, hemophagocytic syndromes, enteric fevers, HIV, bacterial endocarditis, sarcoidosis, acute Chagas disease, acute schistosomiasis, typhus, and amebic liver abscess. Subacute or chronic VL may be confused with brucellosis, prolonged *Salmonella* bacteremia, infectious mononucleosis, myeloproliferative disease, hepatosplenic schistosomiasis, and chronic malaria.

Visceral Leishmaniasis in Immunocompromised Patients

Immunocompromised patients represent a unique spectrum of VL with multiple differences across the clinical picture from presentation to management. Immunosuppressing conditions that have been shown to influence VL include HIV/AIDS, transplant-related immunosuppression, exposure to immunosuppressive drugs such as TNF-α inhibitors or steroids, malignancies or related chemotherapy, and other rare conditions such as genetic disorders in the IL-12 pathway.[91,144,145] Most data in this context are from patients with HIV/AIDS, but there is a growing body of evidence from other etiologies of immunosuppression.

The prevalence of VL in immunosuppression is not fully known. In the Mediterranean, HIV previously accounted for most VL cases, but with the use of ART it is much less common, and other etiologies of immunosuppression have become increasingly recognized.[146–148] In an outbreak of VL in Spain, HIV coinfection was seen in 10% of patients, whereas 13% had another form of immunosuppression.[49] Elsewhere in the world, HIV clearly predominates, with estimated coinfection rates highest in East Africa (20%–40%), followed by Brazil (4%) and India (<1%).[149–151] VL is seen in <1% of transplant patients globally, but increasing rates of transplantation in VL endemic regions such as Brazil and India may result in more cases.[144] Immunosuppressed individuals are often older at diagnosis than immunocompetent patients.

Immunosuppressed patients with VL often have a condition impairing cell-medicated immunity making it harder, if not impossible, for the immune system to control parasite replication, resulting in a wider dissemination and greater burden of mononuclear cells infected with amastigotes. VL also impacts the pathophysiology of HIV infection, causing increased immune activation, which leads to immunosenescence, progression of HIV disease, and poorer $CD4^+$ T-cell recovery with ART.[152–154]

The natural history of VL in immunosuppressed patients differs in several aspects. All immunosuppressed persons are at higher risk of progressing to clinical VL (4-fold in transplant patients and 100- to 2300-fold in HIV-infected patients).[153] In endemic regions, it is often impossible to distinguish activation of latent infection versus an incident case in immunocompromised patients, although patients have been observed to experience reactivation on becoming immunosuppressed years after their last potential VL exposure. Anyone with a history of birth, residence, or travel in a *Leishmania*-endemic area is at risk of late reactivation if he or she becomes immunocompromised. The role of primary preventive chemotherapy among latently infected immunocompromised persons has not been studied. Regardless of etiology, spontaneous resolution is unlikely to occur in immunosuppressed individuals.

Immunosuppressed individuals often present similarly to patients with classic VL, although atypical presentations are more common, correlating with the degree of immunosuppression. In general, HIV-infected patients have more weight loss, weakness, bleeding, and secondary infections but relatively less fever, pallor, and organomegaly.[150,155] When $CD4^+$ counts fall below 50 cells/mm^3, atypical presentations are much more common and include atypical skin lesions similar to PKDL; extensive gastrointestinal tract involvement, which may manifest with chronic diarrhea and malabsorption; intraabdominal lymphadenopathy; airway, pleural, or pericardial involvement; aplastic anemia; and anterior uveitis.[156–159] Compared with patients with HIV coinfection, patients with other types of immunosuppression tend to have more fever, lower leukocyte counts, and less hepatomegaly.[160]

Immunosuppression, particularly due to HIV, influences the diagnosis of VL by increasing the burden of parasites but simultaneously impairing specific antibody production. Parasitologic diagnosis is easier with parasites being found in a wide variety of samples including bronchoalveolar lavage fluid, pleural effusions, or biopsy specimens of the oropharynx, stomach, or intestine. Patients with HIV infection are also more likely to have circulating parasites found in their blood compared with immunocompetent patients. PCR remains highly sensitive in a wide variety of samples. Serologic tests are variable in immunosuppressed individuals, with less sensitivity overall.[144,145,160–162] In immunocompromised patients, the optimal testing regimen is not clear and should involve a multipronged approach, ideally including quantitative PCR of the blood for its utility in monitoring response to therapy.[21]

Treatment and secondary prophylaxis are discussed later in this chapter. Posttreatment relapse is problematic, especially in patients with HIV infection. Rates of relapse vary significantly in heterogeneously designed studies, but more recent rates range from 26% to 37%.[155,163,164,165] Relapse is more common in HIV-coinfected patients not on ART or with a history of prior relapse, high parasite loads at diagnosis, baseline $CD4^+$ counts <100 cells/mm^3, or persistence of low $CD4^+$ counts.[154,163,165] Although relapses can occur at higher $CD4^+$ counts, consideration can be given to stopping secondary prophylaxis in patients on ART if the $CD4^+$ count is >200 cells/mm^3 for at least 6 months, especially in the setting of negative blood PCR.[166] Continued monitoring by PCR could be useful to predict relapse in this context as well. Although ART is essential for sustained prevention of relapse, timing of ART initiation is not clear.[167,168] Whereas relapse is less common in patients without HIV/AIDS, transplant patients still have a rate of approximately 25%.[144]

Finally, death is more common in all immunosuppressed patients, even with treatment. Mortality rates of 25% to 46% are reported in patients with HIV (the higher end of range in patients not on secondary prophylaxis)[150,155,162] and 22% in transplant patients.[144]

Post–Kala-Azar Dermal Leishmaniasis

PKDL follows after active VL caused by *L. donovani* in 5% to 10% of patients within 2 to 4 years after treatment in India and approximately 50% of patients within 0 to 6 months after treatment in Sudan (Fig. 275.6).[169–172] In Bangladesh, active surveillance resulted in higher than previously observed rates, with >25% developing PKDL after therapy.[173] PKDL is rarely seen in areas where *L. infantum* (syn *L. chagasi*) predominates and, when reported, has been seen in patients with concurrent AIDS.[174] Additionally, studies in India have shown that 4% to 29% of patients with PKDL have no history of clinical VL.[33,175,176]

The pathophysiology of PKDL is not conclusively known, but suboptimal therapy, host genetic predisposition, parasite strain difference, and environmental factors such as ultraviolet light exposure or water arsenic levels likely combine to create a Th2-type response in the skin with alternative macrophage activation despite a Th1 response predominating in the viscera.[177,178] This is even more pronounced in nodular lesions, resulting in a higher parasite burden relative to macular lesions.

Skin lesions of PKDL are macular, papular, nodular, or verrucous.[176,179] The lesions are more often macular and chronic in India, whereas in Sudan, they are more frequently papulonodular but generally resolve within 12 months without therapy. Lesions are more typical on sun-exposed skin. Patients generally feel well. The diagnosis is mainly clinical, but amastigotes can be detected in the skin microscopically or with PCR; the latter has high sensitivity even in macular lesions.[173,176] PKDL must be differentiated from syphilis, leprosy, and yaws.

Patients with PKDL have been shown to be infectious to sand flies with no difference in infectiveness seen between nodular and macular

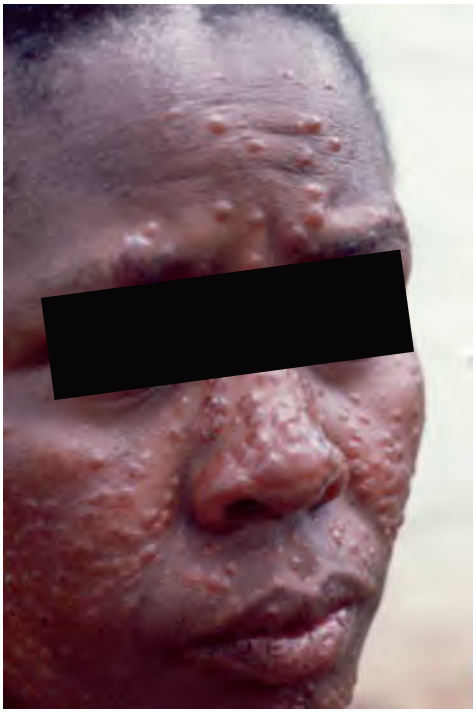

FIG. 275.6 Post–kala-azar dermal leishmaniasis. Papular lesions after treatment of visceral leishmaniasis in a patient in Kenya. *(Courtesy Dr. Tom Simpson, Baltimore, MD.)*

lesions.[33,180] Because of this, patients with PKDL are thought to be reservoirs for anthroponotic infection and may become increasingly important to elimination programs. However, further study is needed regarding the true potential of PKDL in disease transmission, as no VL seroconversion was seen in households with patients with PKDL over a 2-year period.[32] Treatment, discussed next, speeds resolution and is thought to decrease the risk of transmission, but this also remains to be demonstrated.

Treatment

Treatment options for VL and PKDL include amphotericin B formulations, pentavalent antimony (SbV) compounds such as sodium stibogluconate (SSG) or meglumine antimoniate, miltefosine, and paromomycin. Pentamidine is no longer recommended for treatment of VL but is an option for use in secondary prophylaxis and salvage therapy. All are administered parenterally with the exception of oral miltefosine. The precise mechanism of action is not known for any of these therapies; however, they frequently influence NO production in macrophages, and liposomal amphotericin B (L-AmB) and miltefosine promote a Th1-type immune response.[181] Treatment is recommended only for symptomatic individuals at this time. Studies are needed to determine if treatment of latent infection, although theoretically beneficial, impacts disease transmission or prevents disease activation. Patients with VL often have serious bacterial coinfections or tuberculosis that must be treated as well.

There are no standard parasitologic or immunologic tests of cure. Patients respond quickly to efficacious treatment, with resolution of fever, improved sense of well-being, and return of appetite within a week and improved anemia and leukopenia seen in the second week, with normalization by 6 months. Resolution of splenomegaly may take 3 to 6 months. Delayed responses to therapy can be seen, but lack of a rapid clinical response suggests treatment failure. Relapses usually occur within 6 months of initial treatment success.

Liposomal Amphotericin B and Other Formulations

L-AmB (AmBisome; Astellas, Northbrook, IL) is the drug of choice for treatment of VL outside of East Africa. It is licensed for the treatment of VL in the United States, and the approval was based on studies conducted in the Mediterranean in the 1990s.[182,183] The US Food and Drug Administration (FDA)–approved regimen for immunocompetent patients is 3.0 mg/kg/day intravenously (IV) on days 1 to 5, 14, and 21 (total dosage 21 mg/kg).

Cost, availability, and the requirement for a cold chain have limited its use in developing areas, although the WHO has negotiated preferred pricing for developing nations, making it more affordable. In South Asia, L-AmB or an Indian formulation (Fungisome) at doses of 10 to 15 mg/kg, either as a single dose or split over two or three doses, has been shown to be highly effective in treating primary VL in children and adults.[156,184–187] Success with L-AmB has been more difficult to achieve in East Africa, typically requiring significantly higher doses (totaling 24–50 mg/kg) to achieve >90% cure rates.[188,189] In *L. infantum* (syn *L. chagasi*) endemic regions, total doses of approximately 20 mg/kg are generally effective and well tolerated, and a randomized, open-label trial in Brazil demonstrated equivalent cure rates to the prior SbV-based standard-of-care regimen with fewer side effects.[190] L-AmB is generally well tolerated, but side effects include fevers, rigors, nausea, vomiting, hypokalemia, and some nephrotoxicity.

Amphotericin B deoxycholate, a nonlipid formulation, has similar efficacy to L-AmB but must be given over prolonged periods of time and is associated with increased risk of nephrotoxicity and other side effects compared with L-AmB. The recommended dosage is 1 mg/kg/dose every 1 to 2 days for 15 to 20 total doses. Other lipid-associated amphotericin B preparations have been used in the treatment of VL, but the supporting data are much less extensive, and animal studies suggest the liposomal preparation is superior.[191]

Pentavalent Antimony

Pentavalent antimony is still used for the treatment of VL in areas where *Leishmania* isolates remain susceptible and the cost of L-AmB is prohibitive. The dosage regimen recommended for treatment of VL is SbV at 20 mg/kg/day intramuscularly (IM) or IV for 28 days. Intramuscular injections are large volume and painful, and intravenous infusion requires dilution to decrease the risk of local thrombosis. Patients who respond slowly or relapse may respond to a second course.

The use of SbV is second-line treatment in many regions of the world due to poor tolerability or lack of efficacy, with up to 40% resistance seen in parts of India.[192] However, SbV, alone or in combination therapy, continues to be used extensively in East Africa, where they often remain effective for treatment of VL in patients without HIV coinfection.[193,194]

There are multiple preparations of SbV manufactured and sold worldwide. SSG (Pentostam; GlaxoSmithKline, London, UK) and meglumine antimoniate (Glucantime; Sanofi-Aventis, Paris, France) are the two best known commercial products and are considered equivalent in efficacy and tolerability. One SSG generic formulation has been shown to be equivalent to brand-name products,[195,196] but numerous other SbV products vary in content and toxicity profile, even between lots by the same manufacturer. Cardiac deaths have been associated with generic SbV with high osmolarity.[197,198]

Side effects are common and often increase during treatment with SbV. Side effects include anorexia, nausea, vomiting, midepigastric pain, myalgia, large joint arthralgia, headache, asthenia, and malaise. Common laboratory abnormalities include cytopenias and elevations in amylase, lipase, and liver associated enzymes. Although chemical pancreatitis is common, a full treatment course can generally be completed, although we favor pausing therapy until high levels decline (over several days) or stopping therapy for symptomatic pancreatitis.[199] Electrocardiographic changes, including QT prolongation, are common, and arrhythmias, sudden death, and torsades de pointes can occur. Clearance is primarily by the kidneys, and toxicity increases when drug accumulates during renal failure. Patients receiving SbV should be closely monitored clinically along with periodic complete blood counts, serum amylase and lipase, liver enzymes, renal function tests, and serial electrocardiograms.[200] SbV should not be given during pregnancy because a 57% spontaneous abortion rate was observed in one study.[201]

Miltefosine

Miltefosine (Impavido; Profounda, Orlando, FL) is the only oral agent available for the treatment of VL and was licensed by the FDA in 2014

for the treatment of VL caused by *L. donovani*. Initially developed as an antineoplastic agent, miltefosine is associated with nausea, vomiting, and abdominal pain. Whereas early use was promising,[202] prompting use in South Asia's elimination program, a subsequent trial in Nepal showed 86% efficacy, with 13% relapse among 853 patients.[203] Experience in other regions is limited.

Adverse effects include significant nausea, vomiting, dizziness, scrotal pain, nephrotoxicity, and hepatotoxicity. Miltefosine is teratogenic and therefore contraindicated in pregnancy. Adherence to the 28-day oral regimen is challenging due to gastrointestinal distress.[204] Additionally, the recommended weight-based dosing may be inadequate for children[205] and is challenging in persons weighing >60 kg, as doses >150 mg daily are not well tolerated. Resistant parasites have mutations leading to diminished drug accumulation and resistance to the oxidative stress of miltefosine.[206,207]

Paromomycin

Paromomycin (parenteral formulation not commercially available in the United States) is an aminoglycoside with activity against *Leishmania* species. For VL it is administered as 11 mg/kg of base (equivalent to 15 mg/kg paromomycin sulfate) IM for 21 days. Randomized studies in Bangladesh and India have shown 94% to 95% cure rates, with the Indian study showing noninferiority to amphotericin B.[208,209] Results from East Africa were less promising, with efficacy of <85% even when paromomycin was administered longer or at higher doses.[210,211]

Adverse events are more common than with amphotericin and include injection site pain, elevations of aspartate aminotransferase levels, reversible ototoxicity, and nephrotoxicity. Whereas the low cost makes paromomycin attractive, there are concerns about the development of resistance. Use is generally reserved for combination with other agents.

Combination Therapy for Visceral Leishmaniasis

Many experts now advocate combination chemotherapy regimens for VL, as it is a polyparasitic disease syndrome, and using two or more drugs may shorten therapy, decrease toxicity, and prevent the emergence of resistance. In East Africa, SSG with paromomycin is the WHO recommended first-line therapy for uncomplicated VL.[210,212] A multisite, prospective pharmacovigilance study in Ethiopia, Kenya, Sudan, and Uganda demonstrated 95% initial cure, low mortality, and high therapy completion rates with this combination.[213] A phase 2 study in Kenya and Sudan evaluated the combination of L-AmB at 10 mg/kg in a single dose, with either SSG or miltefosine for 10 days versus miltefosine monotherapy for 28 days, but none of the regimens reached >90% efficacy in the formulations tested.[214] Conversely, in Bangladesh and India, combinations of single-dose L-AmB (5 mg/kg) with a short course of miltefosine or paromomycin as well as miltefosine and paromomycin together demonstrated excellent efficacy and safety profiles.[187,215] Further studies are needed with long-term follow-up to assess rates of relapse and PKDL.

Treatment and Secondary Prophylaxis of Visceral Leishmaniasis in Immunocompromised Hosts

Treatment in immunocompromised patients is challenging, and most data come from patients with HIV/AIDS. Cure can be very difficult to achieve and may require multiple courses of therapy. Data are limited on the optimal therapy in this setting.[144,216] A systematic review among patients with HIV coinfection suggests that L-AmB is superior to SbV, with higher rates of clinical improvement and lower mortality.[217] In North America the recommended treatment for immunosuppressed individuals is L-AmB 4.0 mg/kg/day IV on days 1 to 5, 10, 17, 24, 31, and 38 (total dosage 40 mg/kg).[21] Longer courses and higher total doses along with combination therapy are considerations in this population, especially with treatment failure or relapse.

There is evidence that protease inhibitors and efavirenz have in vitro activity against *Leishmania,* but in vivo efficacy is unknown.[218,219] If VL manifests as part of an immune reconstitution inflammatory syndrome, corticosteroids may be necessary in addition to continued ART and pathogen-directed therapy.

Secondary prophylaxis is recommended for the prevention of relapse.[21] Amphotericin B lipid complex and intravenous pentamidine have been studied prospectively for use in secondary prophylaxis, and both decrease relapse substantially compared with no prophylaxis or historical control subjects, respectively.[165,220] Other medications have been looked at retrospectively, including L-AmB and SbV, with the former showing 100% prevention of relapse and death at 6 months in Indian patients.[162] When indicated, options include amphotericin B lipid complex (3 mg/kg IV every 3 weeks), pentamidine (4 mg/kg IV every 2–4 weeks), L-AmB (3–5 mg/kg IV every 3–4 weeks), or SbV (20 mg/kg IM or IV every 3–4 weeks).[21,165,220]

Treatment of Post–Kala-Azar Dermal Leishmaniasis

Chemotherapy in patients with PKDL is frequently unsatisfactory, and robust trials are lacking. Clinical and parasitologic end points in PKDL are poorly defined, although PCR may have an emerging role in future trials.[173,221] Severe PKDL in Sudan is treated with SSG at 20 mg/kg/day for up to 3 months.[222] First-line therapy in the Indian subcontinent is miltefosine for 12 weeks, although concerning rates of relapse have been seen, and a longer course may be required, which presents a significant challenge regarding patient compliance.[221,223] Formulations of amphotericin B have been very promising when used in small numbers of patients, including patients with nodular disease,[224–226] but additional studies are needed to define its role in the treatment of PKDL.

CUTANEOUS LEISHMANIASIS

CL is endemic in widely scattered areas throughout the world (Fig. 275.7). The classic form of Old World CL (OWCL) is the *oriental sore,* also known by a variety of colorful local expressions such as *bouton d'orient, bouton de Crete, bouton de Biskra, Aleppo evil, Baghdad boil,* and *Delhi boil,* in various regions of Africa, India, the Mediterranean littoral region, and Southwest/Central Asia. The spectrum of OWCL disease includes single or multiple, localized, cutaneous ulcers (Fig. 275.8); diffuse CL; uncommon mucosal involvement; and leishmaniasis recidivans (Fig. 275.9). The resulting skin lesions range from troublesome and unsightly to severe and complicated, healing with scarring. Severe cases and facial lesions are very stigmatizing.

The spectrum of New World CL (NWCL) disease includes single or multiple, localized, cutaneous ulcers (Figs. 275.10 to 275.13); diffuse CL; disseminated CL (Fig. 275.14); and mucosal disease (Fig. 275.15) caused by *Leishmania (Viannia) braziliensis* or, less commonly, other related *Leishmania* species.

Epidemiology

CL is a severely neglected tropical disease whose incidence is increasing worldwide. An estimated 12 million people have leishmaniasis; 1 to 1.5 million new cases of CL occur per year, with a mean CL disability-adjusted life-year estimate of 0.6 per 100,000 persons (not including the social stigma associated with scarring of healed CL).[227–229] Approximately 90% of the world's CL cases occur in the high-burden countries of Afghanistan, Algeria, Brazil, Colombia, Iran, Morocco, Pakistan, Saudi Arabia, Syrian Arab Republic, Tunisia, and Turkey; CL cases in these countries tripled between 1998 and 2005.[230] In the Global Burden of Disease study, a 174% change in CL prevalence was seen between 1990 and 2013.[231] CL is an important problem for residents, refugees, settlers, travelers, and military personnel visiting endemic areas. More than 2000 cases of CL have been reported among American troops serving in Iraq and Afghanistan since 2001.[232] Other cases occur among North American travelers after exposure in endemic regions.[233,234] Finally, 90% of the cases of ML occur in three Latin American countries: Bolivia, Brazil, and Peru.

In the Old World, CL is usually a sporadic disease in endemic areas, but it occasionally occurs in an epidemic pattern, particularly when large groups of susceptible people are exposed during road construction, refugee movements, or military activities.[235–237] OWCL is most frequently caused by *L. major, L. tropica,* or *Leishmania aethiopica,* but *L. donovani* and *L. infantum* (syn *L. chagasi*) can also cause simple CL.

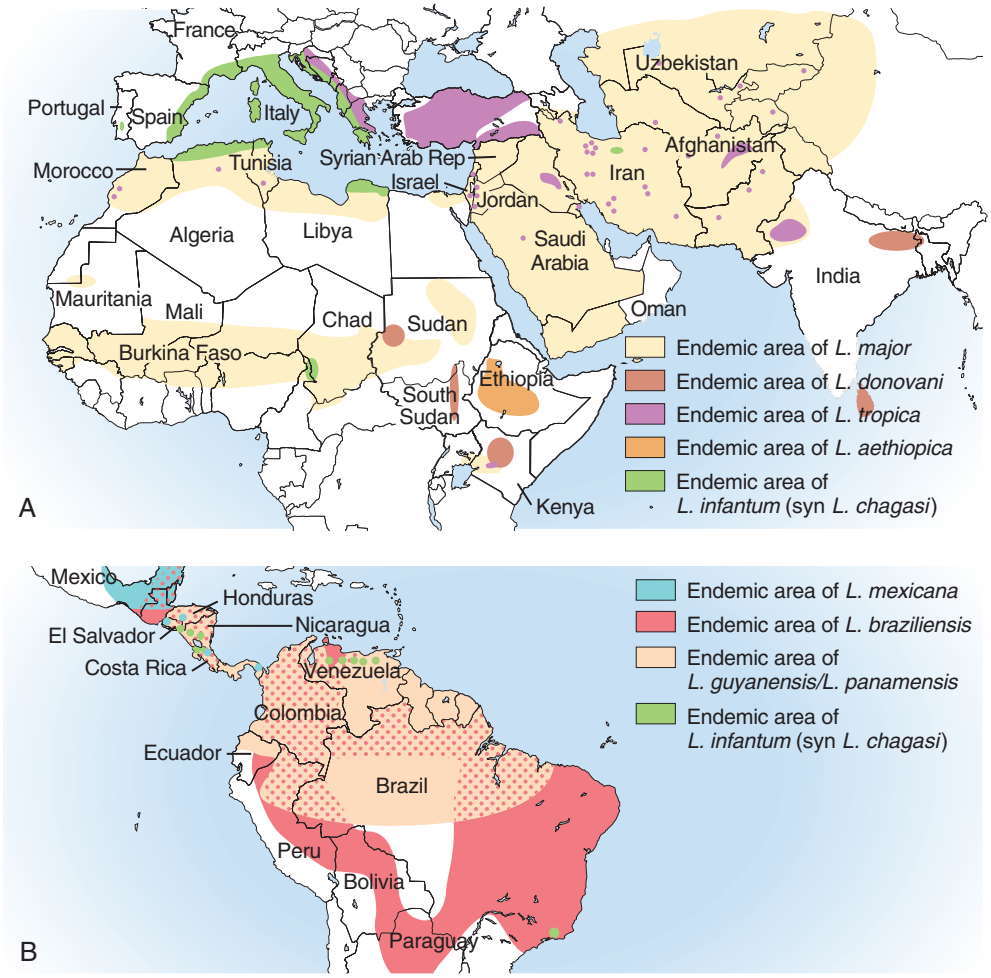

FIG. 275.7 Distribution of cutaneous leishmaniasis (CL). (A) Old World (Eastern Hemisphere). (B) New World (Western Hemisphere). Other species causing CL that are not shown may be found in Table 275.1.

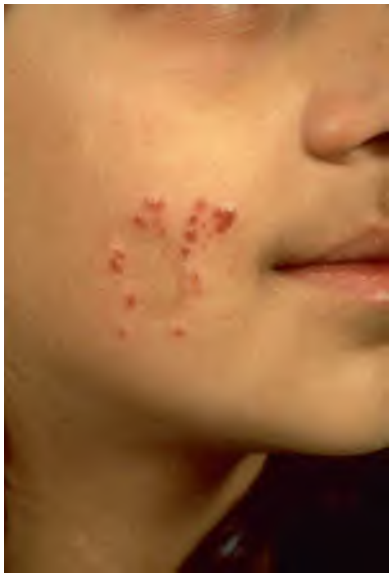

FIG. 275.8 Old World cutaneous leishmaniasis caused by *Leishmania major*. Note the thick crust over the ulcers, alignment with skin creases, and indurated rim.

FIG. 275.9 Leishmaniasis recidivans due to *Leishmania tropica*. Note the central scar from a previous primary ulcer now surrounded by small papules with few parasites present.

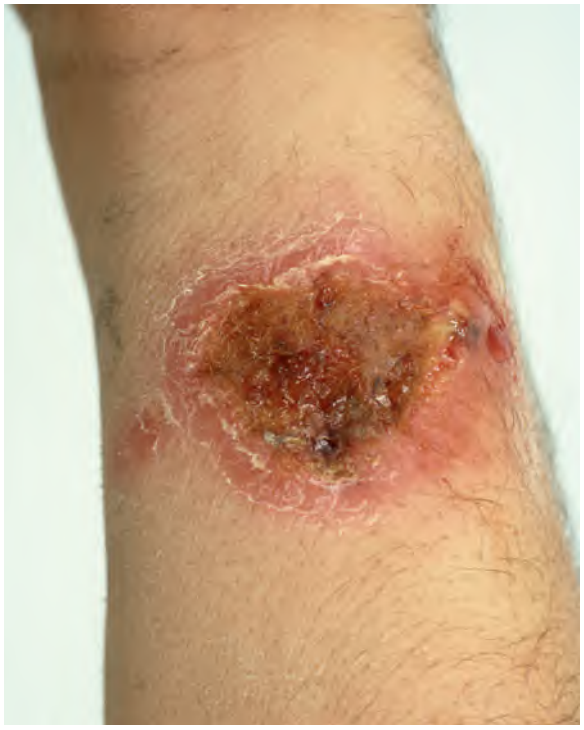

FIG. 275.10 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) panamensis*. Note the large painless ulcer with indurated border; a skin biopsy site is seen on the right side of the lesion.

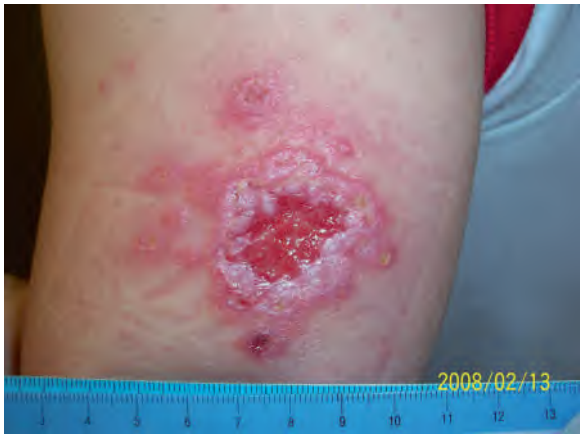

FIG. 275.11 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) guyanensis*. *(Courtesy Dr. Glenn Wortmann, Washington, DC.)*

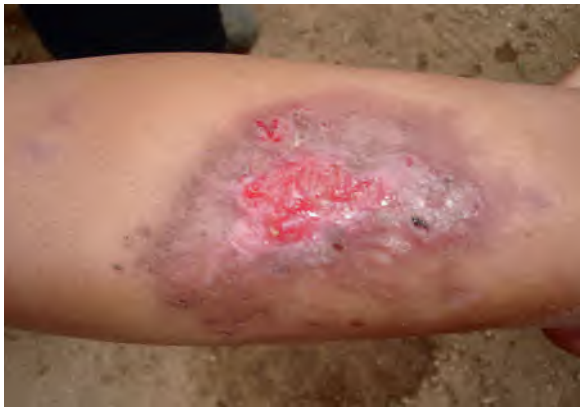

FIG. 275.12 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) peruviana*. *(Courtesy Dr. Alejandro Llanos-Cuentas, Lima, Peru.)*

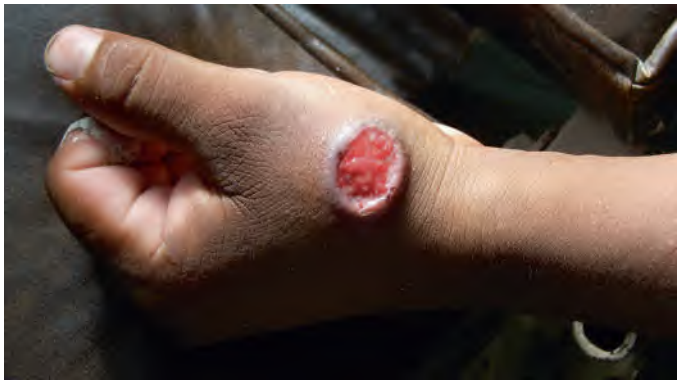

FIG. 275.13 New World cutaneous leishmaniasis caused by *Leishmania mexicana*. Note the clean-based ulcer with a rolled, indurated margin. *(Courtesy Dr. Byron Arana, Guatemala City, Guatemala.)*

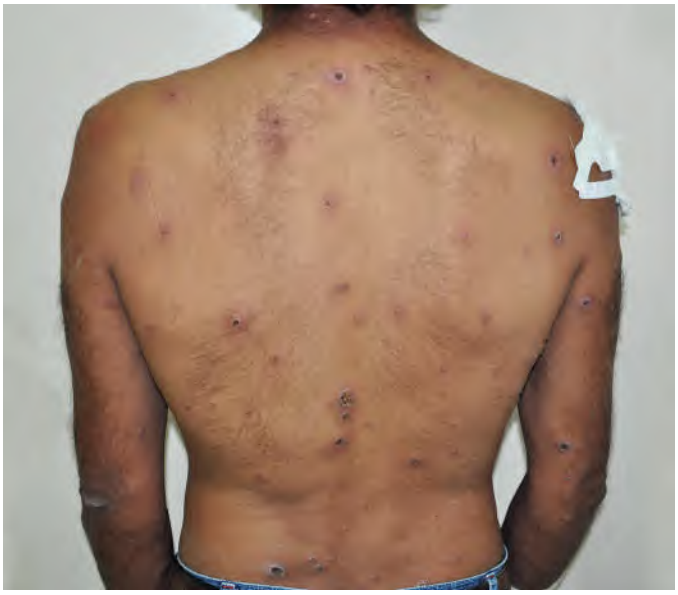

FIG. 275.14 Disseminated cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis*. Note the many pleomorphic lesions, both ulcers and nodules; this patient had 162 total skin lesions of about 4 months' duration. *(Courtesy Dr. Anastacio Q. Sousa, Ceara, Brazil.)*

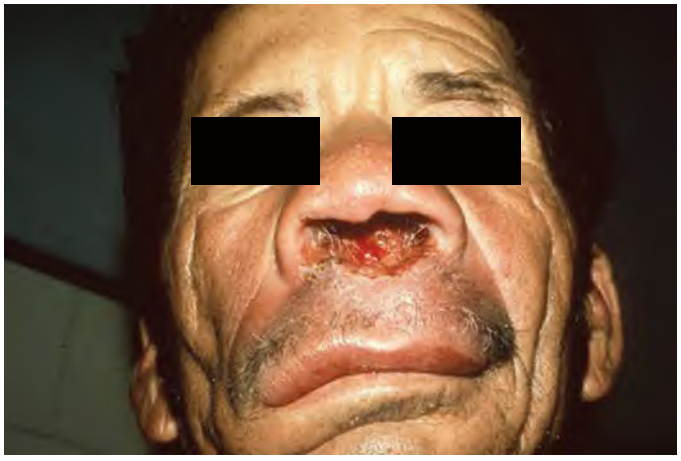

FIG. 275.15 Mucosal leishmaniasis. Note the absence of the nasal septum and full upper lip.

L. major is an infection of desert rodents and affects humans in arid and rural regions of North Africa and Southwest/Central Asia (see Fig. 275.8). *Phlebotomus papatasi* and other *Phlebotomus* species are the vectors.

L. tropica infects dogs and humans in urban areas of Southwest Asia such as Baghdad, Teheran, Kabul, and Damascus, as well as cities in the Mediterranean littoral region, India, and Pakistan. The lesions tend to be crusted and dry, with a predilection for a centrofacial location compared with *L. major*. The vectors include *Phlebotomus sergenti* and *P. papatasi*.

L. aethiopica is endemic in Ethiopia, Kenya, and less commonly southwest Africa, including Namibia. The primary reservoir is the rock hyrax, with rodents as secondary reservoirs. *Phlebotomus longipes* and *Phlebotomus pedifer* are the vectors. Diffuse CL caused by *L. aethiopica* infection is reported from Ethiopia and adjacent areas of Africa.

NWCL is usually a rural zoonosis.[238] The causative species include *L. (V.) braziliensis, Leishmania mexicana, Leishmania (Viannia) panamensis, Leishmania (Viannia) guyanensis, L. amazonensis,* and others (see Table 275.1). *L. infantum* (syn *L. chagasi*) is associated with simple nodular CL in Latin America. The main NWCL reservoirs are forest rodents except in the case of *Leishmania (Viannia) peruviana,* for which dogs are the primary reservoir. The vectors are ground-dwelling or arboreal *Lutzomyia* species. NWCL is an occupational hazard of people living, working, or touring in endemic areas; disease is common in people working at the edge of the forest and among rural settlers. Outbreaks occur when areas of forest are cleared for roads, villages, or farms or when military personnel or tourists enter endemic regions.

L. mexicana is responsible for NWCL from northern Argentina to Texas and Oklahoma, where a small number of autochthonous cases have been reported.[239–241] Lesions typically appear on exposed areas of the extremities (see Fig. 275.13), face, or ears (termed *chiclero's ulcer* when lesions involve the ear). *L. mexicana* is also associated with diffuse CL, an anergic form.

L. amazonensis produces a spectrum of disease in South America that includes simple CL, diffuse CL, ML, and VL.[58]

L. (V.) braziliensis is found in widely scattered areas of Latin America. It is responsible for CL as well as the metastatic complication, ML (see Fig. 275.15). ML caused by *L. (V.) braziliensis* has been diagnosed among American tourists returning from Latin American areas. *L. (V.) panamensis* is found in Panama and adjacent countries (see Fig. 275.10). Of note, Panama and Nicaragua had the highest incidence of CL in the Americas in a more recent study.[242] *L. (V.) guyanensis* is responsible for pian bois or bush yaws in the northern Amazon basin (see Figs. 275.7B and 275.11). *L. (V.) peruviana* is the cause of CL, called *la uta,* in Peru. It typically causes dry lesions (see Fig. 275.12).

New *L. enrietti* species complex parasites, which rarely cause VL as discussed earlier, were identified as causing CL in Ghana's Volta region, Martinique, and Thailand. Lastly, in Australia, *L. enrietti* complex parasites infected red kangaroos, with a proposed vector of midges rather than sand flies.[63]

Pathogenesis and Immunology

The clinical manifestations of CL depend on the virulence factors of the infecting *Leishmania* species and the genetically determined immune responses of their human hosts. At the initial bite site, a papule forms, enlarges to a papulonodule, usually develops central ulceration, and slowly enlarges. Surrounding induration and raised borders are typical.

CL is characterized by a mixed acute and chronic inflammatory infiltrate with infected and uninfected mononuclear phagocytes, lymphocytes, and plasma cells. There are areas of focal necrosis.[243] Early in infection, amastigote-containing macrophages dominate. Eventually, parasitized mononuclear phagocytes are eliminated, lymphocytes become prevalent, and a granulomatous response containing epithelioid cells and giant cells evolves. Lesions heal slowly leaving a flat, atrophic scar. Recovery is associated with a high level of resistance to reinfection by the homologous *Leishmania* species.

Infection occurs when the balance between parasite virulence factors and host cellular immune responses tilts to the parasite, the outcome of proinflammatory and antiinflammatory factors. In localized CL a *Leishmania*-specific Th1 lymphocyte response including IFN-γ, IL-2, and IL-12 is typical.[244] Infected individuals exhibit cutaneous delayed hypersensitivity responses, as evidenced by positive leishmanin skin tests. For durable immunity, memory T cells and $CD8^+$ T lymphocytes have a role.[245,246] Lack of immune control of *Leishmania* is associated with IL-10 and TGF-β. Transcripts from *L. (V.) braziliensis* CL lesions that were upregulated compared with control skin were arginine, C-X-C motif chemokine ligand 9 (CXCL9), and CXCL10.[247,248] Interestingly, RNA-sequencing investigation of CL lesions found many transcripts encoding inhibitory molecules and B-cell functions.[249]

Recently about one-quarter of 52 *Leishmania* (and related *Crithidia* and *Leptomonas*) cultures of multiple species were found to have associated RNA viruses; *Leishmania* RNA virus (LRV) 1 and LRV2 seem to have been acquired about the time that *Leishmania* demonstrated vertebrate parasitism.[250] Varying percentages of *L. (V.) braziliensis, L. (V.) guyanensis,* and *L. amazonensis* isolates from South America have been described as containing a cytoplasmic LRV1. LRV1-infected parasites promoted high levels of inflammatory cytokines through a Toll-like receptor 3–dependent pathway in mice.[251] Among 156 patients, including 109 with CL, 38 with ML, and 5 with mucocutaneous leishmaniasis (78% isolates were *L. [V.] braziliensis*), 71% of patients with ML were LRV1 positive versus 37% patients with CL, suggesting an association of ML and LRV1 in humans. Increased expression of IL-1β, TNF-α, CXCL10, and IL-6 was seen in LRV1-positive *Leishmania* strains compared with LRV1-negative strains.[252] The presence of LRV1-infected *Leishmania* has been associated with an increased rate of SbV or pentamidine failure in the treatment of CL and ML, despite a lack of demonstrable drug resistance.[253,254] Current knowledge suggests some utility of the knowledge of this pathogenic factor in establishing prognosis and stratification for new treatment strategies.

Clinical Manifestations of New World and Old World Cutaneous Leishmaniasis

The incubation period of CL typically varies from 2 weeks to several months but can be as long as several years. A wide variety of skin manifestations, ranging from small papules to papulonodules to dry crusted lesions to large, deep, mutilating ulcers, may be seen. Plaques, satellite lesions, psoriasiform, or verrucous lesions all have been described. The characteristics vary among *Leishmania* species and from one geographic area to another, but overlap occurs. There may be a single lesion, multiple lesions, or widely disseminated disease with hundreds of smaller acneiform lesions. They are usually found on exposed areas of the body. No characteristic of the skin lesion is pathognomonic for CL or can be used to identify the causative *Leishmania* species.

Ulcerative lesions are usually painless, shallow, and circular with well-defined, indurated borders and a central bed of granulation tissue. They gradually increase in size and may develop a "pizza-like" appearance with a raised, circular outer margin, beefy red granulating base, and yellowish exudate on the surface or a thick crusted eschar (see Figs. 275.8 and 275.10). Satellite lesions occur, and they may fuse with the original ulcer. A sporotrichoid chain of lymphatic nodules may be palpated proximal to the lesions, and regional lymphadenopathy may be present. Secondary staphylococcal or streptococcal skin and soft tissue infections can occur. After a variable period, ranging from several months to longer than a year, ulcers heal and leave flat or depressed, atrophic, and depigmented or hyperpigmented scars. The natural history of this healing without intervention ranges from 2 to 6 months for *L. major,* 3 to 9 months for *L. mexicana,* and 6 to 24 months for *L. tropica* and is only 6% at 9 months for *L. (V.) braziliensis*.[255–257]

Several skin manifestations of leishmaniasis are uncommon but should be reviewed. One is PKDL, which is related to VL and was discussed previously. The others are diffuse CL, disseminated CL, and leishmaniasis recidivans.

Diffuse Cutaneous Leishmaniasis

Diffuse CL (anergic CL) is uncommon and is usually associated with infection with *L. mexicana, L. amazonensis,* and sometimes *L. aethiopica*. It is also associated with other *Leishmania* species in hosts with cellular immunocompromise. The initial lesion starts as a localized papule or nodule that does not ulcerate but may coalesce into plaques. Satellite lesions develop around the initial papule, and organisms gradually spread in the skin, resulting in disseminated nodules primarily on the face and

extremities, although eventually all skin can be involved. Diffuse CL has a protracted course and may last for the patient's lifetime, being resistant to treatment.[258–260] Miltefosine leads to an initial favorable response, but relapse follows. Immunotherapy with a combination of heat-killed *L. mexicana* and viable bacillus Calmette-Guérin has been successful in Venezuela with minimal toxicity.[261]

Leishmaniasis Recidivans

Leishmaniasis recidivans is usually associated with *L. tropica* infection. It has been described across North Africa and Southwest Asia.[262] The lesions are often on the face and consist of small papules that surround a scar after initial resolution of the primary CL lesion (see Fig. 275.9). Leishmaniasis recidivans is a relapsing, tuberculoid form of CL characterized by very few parasites, with an intense cell-mediated immune response. In nonendemic areas, it is most often seen in refugees or immigrants from endemic areas.

Disseminated Leishmaniasis

Disseminated leishmaniasis is a syndrome characterized by hundreds of pleomorphic, acneiform, papular, nodular, or ulcerated lesions in multiple noncontiguous sites (see Fig. 275.14) in otherwise immunocompetent patients infected with *L. (V.) braziliensis,* occasionally other species of the *Viannia* subgenera, and *L. amazonensis,* particularly noted in the state of Bahia, Northeast Brazil.[263–267] Parasites are very few, lesion histopathology shows lymphocytes and plasma cell infiltration, and mucosal involvement is seen in up to 44%.[268] This syndrome may be associated with unique strains of *L. (V.) braziliensis* that produce high levels of proinflammatory cytokines such as TNF-α and IFN-γ.[266,269] Disseminated cutaneous lesions have also been observed in a limited number of patients with AIDS or solid-organ transplants.[270,271] Treatment with antimony is unsatisfactory in most cases. An open-label trial of L-AmB in 20 patients with disseminated CL (total dosages 17–37 mg/kg, divided into 7–14 daily doses) demonstrated a cure rate of 65% at 4 months, which increased to 75% among 8 patients receiving ≥30 mg/kg.[268]

Diagnosis

CL is suggested by one or more chronic skin lesions with the appropriate characteristics and a history of exposure in an endemic area. The lesion should be sampled for diagnosis, and generally several diagnostic tests are performed to maximize the likelihood of confirmation of leishmaniasis and to limit the number of procedures.[21] Knowing the infecting species has relevance for the choice of therapeutic agent and regimen; species identification usually requires successful parasite culture; additionally, molecular assays such as PCR in combination with restriction fragment length polymorphism analysis or sequencing can identify subgenera and species.[272]

Obtaining a specimen from an ulcer base requires thorough cleaning and removal of exudate before sampling. Several techniques can yield an adequate sample, including scraping the base or ulcer center, brushing (i.e., cytology brush) the ulcer, obtaining touch impressions (such as using tape or glass slide) from ulcer base, and aspirating the indurated margin.[56,273] Skin biopsy may be useful to allow assessment of diagnoses other than leishmaniasis; in this case the indurated edge of the lesion is preferred for sampling (see biopsy site in Fig. 275.10). Material obtained by scrapings, brushings, and touch preparations made from biopsy samples are stained with a Wright-Giemsa or Giemsa preparation and examined under oil-immersion microscopy for amastigotes. Portions of the lesion biopsy may also be used for histologic examination using hematoxylin and eosin staining along with standard mycobacterial and fungal culture as clinically indicated.

The sensitivity of direct parasite identification and culture varies with the type and duration of the lesion and the infecting *Leishmania* species. The combined sensitivity of these methods ranges from 50% to 90%.[274] Molecular assays performed on lesion samples have better sensitivity and are faster tests; however, there is no single standard assay, and the performance characteristics vary.[21]

Current immunologic tests for CL diagnosis are generally not recommended. Antileishmanial antibodies are detectable in the serum of only a few patients with CL, but actual sensitivity depends on the assay used, and the titers are usually low. The leishmanin skin test is usually positive in patients with localized CL but is not available in North America.

The differential diagnosis includes sporotrichosis, blastomycosis, chromoblastomycosis, mycobacterial infection, cutaneous myiasis, syphilis, yaws, leprosy, sarcoidosis, lupus vulgaris, pyoderma gangrenosum, vasculitis, fixed drug eruption, and neoplasms of the skin. Rarely, lesions may assume a keloidal form and give the appearance of lobomycosis. In returning travelers the competing diagnosis is tropical pyoderma, a chronic ulcerative lesion caused by typical gram-positive bacteria often at sites of minor trauma (i.e., excoriated insect bites).

Treatment

The treatment of CL syndromes is best approached by considering the extent and severity of lesions in each individual, the confirmed or most likely infecting parasite strain, the likelihood of natural healing, and the probability of late manifestations of ML. Because most cases of CL will eventually resolve, and treatment, especially systemic, is not without risk, the first management decision in a patient with CL is whether to recommend specific treatment or not.[275] Table 275.2 lists criteria that should be considered in making the initial treatment decision and the relative need for a systemic versus local treatment option. Clinical trials in OWCL and NWCL have been reviewed, and North American clinical practice guidelines have been published.[21,276,277–279] Miltefosine is the only drug approved by the FDA for the treatment of CL due to *L. (V.)*

TABLE 275.2 Continuum of Cutaneous Leishmaniasis Management

SIMPLE CL	COMPLEX CL
Species unlikely associated with ML	New World CL species associated with ML, particularly *Viannia* subgenera in mucosal belt of Bolivia, Brazil, Peru
No mucosal involvement	Local subcutaneous nodules Large regional adenopathy
Single or few lesions	>4 lesions of significant size (generally >1 cm)
Small size (<1 cm)	Individual lesion ≥5 cm
Location feasible for local treatment	Size or location of lesion for which local treatment is not feasible
Nonexposed skin	Lesions on face, including ears, eyelids, lips; fingers, toes, genitalia
Immunocompetent host	Suppressed cell-mediated immune response
Lesions resolve without prior therapy, not ML-associated species	Clinical failure of local therapy (2–3 months posttreatment)
	Unusual syndromes of leishmaniasis recidivans, diffuse cutaneous leishmaniasis, disseminated cutaneous leishmaniasis
MANAGEMENT OPTIONS: SIMPLE CL[a]	**MANAGEMENT OPTIONS: COMPLEX CL[a]**
Watchful waiting (wound care) Local therapy Cryotherapy Thermotherapy Topical paromomycin Intralesional SbV or pentamidine Photodynamic therapy Systemic therapy—azoles	Systemic therapy Miltefosine SbV Liposomal amphotericin B Amphotericin B deoxycholate Pentamidine Adjuvant immunomodulatory therapies, including imiquimod, pentoxifylline, CpG DNA

[a]Not all inclusive.

CL, Cutaneous leishmaniasis; *ML,* mucosal leishmaniasis; *SbV,* pentavalent antimony

Modified from Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. *2016;63:e202–e264.*

braziliensis, L. (V.) guyanensis, and *L. (V.) panamensis*. The ThermoMed device (Thermosurgery Technologies, Inc., Phoenix, AZ) is cleared by the FDA for treatment of CL. In addition, several other treatments have been used successfully, albeit off-label, for the treatment of CL.

In all forms of CL, attention should be directed to local wound care. CL is both a parasitic infection and an open wound. Ulcers should be washed daily in soap and water, and then a moisturizing ointment (i.e., white petrolatum) may be applied. Antibiotics should be administered if there is evidence of cellulitis, painful or tender areas, or purulence.

Systemic treatment is recommended for immunocompromised patients with CL due to concerns about further dissemination (mainly skin). Data to determine the best treatment strategy in this situation are very limited, and further research is encouraged.

One of the challenges of CL treatment recommendations is that they are based on many poor quality studies, including diverse treatment courses and outcome measures, and the results are not necessarily generalizable between various species or in the same species between geographic areas. This has spurred many systematic reviews and meta-analyses of CL treatment modalities, some of which are mentioned here, as well as clinical practice guidelines.

Old World Cutaneous Leishmaniasis

Treatment of CL acquired in the Old World has been reviewed more recently; additionally see the IDSA-ASTMH guidelines supplemental material, which includes a searchable spreadsheet of treatment trials for OWCL.[21,277,279] Key factors affecting treatment decisions are host factors, patient preferences, location and number of lesions, spontaneous healing status, metastatic potential of a given species, and availability of and technical experience in delivering local therapies. Local therapies for OWCL including intralesional SbV (available in Canada, but not in the United States), paromomycin-based topical creams and ointments (generally studied for treatment of ulcerative lesions), and thermotherapy and cryotherapy have long been favored in endemic regions, although they are less commonly used in nonendemic countries.[280]

The interpretation of clinical trial results with topical paromomycin-based products is confused by the variety of formulations used. However, the combination of 15% paromomycin plus 12% methylbenzethonium chloride in soft white paraffin, administered twice daily for 28 days, is better than placebo in *L. major* infections but has less benefit in *L. tropica* infections.[279,281,282] WR 279,396 (15% paromomycin, 0.5% gentamicin) cream has been associated with 81% to 94% response rates in phase 2 and 3 clinical trials (for *L. major* and *L. [V.] panamensis*) but is no longer available; currently 15% paromomycin cream can be used in military beneficiaries under an expanded access protocol.[283–285] A meta-analysis of 14 studies comprising 279 patients treated with topical paromomycin found that clinical cure was 2.6 times more likely in patients treated with paromomycin compared with placebo; in OWCL, efficacy was similar to that of intralesional SbV, whereas in NWCL, topical paromomycin was inferior to parenteral SbV.[281]

Locally applied heat therapy administered with the ThermoMed device is generally applied in one treatment session and results in an initial second-degree burn that heals with good cosmetic effect. Heat is delivered to the skin lesion using localized radiofrequency waves, and the 50°C temperature requires advance application of local anesthesia. In a meta-analysis including eight trials (622 thermotherapy-treated subjects), efficacy was estimated as 73%.[286] The practical limitation to a single or a few lesions and access to the expensive delivery modality limit its impact.

Cryotherapy, delivering liquid nitrogen treatment to the CL lesion and surrounding 1 to 2 mm of skin with a freeze-thaw-freeze cycle, is a reasonable choice for lesions <1 cm, few in number and early in development (<3 months). It may leave permanent hypopigmentation at the site in dark-skinned individuals. Treatment is repeated multiple times, usually at 2- to 3-week intervals. In a meta-analysis of eight studies the per-lesion efficacy of cryotherapy was estimated as 67% including both OWCL and NWCL.[287] Combining cryotherapy with intralesional SbV may result in higher cure rates.[288,289] Other local treatment modalities include intralesional injection of SbV (efficacy was 77% in a systematic review),[290] intralesional amphotericin,[291,292] photodynamic therapy,[293] and laser ablation. Further details about local therapy can be found in the IDSA-ASTMH leishmaniasis clinical practice guidelines.[21]

When systemic treatment is indicated (complex CL) (see Table 275.2), options include oral off-label treatment with azoles or miltefosine and parenteral treatment with SbV (either SSG or meglumine antimoniate, both investigational new drugs). Although case series of off-label use of L-AmB suggest efficacy, the evidence base is poorly developed.[21,294,295,296] Healing of cutaneous lesions occurs slowly over a period of weeks and is often incomplete at the end of the treatment course; 3 months is the target to healing—with no relapses over the next 6 to 12 months.

Oral systemic treatment in OWCL with several agents has been studied. Miltefosine given at 2.5 mg/kg/day for 28 days was shown to be as efficacious (81% in 32 patients) as parenteral SbV at 20 mg/kg/day for 14 days for the treatment of *L. major*.[297] In a case series of 34 Dutch military soldiers with *L. major,* 88% were considered healed, although molecular testing showed persistent parasites, making the point that the goal of therapy is clinical healing and not necessarily parasitologic cure.[236] In two small case series (21 subjects) with likely *L. tropica* infections, efficacy with miltefosine treatment was >80%.[298,299]

Azoles in general have modest efficacy in the treatment of OWCL, and the evidence base is characterized by few controlled trials, small numbers of subjects, and various doses and durations. In a systematic review, among 24 OWCL studies a pooled azole efficacy of 62%, including a 53% efficacy in *L. major* and 15% in *L. tropica* was observed.[300] Fluconazole, 200 mg daily for 6 weeks, cured 59% after 3 months compared with 21% in a no-treatment control arm in patients with CL caused by *L. major* in Saudi Arabia.[301] *L. major* in other locations (e.g., Iran or Iraq) seems to be less sensitive. Fluconazole 400 mg daily for 6 weeks was nearly five times better than fluconazole 200 mg for treatment of *L. major* CL in Iran.[302] Ketoconazole (which carries a black box warning from the FDA) at 600 mg daily for 1 month cured 89% of 64 patients with *L. major* CL[303] but none of 32 patients with *L. tropica* CL.[304] A Cochrane systematic review of OWCL suggested, albeit with low confidence due to poor study design, that itraconazole 200 mg/day orally for 6 to 8 weeks versus placebo has a relative risk for healing of 3.7; itraconazole was associated in some studies with a better outcome in *L. major* than *L. tropica*.[277,305–307]

New World Cutaneous Leishmaniasis

Treatment of NWCL has also been reviewed.[276,278,308] In NWCL, species identification may allow a more diverse consideration of treatment options when *L. (V.) braziliensis* is not identified. Generally current practice is to treat systemically for *Viannia* species that are associated with ML (complicated CL) (see Table 275.2). Additionally, NWCL due to *L. (V.) braziliensis* is less likely to heal spontaneously, with rates of 6% at 9 months.[255] The dosing in NWCL is the same as OWCL, and in general local therapy is not used south of Nicaragua, although studies are assessing this further.[309–311]

Pentavalent Antimony

Parenteral SbV, usually given at 20 mg/kg/day for 20 days, is the most common medical treatment in the Americas and produced an overall cure rate of 76% (range, 58%–100%) in >1000 patients entered into selected controlled trials.[308] Various clinical, epidemiologic, and laboratory criteria have been evaluated to find correlates of therapeutic success.[312–315] Young age (presumably reflecting a nonimmune or less immune status), other nonimmune status (e.g., tourists and new immigrants to endemic locations), intrinsic SbV susceptibility, and geographic location (presumably reflecting *Leishmania* species or strain variations) all have been reported to correlate with therapeutic failure. Reported SbV efficacy tended to be lower in Brazil and higher in Colombia, and *L. (V.) braziliensis* and *L. (V.) peruviana* respond less well than *L. (V.) guyanensis*.

Liposomal Amphotericin B

The efficacy of L-AmB for NWCL has not been systematically assessed in a controlled study, but efficacy in the 84% to 85% range was initially reported in small series of nonimmune travelers.[296,316,317] In a European case series, the rate of CL response was 46%, primarily due to lower response rates in *Viannia* species.[295] The optimal L-AmB dose and regimen is not defined for CL.[318] Use of the FDA-approved regimen for

VL of 3.0 mg/kg/day for days 1 to 5 and subsequent doses on days 14 and 21 (21 mg/kg total dosage) is reasonable pending more data but inconvenient. Collapsing the VL regimen to 3.0 mg/kg/day for days 1 to 5, boosting on days 8 and 9[296] or solely on day 10,[316] has been reported for CL treatment. Where resources are less limited, off-label use of L-AmB for CL is appealing because the drug is available, it is approved by the FDA for VL, and clinicians are familiar with its use. L-AmB is expensive and less used in endemic countries; amphotericin B deoxycholate is often used with good results in patients who fail an initial course of SbV.

Pentamidine

Pentamidine has a unique niche in the treatment of CL caused by *L. (V.) guyanensis* in French Guiana and Surinam. Cure rates as high as 90% are reported with just two intramuscular injections of 4 mg/kg, although a trial in Surinam suggested a lower healing rate.[319–321] Pentamidine has not been considered as efficacious in other geographic locations or in other *Leishmania* parasites.[308] However, a comparison of intralesional antimony (five injections) and intralesional pentamidine (120 μg/mm^2 lesion area) for patients with single-lesion Bolivian *L. (V.) braziliensis* showed equivalent cure rates.[310]

Azoles

In the New World, ketoconazole (black box warning), 600 mg daily for 28 days, cured 89% (8 of 9) of patients with *L. mexicana* in Guatemala[322] and 76% (16 of 21) of patients with *L. (V.) panamensis* in Panama.[323] Ketoconazole is not efficacious for *L. (V.) braziliensis* in Guatemala.[322] The treatment of *L. (V.) braziliensis* CL in Brazil with up to 8 mg/kg/day fluconazole for 28 days was not effective, with only 22% healing at 2 months.[324] In a systematic review of azole therapy for CL, the NWCL pooled azole efficacy was 66%, with *L. mexicana* response of 89%.[300]

Miltefosine

The efficacy of miltefosine in treatment of NWCL differs from region to region and must be evaluated in different settings to determine utility.[325] The FDA approved miltefosine for treatment of CL in adults and adolescents at least 12 years of age who weigh at least 30 kg. The FDA-approved regimen for persons who weigh at least 45 kg is one 50-mg capsule three times a day (total 150 mg/day) for 28 consecutive days. Increasing beyond this 150-mg daily dose is poorly tolerated, and efficacy may correlate with doses that are ≥2.5 mg/kg/day, which limits it to persons weighing ≤75 kg.[205] The FDA-approved indication is CL caused by three New World species in the *Viannia* subgenus—*L. (V.) braziliensis, L. (V.) panamensis,* and *L. (V.) guyanensis*. Even for these species, the effectiveness of miltefosine has been variable in different geographic regions. For example, in regions of Guatemala, the per-protocol cure rates were 53% (20 of 38) for miltefosine (and only 33% in *L. [V.] braziliensis*) and 21% (4 of 19) for placebo; yet for *L. (V.) braziliensis* CL in Colombia, miltefosine group cure rates were 80% (32 of 40).[326] In Bahia, Brazil, 75% (49 of 60) of patients with presumed *L. (V.) braziliensis* treated with miltefosine were cured at 6 months versus 53% (18 of 30) of patients treated with parenteral SbV 20 mg/kg/day for 20 days.[327]

MUCOSAL LEISHMANIASIS

Epidemiology

ML is a metastatic complication usually associated with NWCL and is the primary reason that NWCL is treated systemically. About 2% to 10% of persons infected with *L. (V.) braziliensis,* or, less commonly, *L. (V.) panamensis, L. (V.) guyanensis,* or *L. amazonensis*[58,328] develop mucous membrane involvement of the nose, oral cavity, pharynx, or larynx during or months to years after their skin lesions have healed.[329,330,331] A study following >3000 subjects with CL found a lifetime risk for ML of 13%.[332] Mucosal involvement usually occurs after a resolved primary ulcer but can be concurrent; additionally up to 21% of persons never recall a CL lesion.[333] The time between the primary lesion and mucosal involvement may be as short as 1 month or as long as 2 decades.[329,334] Although it is well known that the risk of ML associated with *L. (V.) braziliensis* is much higher in Bolivia, Brazil, and Peru, the distribution of the parasite extends from Mexico to Argentina.[335] ML is relatively uncommon north of Costa Rica. Whether this difference is related to different parasite strains, host immune response differences, or even differences in sand fly vectors such as components of sand fly saliva awaits clarification.

Mucosal involvement is seen occasionally because of the contiguous spread of cutaneous lesions caused by *L. tropica* or other *Leishmania* species. ML has also been described in Sudan associated with both *L. major* and *L. donovani*. In Sudan, ML is sporadic, involves the upper respiratory tract and oral mucosa, is usually a primary disease (but has been seen concurrently with or after VL), and may be caused by parasites that are genetically different from parasites associated with VL.[336–338] Mucosal involvement with lesions described as nodular or tumor-like masses has also been described with *L. infantum* (syn *L. chagasi*),[339] which is usually not associated with prior cutaneous lesions and is often associated with immunocompromised patients.[340,341]

Pathogenesis and Immunology

Various theories have been advanced to explain mucosal involvement including lower temperature, which favors parasite growth; failure of cell-mediated immune responses to be effective in mucosal tissue; local trauma; and capillary plexus trapping of amastigotes in the nose. Spontaneous cure of ML has been reported, but it is thought to be rare.[342] Risk factors associated with the development of ML are male sex, young adult age, severe malnutrition, and duration of primary ulcer greater than 4 months.[343] Host immune response,[344] racial background,[345] parasite strain differences,[346] and familial clustering[347] are associated with increased risk of ML, but the associations are not seen in all studies.

Histopathologically, chronic mucosal lesions are characterized by an intense mononuclear cell infiltrate with few parasites. In patients with ML, peripheral blood mononuclear cells proliferate and produce IFN-γ in response to leishmanial antigens in vitro, and their leishmanin skin tests are usually strongly positive.[348] CD8$^+$ T cells increase in the lesions and express high levels of perforin and granzyme suggesting cytolytic activity.[349] Th17$^+$ cells are increased in ML lesions and associated with areas of neutrophils.[350]

Clinical Manifestations

The initial symptoms are often nasal stuffiness, discharge, discomfort, blockage, or epistaxis. Over time a small nodule develops on the inferior turbinate or septum. Anterior septal ulceration appears early and may progress to perforation and eventual destruction of the septum, resulting in nasal collapse, sometimes called a *tapir nose*. Perforation can also occur through the soft palate. The upper lip may be involved in addition to the buccal, pharyngeal, or laryngeal mucosa.[342] When hypertrophy predominates, resulting in a protuberant nose and lips, the condition is called *espundia,* a term likely derived from the Spanish word for sponge (see Fig. 275.15).[351] If ulceration predominates, there is severe mutilation. A clinical grading system for ML lesions has been published.[352] A series of 327 persons with ML in Brazil found that 97% had involvement of the nasal cavity, 8% had involvement of pharynx, 7% had involvement of oral cavity, and 1% had involvement of larynx.[333] Any patient with possible ML who complains of a change in voice or is observed to have dysphonia requires a thorough examination of the epiglottis, vocal cords, and surrounding laryngeal tissue. Occasionally, severe inflammation can involve the epiglottis and threaten the airway. In this setting, high-dose intravenous steroids must be given 1 or 2 days before the initiation of chemotherapy for ML. Acute airway obstruction can occur with the increase in inflammation that usually follows treatment of ML.[353] On rare occasions, mucosal lesions or fibrosis or both are so extensive that the individual is unable to eat or experiences fatal aspiration pneumonia.

The IDSA-ASTMH clinical practice guidelines recommend that all persons at risk for ML should have a thorough naso-oropharyngeal examination, even if no symptoms are present; if symptoms are present, referral to an otolaryngologist should be done to permit fiberoptic endoscopy and biopsy of any visualized lesions.[21] Additionally, periodic careful ML examinations should be done up to 2 years after CL treatment for ML-risk species and for any later consistent symptoms. Computed tomography with or without fluorodeoxyglucose positron emission

tomography to follow lesions during and after treatment may provide additional assessment techniques.[354,355]

Diagnosis

The clinical suspicion of ML is raised by visualizing abnormal mucosal lesions or when any nasal, oral, pharyngeal, or laryngeal symptoms are vocalized or elicited in a patient with a past history of CL, a scar consistent with CL, or residence or travel to known endemic areas, especially in the highest-risk countries of Bolivia, Brazil, and Peru. Most of the approach to the diagnosis of CL applies here, specifically sample abnormal-appearing tissue, with the recognition that ML is pauciparasitic, which necessitates using molecular assays (see Fig. 275.3).

A confirmed parasitologic diagnosis of ML is preferred. A clinical diagnosis of ML can have a high pretest positive predictive value in an endemic area on the basis of typical clinical findings, a characteristic scar representing previous cutaneous infection, a positive leishmanin skin test result, or the presence of antileishmanial antibodies in serum.

The differential diagnosis of ML includes paracoccidioidomycosis, syphilis, tertiary yaws, histoplasmosis, sarcoidosis, leprosy, tuberculosis, granulomatosis with polyangiitis, basal cell carcinoma, nasal cocaine use, lethal midline granuloma, and T-cell lymphoma. The polyp-like nasal lesions that occur in some patients with ML may occasionally mimic rhinosporidiosis.

Treatment

Randomized trials to assess optimal treatment for ML are lacking.[356] All persons with New World ML should receive treatment, and the selection of agent, duration, and dose should be tailored to the individual patient.[21] SbV has been used for the treatment of New World ML for decades, but the response is variable (51%–88%), and relapses are common.[333,357] Early disease responds better than late-stage disease. Treatment regimens have varied, but a dose of SbV of 20 mg/kg/day for 28 days is typically administered, with some patients requiring longer courses. Patients who do not clinically respond and patients who relapse (most in the first year) can be treated with amphotericin B deoxycholate.[358,359] Depending on the patient's tolerance, 1.0 mg/kg is given IV daily or every other day for 30 doses. L-AmB has been used successfully in a small number of cases.[360,361] A retrospective series of 29 cases (predominantly *L. [V.] braziliensis*) with contraindications to the use of antimony reported an L-AmB–associated cure rate of 93%.[362] The total dosage is not well established, but ranges of 20 to 40 mg/kg have been reported.

Miltefosine given in a 28-day course is approved in the United States for the treatment of ML secondary to *L. (V.) braziliensis*. A nonrandomized trial of Bolivian ML caused by *L. (V.) braziliensis* treated with miltefosine 2.5 mg/kg/day for 28 days cured 62% (49 of 79) of patients at 12 months of follow-up in an intention-to-treat analysis, including 58% (21 of 36) with more extensive disease (palate, pharynx, and larynx).[363] In a follow-up study of 6 weeks of miltefosine with an extended follow-up of 21 patients for 24 months, 71% receiving primary treatment with miltefosine were cured, and 7% had definite or probable relapse.[364] In a case series of 7 *L. infantum* (syn *L. chagasi*) ML infections, miltefosine was uniformly effective.[365]

Other adjunctive approaches have been used with success. Oral pentoxifylline, an inhibitor of TNF-α, combined with SbV resulted in 100% cure (11 of 11) compared with a 58% cure (7 of 12) in the SbV-only trial arm of Brazilian ML. Time to healing was shorter in the combination group as well.[366] Plastic surgery may be necessary to ameliorate the sequelae of ML. It should be delayed for a year after successful therapy.

PREVENTION

For anthroponotic leishmaniasis (*L. donovani* or *L. tropica*) where humans are thought to be the reservoir, differences in time to diagnosis factor into transmission rates. Improved basic health system functioning combined with improved testing (better specificity in early infection) could decrease incidence.[367] There are several approaches to prevention for individuals and communities as public health interventions. For the individual, there is no form of chemoprophylaxis or active (vaccine) or passive (immunoglobulin) immunoprophylaxis for travelers. Standard personal protective measures such as *N,N*-diethyl-meta-toluamide–based insect repellents and permethrin or other insecticides applied to clothing and insecticide-impregnated bed nets provide protection against sand flies if used correctly.[368] Sand flies are one-third the size of a mosquito and can travel up to 960 m in 36 hours, although 200 m is more common.[369] Mosquito protecting bed nets with 156 holes/in^2 treated with insecticide were better than untreated nets[370]; they were effective in preventing CL in Afghanistan and the Syrian Arab Republic and were associated with decreased rates of VL in Bangladesh, Nepal, and Syria.[371] However, results of insecticide-treated bed net studies are variable, as in Morocco, long-lasting insecticide-treated nets were not superior to standard-of-care environmental management.[372–374] Insecticide-impregnated fabrics used as curtains, uniforms, and bedsheets were associated with reduced burden of leishmaniasis.[375,376] It has been suggested that there is not enough evidence to determine if indoor residual spraying is advantageous.[375]

For community-based efforts in endemic areas, vector control and reservoir control are effective, depending on local sand fly behavior and transmission dynamics. Residual insecticides applied in houses and other buildings have yielded good results in sites where peridomestic transmission occurs. However, spraying is necessary at intervals, sand flies may become resistant, and there is concern about the environmental impact. Of note, the cessation of dichlorodiphenyltrichloroethane spraying for malaria in Bangladesh, India, southern Iran, and Peru was followed by major epidemics and a resurgence of leishmaniasis.[377–381] In areas where transmission occurs away from dwellings, residual insecticides are of no benefit.

Reservoir control is another option in areas with domestic animal or human reservoirs. In *L. infantum* (syn *L. chagasi*) areas, domestic and wild dogs are thought to be a primary reservoir. Immunizing dogs, preventing infection through insecticide dog collars, and culling feral dogs should interrupt transmission and reduce human disease. In northeastern Brazil, infected domestic dogs have been identified by mass serologic testing and exterminated, but the efficacy of the program has not been proven, and it is poorly accepted.[382] Other studies suggest that insecticide-impregnated collars may protect dogs from sand fly bites and reduce the risk of human disease.[383,384] In sites where leishmaniasis is a zoonosis involving sylvatic mammals, reservoir control is rarely possible. In areas of anthroponotic transmission, case identification and treatment is important in control. Persons living in the same household as active cases and asymptomatic human infections in the community are a transmission risk.[385,386] In countries where VL has been spread among intravenous drug users, needle exchange programs might limit transmission. Also, for transfusion-associated infection, leukodepletion effectively reduces transmission risk.[387]

Although no commercial vaccine is currently available, there is a rationale to expect one in the future. The spontaneous resolution of human infection is associated with high-level immunity against the homologous infecting *Leishmania* species.[388] Mothers living in endemic areas have exposed the buttocks of their children to sand flies to ensure that leishmanial infection occurs at an inconspicuous site, thus protecting them from later disfiguring facial lesions. In Iran, Israel, and Uzbekistan, leishmanization has been performed by inoculating live *L. major* promastigotes taken from culture. Although this practice was effective in preventing naturally acquired disease, it was discontinued because some of the resulting lesions were slow to heal, others became secondarily infected, and parasites persisted at the site of inoculation even after the lesions healed.[389,390] Leishmanization cannot be used on a large scale or in HIV-endemic areas. Because *Leishmania* organisms lose their infectivity when subcultured and cannot be lyophilized, they must be kept cryopreserved; hence their delivery is impractical. The first generation of *Leishmania* vaccines included whole killed parasites with or without bacillus Calmette-Guérin as adjuvant.[391] Second-generation efforts have focused on identifying protective leishmanial immunogens, polyprotein vaccines, recombinant antigens, and effective adjuvants. Third-generation efforts are focused on the development of genetically engineered, live, avirulent parasites.[21,392–396] More recently interest has increased in the use of sand fly salivary molecules in leishmaniasis vaccines.[397,398]

In 2005 the governments of Bangladesh, India, and Nepal signed a memorandum of understanding to eliminate VL from the region. This ambitious elimination goal has five pillars: early diagnosis and complete

treatment, integrated vector management and vector surveillance, effective disease surveillance through passive and active case detection, social mobilization and building partnerships, and clinical and operational research.[399,400] This effort has led to significant declines in VL incidence but with lower rates faces challenges and will require sustained program implementation, coordination, and political support.

Key References

The complete reference list is available online at Expert Consult.

5. Akhoundi M, Kuhls K, Cannet A, et al. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis*. 2016;10:e0004349.
8. Lestinova T, Rohousova I, Sima M, et al. Insights into the sand fly saliva: blood-feeding and immune interactions between sand flies, hosts, and *Leishmania*. *PLoS Negl Trop Dis*. 2017;11:e0005600.
13. McCall LI, Zhang WW, Matlashewski G. Determinants for the development of visceral leishmaniasis disease. *PLoS Pathog*. 2013;9:e1003053.
14. Zhang WW, Ramasamy G, McCall LI, et al. Genetic analysis of *Leishmania donovani* tropism using a naturally attenuated cutaneous strain. *PLoS Pathog*. 2014;10:e1004244.
21. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clin Infect Dis*. 2016;63:e202–e264.
30. World Health Organization. Global leishmaniasis update, 2006-2015: a turning point in leishmaniasis surveillance. *Wkly Epidemiol Rec*. 2017;92:557–565.
33. Hirve S, Boelaert M, Matlashewski G, et al. Transmission dynamics of visceral leishmaniasis in the Indian subcontinent – a systematic literature review. *PLoS Negl Trop Dis*. 2016;10:e0004896.
40. Bhattacharya SK, Dash AP. Elimination of kala-azar from the Southeast Asia region. *Am J Trop Med Hyg*. 2017;96:802–804.
66. Kumar R, Bhatia M, Pai K. Role of cytokines in the pathogenesis of visceral leishmaniasis. *Clin Lab*. 2017;63:1549–1559.
127. de Ruiter CM, van der Veer C, Leeflang MM, et al. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. *J Clin Microbiol*. 2014;52:3147–3155.
128. Sudarshan M, Singh T, Chakravarty J, et al. A correlative study of splenic parasite score and peripheral blood parasite load estimation by quantitative PCR in visceral leishmaniasis. *J Clin Microbiol*. 2015;53:3905–3907.
137. Boelaert M, Verdonck K, Menten J, et al. Rapid tests for the diagnosis of visceral leishmaniasis in patients with suspected disease. *Cochrane Database Syst Rev*. 2014;(6):Cd009135.
144. Gajurel K, Dhakal R, Deresinski S. Leishmaniasis in solid organ and hematopoietic stem cell transplant recipients. *Clin Transplant*. 2017;31.
163. Abongomera C, Diro E, Vogt F, et al. The risk and predictors of visceral leishmaniasis relapse in human immunodeficiency virus-coinfected patients in Ethiopia: a retrospective cohort study. *Clin Infect Dis*. 2017;65:1703–1710.
165. Diro E, Ritmeijer K, Boelaert M, et al. Long-term clinical outcomes in visceral leishmaniasis/HIV-coinfected patients during and after pentamidine secondary prophylaxis in Ethiopia: a single-arm clinical trial. *Clin Infect Dis*. 2018;66:444–451.
178. Zijlstra EE. The immunology of post-kala-azar dermal leishmaniasis (PKDL). *Parasit Vectors*. 2016;9:464.
190. Romero GA, Costa DL, Costa CH, et al. Efficacy and safety of available treatments for visceral leishmaniasis in Brazil: a multicenter, randomized, open label trial. *PLoS Negl Trop Dis*. 2017;11:e0005706.
203. Ostyn B, Hasker E, Dorlo TP, et al. Failure of miltefosine treatment for visceral leishmaniasis in children and men in South-East Asia. *PLoS ONE*. 2014;9:e100220.
268. Machado PR, Rosa ME, Guimaraes LH, et al. Treatment of disseminated leishmaniasis with liposomal amphotericin B. *Clin Infect Dis*. 2015;61:945–949.
275. Morizot G, Kendjo E, Mouri O, et al. Travelers with cutaneous leishmaniasis cured without systemic therapy. *Clin Infect Dis*. 2013;57:370–380.
277. Heras-Mosteiro J, Monge-Maillo B, Pinart M, et al. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2017;(12):CD005067.
278. Gonzalez U, Pinart M, Rengifo-Pardo M, et al. Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2009;(2):CD004834.
279. Gonzalez U, Pinart M, Reveiz L, et al. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2008;(4):CD005067.
281. Kim DH, Chung HJ, Bleys J, et al. Is paromomycin an effective and safe treatment against cutaneous leishmaniasis? A meta-analysis of 14 randomized controlled trials. *PLoS Negl Trop Dis*. 2009;3:e381.
286. Cardona-Arias JA, Velez ID, Lopez-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: a meta-analysis of controlled clinical trials. *PLoS ONE*. 2015;10:e0122569.
287. Lopez-Carvajal L, Cardona-Arias JA, Zapata-Cardona MI, et al. Efficacy of cryotherapy for the treatment of cutaneous leishmaniasis: meta-analyses of clinical trials. *BMC Infect Dis*. 2016;16:360.
290. Brito NC, Rabello A, Cota GF. Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: a systematic review. *PLoS ONE*. 2017;12:e0184777.
295. Guery R, Henry B, Martin-Blondel G, et al. Liposomal amphotericin B in travelers with cutaneous and muco-cutaneous leishmaniasis: not a panacea. *PLoS Negl Trop Dis*. 2017;11:e0006094.
300. Galvao EL, Rabello A, Cota GF. Efficacy of azole therapy for tegumentary leishmaniasis: a systematic review and meta-analysis. *PLoS ONE*. 2017;12:e0186117.
308. Tuon FF, Amato VS, Graf ME, et al. Treatment of New World cutaneous leishmaniasis – a systematic review with a meta-analysis. *Int J Dermatol*. 2008;47:109–124.
316. Solomon M, Pavlotzky F, Barzilai A, et al. Liposomal amphotericin B in comparison to sodium stibogluconate for *Leishmania braziliensis* cutaneous leishmaniasis in travelers. *J Am Acad Dermatol*. 2013;68:284–289.
322. Navin TR, Arana BA, Arana FE, et al. Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) versus ketoconazole for treating cutaneous leishmaniasis in Guatemala. *J Infect Dis*. 1992;165:528–534.
326. Soto J, Arana BA, Toledo J, et al. Miltefosine for new world cutaneous leishmaniasis. *Clin Infect Dis*. 2004;38:1266–1272.
331. Blum J, Lockwood DN, Visser L, et al. Local or systemic treatment for New World cutaneous leishmaniasis? Re-evaluating the evidence for the risk of mucosal leishmaniasis. *Int Health*. 2012;4:153–163.
333. Cincura C, de Lima CM, Machado PR, et al. Mucosal leishmaniasis: a retrospective study of 327 cases from an endemic area of *Leishmania* (*Viannia*) *braziliensis*. *Am J Trop Med Hyg*. 2017;97:761–766.
352. Lessa HA, Lessa MM, Guimaraes LH, et al. A proposed new clinical staging system for patients with mucosal leishmaniasis. *Trans R Soc Trop Med Hyg*. 2012;106:376–381.
363. Soto J, Toledo J, Valda L, et al. Treatment of Bolivian mucosal leishmaniasis with miltefosine. *Clin Infect Dis*. 2007;44:350–356.
366. Machado PR, Lessa H, Lessa M, et al. Oral pentoxifylline combined with pentavalent antimony: a randomized trial for mucosal leishmaniasis. *Clin Infect Dis*. 2007;44:788–793.
375. Stockdale L, Newton R. A review of preventative methods against human leishmaniasis infection. *PLoS Negl Trop Dis*. 2013;7:e2278.
376. Gonzalez U, Pinart M, Sinclair D, et al. Vector and reservoir control for preventing leishmaniasis. *Cochrane Database Syst Rev*. 2015;(8):CD008736.

References

1. Rougeron V, De Meeus T, Banuls AL. A primer for *Leishmania* population genetic studies. *Trends Parasitol*. 2015;31:52–59.
2. Dumetz F, Imamura H, Sanders M, et al. Modulation of aneuploidy in *Leishmania donovani* during adaptation to different *in vitro* and *in vivo* environments and its impact on gene expression. *MBio*. 2017;8.
3. Lainson R, Shaw J. Evolution, classification and geographic distribution. In: Peters W, Killick-Kendrick R, eds. *The Leishmaniases in Biology and Medicine*. Vol 1. London: Academic Press; 1987:1–120.
4. Llanes A, Restrepo CM, Vecchio GD, et al. The genome of *Leishmania panamensis*: insights into genomics of the *L.* (*Viannia*) subgenus. *Sci Rep*. 2015;5:8550.
5. Akhoundi M, Kuhls K, Cannet A, et al. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis*. 2016;10:e0004349.
6. Peacock CS, Seeger K, Harris D, et al. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nat Genet*. 2007;39:839–847.
7. Schonian G, Kuhls K, Mauricio IL. Molecular approaches for a better understanding of the epidemiology and population genetics of *Leishmania*. *Parasitology*. 2011;138:405–425.
8. Lestinova T, Rohousova I, Sima M, et al. Insights into the sand fly saliva: blood-feeding and immune interactions between sand flies, hosts, and *Leishmania*. *PLoS Negl Trop Dis*. 2017;11:e0005600.
9. Valverde JG, Paun A, Inbar E, et al. Increased transmissibility of *Leishmania donovani* from the mammalian host to vector sand flies after multiple exposures to sand fly bites. *J Infect Dis*. 2017;215:1285–1293.
10. Doehl JSP, Bright Z, Dey S, et al. Skin parasite landscape determines host infectiousness in visceral leishmaniasis. *Nat Commun*. 2017;8:57.
11. Goncalves-de-Albuquerque SD, Pessoa ESR, Trajano-Silva LA, et al. The equivocal role of Th17 cells and neutrophils on immunopathogenesis of leishmaniasis. *Front Immunol*. 2017;8:1437.
12. Terrazas C, Varikuti S, Kimble J, et al. IL-17A promotes susceptibility during experimental visceral leishmaniasis caused by *Leishmania donovani*. *FASEB J*. 2016;30:1135–1143.
13. McCall LI, Zhang WW, Matlashewski G. Determinants for the development of visceral leishmaniasis disease. *PLoS Pathog*. 2013;9:e1003053.
14. Zhang WW, Ramasamy G, McCall LI, et al. Genetic analysis of *Leishmania donovani* tropism using a naturally attenuated cutaneous strain. *PLoS Pathog*. 2014;10:e1004244.
15. Rogers MB, Hilley JD, Dickens NJ, et al. Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*. *Genome Res*. 2011;21:2129–2142.
16. Atayde VD, Hassani K, da Silva Lira Filho A, et al. *Leishmania* exosomes and other virulence factors: impact on innate immune response and macrophage functions. *Cell Immunol*. 2016;309:7–18.
17. Seguin O, Descoteaux A. *Leishmania*, the phagosome, and host responses: the journey of a parasite. *Cell Immunol*. 2016;309:1–6.
18. Mouri O, Morizot G, Van der Auwera G, et al. Easy identification of *Leishmania* species by mass spectrometry. *PLoS Negl Trop Dis*. 2014;8:e2841.
19. Lachaud L, Fernandez-Arevalo A, Normand AC, et al. Identification of *Leishmania* by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry using a free web-based application and a dedicated mass-spectral library. *J Clin Microbiol*. 2017;55:2924–2933.
20. Evers DL, Frye FA, Weina PJ. A simple empirical algorithm to distinguish among *Leishmania braziliensis*, *major*, and *tropica* species by MALDI-TOF mass spectrometry. *J Microbiol Methods*. 2017.
21. Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clin Infect Dis*. 2016;63:e202–e264.
22. Alam MZ, Kuhls K, Schweynoch C, et al. Multilocus microsatellite typing (MLMT) reveals genetic homogeneity of Leishmania donovani strains in the Indian subcontinent. *Infect Genet Evol*. 2009;9:24–31.
23. Schonian G, Schnur L, el Fari M, et al. Genetic heterogeneity in the species *Leishmania tropica* revealed by different PCR-based methods. *Trans R Soc Trop Med Hyg*. 2001;95:217–224.
24. Aebischer T. Recurrent cutaneous leishmaniasis: a role for persistent parasites? *Parasitol Today*. 1994;10:25–28.
25. Mendonca MG, de Brito ME, Rodrigues EH, et al. Persistence of leishmania parasites in scars after clinical cure of American cutaneous leishmaniasis: is there a sterile cure? *J Infect Dis*. 2004;189:1018–1023.
26. Schubach A, Marzochi MC, Cuzzi-Maya T, et al. Cutaneous scars in American tegumentary leishmaniasis patients: a site of *Leishmania* (*Viannia*) *braziliensis* persistence and viability eleven years after antimonial therapy and clinical cure. *Am J Trop Med Hyg*. 1998;58:824–827.
27. Dereure J, Duong Thanh H, Lavabre-Bertrand T, et al. Visceral leishmaniasis. Persistence of parasites in lymph nodes after clinical cure. *J Infect*. 2003;47:77–81.
28. Kuhls K, Alam MZ, Cupolillo E, et al. Comparative microsatellite typing of new world leishmania infantum reveals low heterogeneity among populations and its recent old world origin. *PLoS Negl Trop Dis*. 2011;5:e1155.
29. Alvar J, Velez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE*. 2012;7:e35671.
30. World Health Organization. Global leishmaniasis update, 2006-2015: a turning point in leishmaniasis surveillance. *Wkly Epidemiol Rec*. 2017;92:557–565.
31. Courtenay O, Peters NC, Rogers ME, et al. Combining epidemiology with basic biology of sand flies, parasites, and hosts to inform leishmaniasis transmission dynamics and control. *PLoS Pathog*. 2017;13:e1006571.
32. Das VN, Pandey RN, Siddiqui NA, et al. Longitudinal study of transmission in households with visceral leishmaniasis, asymptomatic infections and PKDL in highly endemic villages in Bihar, India. *PLoS Negl Trop Dis*. 2016;10:e0005196.
33. Hirve S, Boelaert M, Matlashewski G, et al. Transmission dynamics of visceral leishmaniasis in the Indian subcontinent – a systematic literature review. *PLoS Negl Trop Dis*. 2016;10:e0004896.
34. Michel G, Pomares C, Ferrua B, et al. Importance of worldwide asymptomatic carriers of *Leishmania infantum* (*L. chagasi*) in human. *Acta Trop*. 2011;119:69–75.
35. dos Santos Marques LH, da Rocha IA, Reis IA, et al. *Leishmania infantum*: illness, transmission profile and risk factors for asymptomatic infection in an endemic metropolis in Brazil. *Parasitology*. 2017;144:546–556.
36. Kaushal H, Bhattacharya SK, Verma S, et al. Serological and molecular analysis of *Leishmania* infection in healthy individuals from two districts of West Bengal, India, endemic for visceral leishmaniasis. *Am J Trop Med Hyg*. 2017;96:1448–1455.
37. Lemma W, Tekie H, Yared S, et al. Sero-prevalence of *Leishmania donovani* infection in labour migrants and entomological risk factors in extra-domestic habitats of Kafta-Humera lowlands - kala-azar endemic areas in the northwest Ethiopia. *BMC Infect Dis*. 2015;15:99.
38. Maia Z, Viana V, Muniz E, et al. Risk factors associated with human visceral leishmaniasis in an urban area of Bahia, Brazil. *Vector Borne Zoonotic Dis*. 2016;16:368–376.
39. Singh OP, Hasker E, Sacks D, et al. Asymptomatic *Leishmania* infection: a new challenge for *Leishmania* control. *Clin Infect Dis*. 2014;58:1424–1429.
40. Bhattacharya SK, Dash AP. Elimination of kala-azar from the Southeast Asia region. *Am J Trop Med Hyg*. 2017;96:802–804.
41. Al-Salem W, Herricks JR, Hotez PJ. A review of visceral leishmaniasis during the conflict in South Sudan and the consequences for East African countries. *Parasit Vectors*. 2016;9:460.
42. Baleela R, Llewellyn MS, Fitzpatrick S, et al. *Leishmania donovani* populations in Eastern Sudan: temporal structuring and a link between human and canine transmission. *Parasit Vectors*. 2014;7:496.
43. Lun ZR, Wu MS, Chen YF, et al. Visceral leishmaniasis in China: an endemic disease under control. *Clin Microbiol Rev*. 2015;28:987–1004.
44. Mahdy MA, Al-Mekhlafi AM, Abdul-Ghani R, et al. First molecular characterization of *Leishmania* species causing visceral leishmaniasis among children in Yemen. *PLoS ONE*. 2016;11:e0151265.
45. Faulde MK, Erkens K, Dieterle R. [Epidemiology and prevention of leishmaniasis in northern Afghanistan]. *Hautarzt*. 2015;66:347–354.
46. Todd CS, Mansoor GF, Buhler C, et al. Prevalence of zoonotic and vector-borne infections among Afghan National Army recruits in Afghanistan. *Vector Borne Zoonotic Dis*. 2016;16:501–506.
47. Alvar J, Canavate C, Gutierrez-Solar B, et al. Leishmania and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev*. 1997;10:298–319.
48. Alvar J, Aparicio P, Aseffa A, et al. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev*. 2008;21:334–359. table of contents.
49. Arce A, Estirado A, Ordobas M, et al. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. *Euro Surveill*. 2013;18:20546.
50. Seva ADP, Martcheva M, Tuncer N, et al. Efficacies of prevention and control measures applied during an outbreak in Southwest Madrid, Spain. *PLoS ONE*. 2017;12:e0186372.
51. Conti RV, Moura Lane VF, Montebello L, et al. Visceral leishmaniasis epidemiologic evolution in timeframes, based on demographic changes and scientific achievements in Brazil. *J Vector Borne Dis*. 2016;53:99–104.
52. Cruz I, Morales MA, Noguer I, et al. Leishmania in discarded syringes from intravenous drug users. *Lancet*. 2002;359:1124–1125.
53. Grinnage-Pulley T, Scott B, Petersen CA. A mother's gift: congenital transmission of *Trypanosoma* and *Leishmania* species. *PLoS Pathog*. 2016;12:e1005302.
54. Jimenez-Marco T, Fisa R, Girona-Llobera E, et al. Transfusion-transmitted leishmaniasis: a practical review. *Transfusion*. 2016;56(suppl 1):S45–S51.
55. Symmers WS. Leishmaniasis acquired by contagion: a case of marital infection in Britain. *Lancet*. 1960;1:127–132.
56. Turchetti AP, Souza TD, Paixao TA, et al. Sexual and vertical transmission of visceral leishmaniasis. *J Infect Dev Ctries*. 2014;8:403–407.
57. Aleixo JA, Nascimento ET, Monteiro GR, et al. Atypical American visceral leishmaniasis caused by disseminated *Leishmania amazonensis* infection presenting with hepatitis and adenopathy. *Trans R Soc Trop Med Hyg*. 2006;100:79–82.
58. Barral A, Pedral-Sampaio D, Grimaldi Junior G, et al. Leishmaniasis in Bahia, Brazil: evidence that *Leishmania amazonensis* produces a wide spectrum of clinical disease. *Am J Trop Med Hyg*. 1991;44:536–546.
59. Alborzi A, Rasouli M, Shamsizadeh A. *Leishmania tropica*-isolated patient with visceral leishmaniasis in southern Iran. *Am J Trop Med Hyg*. 2006;74:306–307.
60. Sacks DL, Kenney RT, Kreutzer RD, et al. Indian kala-azar caused by *Leishmania tropica*. *Lancet*. 1995;345:959–961.
61. Magill AJ, Grogl M, Gasser RA Jr, et al. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med*. 1993;328:1383–1387.
62. Liautaud B, Vignier N, Miossec C, et al. First case of visceral leishmaniasis caused by *Leishmania martiniquensis*. *Am J Trop Med Hyg*. 2015;92:317–319.
63. Cotton JA. The expanding world of human leishmaniasis. *Trends Parasitol*. 2017;33:341–344.
64. Christophers SR. On a Parasite Found in Persons Suffering from Enlargement of the Spleen in India. Scientific Memoirs, New Series, no. 11. Calcutta: Government of India Printing; 1904.
65. Lubitz JM. Pathology of kala-azar; report of a case. *Am J Trop Med Hyg*. 1948;28:275–286.
66. Kumar R, Bhatia M, Pai K. Role of cytokines in the pathogenesis of visceral leishmaniasis. *Clin Lab*. 2017;63:1549–1559.
67. de Oliveira FA, Barreto AS, Bomfim LG, et al. Soluble CD40 ligand in sera of subjects exposed to *Leishmania infantum* infection reduces the parasite load in macrophages. *PLoS ONE*. 2015;10:e0141265.
68. Nascimento MS, Ferreira MD, Quirino GF, et al. NOD2-RIP2-mediated signaling helps shape adaptive immunity in visceral leishmaniasis. *J Infect Dis*. 2016;214:1647–1657.
69. Murray HW, Luster AD, Zheng H, et al. Gamma interferon-regulated chemokines in *Leishmania donovani* infection in the liver. *Infect Immun*. 2017;85.
70. Murray HW, Mitchell-Flack M, Zheng H, et al. Granzyme-mediated regulation of host defense in the liver in experimental *Leishmania donovani* infection. *Infect Immun*. 2015;83:702–712.
71. Kong F, Saldarriaga OA, Spratt H, et al. Transcriptional profiling in experimental visceral leishmaniasis reveals a broad splenic inflammatory environment that conditions macrophages toward a disease-promoting phenotype. *PLoS Pathog*. 2017;13:e1006165.
72. Gupta P, Srivastav S, Saha S, et al. *Leishmania donovani* inhibits macrophage apoptosis and pro-inflammatory response through AKT-mediated regulation of beta-catenin and FOXO-1. *Cell Death Differ*. 2016;23:1815–1826.
73. Thomas SA, Nandan D, Kass J, et al. Countervailing, time-dependent effects on host autophagy promotes intracellular survival of *Leishmania*. *J Biol Chem*. 2017.
74. Vijayamahantesh, Amit A, Dikhit MR, et al. Adenosine generated by ectonucleotidases modulates the host immune system during visceral leishmaniasis. *Cytokine*. 2017;91:170–179.

75. Giri J, Srivastav S, Basu M, et al. *Leishmania donovani* exploits myeloid cell leukemia 1 (MCL-1) protein to prevent mitochondria-dependent host cell apoptosis. *J Biol Chem*. 2016;291:3496–3507.
76. Sharma S, Davis RE, Srivastva S, et al. A subset of neutrophils expressing markers of antigen-presenting cells in human visceral leishmaniasis. *J Infect Dis*. 2016;214:1531–1538.
77. Sharma S, Srivastva S, Davis RE, et al. The phenotype of circulating neutrophils during visceral leishmaniasis. *Am J Trop Med Hyg*. 2017;97:767–770.
78. Omachi S, Fujii W, Azuma N, et al. B-cell activating factor deficiency suppresses splenomegaly during *Leishmania donovani* infection. *Biochem Biophys Res Commun*. 2017;489:528–533.
79. Gardinassi LG, Dotz V, Hipgrave Ederveen A, et al. Clinical severity of visceral leishmaniasis is associated with changes in immunoglobulin G Fc N-glycosylation. *MBio*. 2014;5:e01844.
80. Zackay A, Cotton JA, Sanders M, et al. Genome wide comparison of Ethiopian *Leishmania donovani* strains reveals differences potentially related to parasite survival. *PLoS Genet*. 2018;14:e1007133.
81. Wilson ME, Jeronimo SM, Pearson RD. Immunopathogenesis of infection with the visceralizing *Leishmania* species. *Microb Pathog*. 2005;38:147–160.
82. Jamieson SE, Miller EN, Peacock CS, et al. Genome-wide scan for visceral leishmaniasis susceptibility genes in Brazil. *Genes Immun*. 2007;8:84–90.
83. Miller EN, Fadl M, Mohamed HS, et al. Y chromosome lineage- and village-specific genes on chromosomes 1p22 and 6q27 control visceral leishmaniasis in Sudan. *PLoS Genet*. 2007;3:e71.
84. El-Safi S, Kheir MM, Bucheton B, et al. Genes and environment in susceptibility to visceral leishmaniasis. *C R Biol*. 2006;329:863–870.
85. Mohamed HS, Ibrahim ME, Miller EN, et al. SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. *Eur J Hum Genet*. 2004;12:66–74.
86. Bucheton B, Abel L, El-Safi S, et al. A major susceptibility locus on chromosome 22q12 plays a critical role in the control of kala-azar. *Am J Hum Genet*. 2003;73:1052–1060.
87. Mohamed HS, Ibrahim ME, Miller EN, et al. Genetic susceptibility to visceral leishmaniasis in The Sudan: linkage and association with IL4 and IFNGR1. *Genes Immun*. 2003;4:351–355.
88. Fakiola M, Strange A, Cordell HJ, et al. Common variants in the HLA-DRB1-HLA-DQA1 HLA class II region are associated with susceptibility to visceral leishmaniasis. *Nat Genet*. 2013;45:208–213.
89. Mishra A, Antony JS, Gai P, et al. Mannose-binding Lectin (MBL) as a susceptible host factor influencing Indian visceral leishmaniasis. *Parasitol Int*. 2015;64:591–596.
90. Mishra A, Nizamuddin S, Arekatla G, et al. IL10 variant g.5311A is associated with visceral leishmaniasis in Indian population. *PLoS ONE*. 2015;10:e0124559.
91. Parvaneh N, Barlogis V, Alborzi A, et al. Visceral leishmaniasis in two patients with IL-12p40 and IL-12Rbeta1 deficiencies. *Pediatr Blood Cancer*. 2017;64.
92. Weirather JL, Duggal P, Nascimento EL, et al. Comprehensive candidate gene analysis for symptomatic or asymptomatic outcomes of *Leishmania infantum* infection in Brazil. *Ann Hum Genet*. 2017;81:41–48.
93. Marquet S, Bucheton B, Reymond C, et al. Exome sequencing identifies two variants of the alkylglycerol monooxygenase gene as a cause of relapses in visceral leishmaniasis in children, in Sudan. *J Infect Dis*. 2017;216:22–28.
94. Maurya R, Bhattacharya P, Ismail N, et al. Differential role of leptin as an immunomodulator in controlling visceral leishmaniasis in normal and leptin-deficient mice. *Am J Trop Med Hyg*. 2016;95:109–119.
95. Saha P, Ganguly S, Chatterjee M, et al. Asymptomatic leishmaniasis in kala-azar endemic areas of Malda district, West Bengal, India. *PLoS Negl Trop Dis*. 2017;11:e0005391.
96. Orsini M, Canela JR, Disch J, et al. High frequency of asymptomatic *Leishmania* spp. infection among HIV-infected patients living in endemic areas for visceral leishmaniasis in Brazil. *Trans R Soc Trop Med Hyg*. 2012;106:283–288.
97. Mouri O, Benhamou M, Leroux G, et al. Spontaneous remission of fully symptomatic visceral leishmaniasis. *BMC Infect Dis*. 2015;15:445.
98. Abongomera C, Ritmeijer K, Vogt F, et al. Development and external validation of a clinical prognostic score for death in visceral leishmaniasis patients in a high HIV co-infection burden area in Ethiopia. *PLoS ONE*. 2017;12:e0178996.
99. Daher EF, Soares DS, Filho SL, et al. Hyponatremia and risk factors for death in human visceral leishmaniasis: new insights from a cross-sectional study in Brazil. *BMC Infect Dis*. 2017;17:168.
100. Das A, Karthick M, Dwivedi S, et al. Epidemiologic correlates of mortality among symptomatic visceral leishmaniasis cases: findings from situation assessment in high endemic foci in India. *PLoS Negl Trop Dis*. 2016;10:e0005150.
101. Kämink SS, Collin SM, Harrison T, et al. A clinical severity scoring system for visceral leishmaniasis in immunocompetent patients in South Sudan. *PLoS Negl Trop Dis*. 2017;11:e0005921.
102. Most H, Lavietes PH. Kala azar in American military personnel; report of 30 cases. *Medicine (Baltimore)*. 1947;26:221–284.
103. Zijlstra EE, el-Hassan AM. Leishmaniasis in Sudan. Visceral leishmaniasis. *Trans R Soc Trop Med Hyg*. 2001;95(suppl 1):S27–S58.
104. Pearson RD, Cox G, Jeronimo SM, et al. Visceral leishmaniasis: a model for infection-induced cachexia. *Am J Trop Med Hyg*. 1992;47(1 Pt 2):8–15.
105. Barati M, Sharifi I, Daie Parizi M, et al. Bacterial infections in children with visceral leishmaniasis: observations made in Kerman province, southern Iran, between 1997 and 2007. *Ann Trop Med Parasitol*. 2008;102:635–641.
106. Kadivar MR, Kajbaf TZ, Karimi A, et al. Childhood visceral leishmaniasis complicated by bacterial infections. *East Mediterr Health J*. 2000;6:879–883.
107. Andrade TM, Carvalho EM, Rocha H. Bacterial infections in patients with visceral leishmaniasis. *J Infect Dis*. 1990;162:1354–1359.
108. Chandra H, Chandra S. Case of hemophagocytic lymphohistiocytosis with leishmaniasis. *Trop Parasitol*. 2015;5:135–136.
109. Daher EF, Lima LL, Vieira AP, et al. Hemophagocytic syndrome in children with visceral leishmaniasis. *Pediatr Infect Dis J*. 2015;34:1311–1314.
110. Atta AM, Carvalho EM, Jeronimo SM, et al. Serum markers of rheumatoid arthritis in visceral leishmaniasis: rheumatoid factor and anti-cyclic citrullinated peptide antibody. *J Autoimmun*. 2007;28:55–58.
111. Pearson RD, de Alencar JE, Romito R, et al. Circulating immune complexes and rheumatoid factors in visceral leishmaniasis. *J Infect Dis*. 1983;147:1102.
112. Pagliano P, Rossi M, Rescigno C, et al. Mediterranean visceral leishmaniasis in HIV-negative adults: a retrospective analysis of 64 consecutive cases (1995-2001). *J Antimicrob Chemother*. 2003;52:264–268.
113. Duvic C, Nedelec G, Debord T, et al. [Important parasitic nephropathies: update from the recent literature]. *Nephrologie*. 1999;20:65–74.
114. Caravaca F, Munoz A, Pizarro JL, et al. Acute renal failure in visceral leishmaniasis. *Am J Nephrol*. 1991;11:350–352.
115. Dutra M, Martinelli R, de Carvalho EM, et al. Renal involvement in visceral leishmaniasis. *Am J Kidney Dis*. 1985;6:22–27.
116. Duarte MI, Silva MR, Goto H, et al. Interstitial nephritis in human kala-azar. *Trans R Soc Trop Med Hyg*. 1983;77:531–537.
117. Leblond V, Beaufils H, Ginsburg C, et al. Collapsing focal segmental glomerulosclerosis associated with visceral leishmaniasis. *Nephrol Dial Transplant*. 1994;9:1353.
118. Liborio AB, Rocha NA, Oliveira MJ, et al. Acute kidney injury in children with visceral leishmaniasis. *Pediatr Infect Dis J*. 2012;31:451–454.
119. Daher EF, Evangelista LF, Silva Junior GB, et al. Clinical presentation and renal evaluation of human visceral leishmaniasis (kala-azar): a retrospective study of 57 patients in Brazil. *Braz J Infect Dis*. 2008;12:329–332.
120. Lima Verde FA, Lima Verde FA, Lima Verde IA, et al. Evaluation of renal function in human visceral leishmaniasis (kala-azar): a prospective study on 50 patients from Brazil. *J Nephrol*. 2007;20:430–436.
121. Kager PA, Rees PH, Manguyu FM, et al. Splenic aspiration; experience in Kenya. *Trop Geogr Med*. 1983;35:125–131.
122. Chulay JD, Bryceson AD. Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with visceral leishmaniasis. *Am J Trop Med Hyg*. 1983;32:475–479.
123. da Silva MR, Stewart JM, Costa CH. Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. *Am J Trop Med Hyg*. 2005;72:811–814.
124. Torpiano P, Pace D. Leishmaniasis: diagnostic issues in Europe. *Expert Rev Anti Infect Ther*. 2015;13:1123–1138.
125. Myles O, Wortmann GW, Cummings JF, et al. Visceral leishmaniasis: clinical observations in 4 US army soldiers deployed to Afghanistan or Iraq, 2002-2004. *Arch Intern Med*. 2007;167:1899–1901.
126. Lopez-Velez R, Laguna F, Alvar J, et al. Parasitic culture of buffy coat for diagnosis of visceral leishmaniasis in human immunodeficiency virus-infected patients. *J Clin Microbiol*. 1995;33:937–939.
127. de Ruiter CM, van der Veer C, Leeflang MM, et al. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. *J Clin Microbiol*. 2014;52:3147–3155.
128. Sudarshan M, Singh T, Chakravarty J, et al. A correlative study of splenic parasite score and peripheral blood parasite load estimation by quantitative PCR in visceral leishmaniasis. *J Clin Microbiol*. 2015;53:3905–3907.
129. Sudarshan M, Sundar S. Parasite load estimation by qPCR differentiates between asymptomatic and symptomatic infection in Indian visceral leishmaniasis. *Diagn Microbiol Infect Dis*. 2014;80:40–42.
130. Hossain F, Ghosh P, Khan MA, et al. Real-time PCR in detection and quantitation of *Leishmania donovani* for the diagnosis of visceral leishmaniasis patients and the monitoring of their response to treatment. *PLoS ONE*. 2017;12:e0185606.
131. Koltas IS, Eroglu F, Uzun S, et al. A comparative analysis of different molecular targets using PCR for diagnosis of old world leishmaniasis. *J Parasitol Res*. 2016;164:43–48.
132. Machado de Assis TS, Azeredo-da-Silva AL, Werneck GL, et al. Cost-effectiveness analysis of diagnostic tests for human visceral leishmaniasis in Brazil. *Trans R Soc Trop Med Hyg*. 2016;110:464–471.
133. Abbasi I, Kirstein OD, Hailu A, et al. Optimization of loop-mediated isothermal amplification (LAMP) assays for the detection of *Leishmania* DNA in human blood samples. *Acta Trop*. 2016;162:20–26.
134. Mondal D, Ghosh P, Khan MA, et al. Mobile suitcase laboratory for rapid detection of *Leishmania donovani* using recombinase polymerase amplification assay. *Parasit Vectors*. 2016;9:281.
135. Verma S, Singh R, Sharma V, et al. Development of a rapid loop-mediated isothermal amplification assay for diagnosis and assessment of cure of *Leishmania* infection. *BMC Infect Dis*. 2017;17:223.
136. Bhattacharyya T, Boelaert M, Miles MA. Comparison of visceral leishmaniasis diagnostic antigens in African and Asian *Leishmania donovani* reveals extensive diversity and region-specific polymorphisms. *PLoS Negl Trop Dis*. 2013;7:e2057.
137. Boelaert M, Verdonck K, Menten J, et al. Rapid tests for the diagnosis of visceral leishmaniasis in patients with suspected disease. *Cochrane Database Syst Rev*. 2014;(6):Cd009135.
138. Osman HA, Mahamoud A, Abass EM, et al. Local production of a liquid direct agglutination test as a sustainable measure for control of visceral leishmaniasis in Sudan. *Am J Trop Med Hyg*. 2016;94:982–986.
139. Hartzell JD, Aronson NE, Weina PJ, et al. Positive rK39 serologic assay results in US servicemen with cutaneous leishmaniasis. *Am J Trop Med Hyg*. 2008;79:843–846.
140. Gao CH, Yang YT, Shi F, et al. Development of an immunochromatographic test for diagnosis of visceral leishmaniasis based on detection of a circulating antigen. *PLoS Negl Trop Dis*. 2015;9:e0003902.
141. Jamal F, Shivam P, Kumari S, et al. Identification of *Leishmania donovani* antigen in circulating immune complexes of visceral leishmaniasis subjects for diagnosis. *PLoS ONE*. 2017;12:e0182474.
142. Singh OP, Sundar S. Whole blood assay and visceral leishmaniasis: challenges and promises. *Immunobiology*. 2014;219:323–328.
143. Carrillo E, Carrasco-Anton N, Lopez-Medrano F, et al. Cytokine release assays as tests for exposure to *Leishmania*, and for confirming cure from leishmaniasis, in solid organ transplant recipients. *PLoS Negl Trop Dis*. 2015;9:e0004179.
144. Gajurel K, Dhakal R, Deresinski S. Leishmaniasis in solid organ and hematopoietic stem cell transplant recipients. *Clin Transplant*. 2017;31.
145. van Griensven J, Carrillo E, Lopez-Velez R, et al. Leishmaniasis in immunosuppressed individuals. *Clin Microbiol Infect*. 2014;20:286–299.
146. Herrador Z, Gherasim A, Jimenez BC, et al. Epidemiological changes in leishmaniasis in Spain according to hospitalization-based records, 1997-2011: raising awareness towards leishmaniasis in non-HIV patients. *PLoS Negl Trop Dis*. 2015;9:e0003594.
147. Monge-Maillo B, Norman FF, Cruz I, et al. Visceral leishmaniasis and HIV coinfection in the Mediterranean region. *PLoS Negl Trop Dis*. 2014;8:e3021.
148. Fletcher K, Issa R, Lockwood DN. Visceral leishmaniasis and immunocompromise as a risk factor for the development of visceral leishmaniasis: a changing pattern at the Hospital for Tropical Diseases, London. *PLoS ONE*. 2015;10:e0121418.
149. Diro E, Lynen L, Ritmeijer K, et al. Visceral leishmaniasis and HIV coinfection in East Africa. *PLoS Negl Trop Dis*. 2014;8:e2869.
150. Leite de Sousa-Gomes M, Romero GAS, Werneck GL. Visceral leishmaniasis and HIV/AIDS in Brazil: are we aware enough? *PLoS Negl Trop Dis*. 2017;11:e0005772.

151. Singh S. Changing trends in the epidemiology, clinical presentation, and diagnosis of *Leishmania*-HIV co-infection in India. *Int J Infect Dis*. 2014;29:103–112.
152. Casado JL, Abad-Fernandez M, Moreno S, et al. Visceral leishmaniasis as an independent cause of high immune activation, T-cell senescence, and lack of immune recovery in virologically suppressed HIV-1-coinfected patients. *HIV Med*. 2015;16:240–248.
153. Pagliano P, Esposito S. Visceral leishmaniosis in immunocompromised host: an update and literature review. *J Chemother*. 2017;29:261–266.
154. Silva-Freitas ML, Cota GF, Machado-de-Assis TS, et al. Immune activation and bacterial translocation: a link between impaired immune recovery and frequent visceral leishmaniasis relapses in HIV-infected patients. *PLoS ONE*. 2016;11:e0167512.
155. Coutinho JV, Santos FS, Ribeiro RD, et al. Visceral leishmaniasis and leishmaniasis-HIV coinfection: comparative study. *Rev Soc Bras Med Trop*. 2017;50:670–674.
156. Lucero E, Collin SM, Gomes S, et al. Effectiveness and safety of short course liposomal amphotericin B (AmBisome) as first line treatment for visceral leishmaniasis in Bangladesh. *PLoS Negl Trop Dis*. 2015;9:e0003699.
157. Davies O, Allen F, Gruener AM, et al. Uveitis secondary to leishmaniasis immune reconstitution syndrome in a HIV-positive patient. *Int J STD AIDS*. 2016;27:598–600.
158. Diro E, van Griensven J, Mohammed R, et al. Atypical manifestations of visceral leishmaniasis in patients with HIV in north Ethiopia: a gap in guidelines for the management of opportunistic infections in resource poor settings. *Lancet Infect Dis*. 2015;15:122–129.
159. Van Os L, Cnops L, Van Esbroeck M, et al. Slowly progressive keratouveitis in a patient with known systemic leishmaniasis and HIV. *Ocul Immunol Inflamm*. 2015;23:248–251.
160. Ramos JM, Leon R, Merino E, et al. Is visceral leishmaniasis different in immunocompromised patients without human immunodeficiency virus? A comparative, multicenter retrospective cohort analysis. *Am J Trop Med Hyg*. 2017;97:1127–1133.
161. Barbosa Junior WL, Ramos de Araujo PS, Dias de Andrade L, et al. Rapid tests and the diagnosis of visceral leishmaniasis and human immunodeficiency virus/acquired immunodeficiency syndrome coinfection. *Am J Trop Med Hyg*. 2015;93:967–969.
162. Goswami RP, Goswami RP, Basu A, et al. Protective efficacy of secondary prophylaxis against visceral leishmaniasis in human immunodeficiency virus coinfected patients over the past 10 years in eastern India. *Am J Trop Med Hyg*. 2017;96:285–291.
163. Abongomera C, Diro E, Vogt F, et al. The risk and predictors of visceral leishmaniasis relapse in human immunodeficiency virus-coinfected patients in Ethiopia: a retrospective cohort study. *Clin Infect Dis*. 2017;65:1703–1710.
164. Cipriano P, Miranda AC, Antunes I, et al. Visceral leishmaniasis in HIV-infected patients: the challenge of relapse and treatment failure. *Acta Med Port*. 2017;30:443–448.
165. Diro E, Ritmeijer K, Boelaert M, et al. Long-term clinical outcomes in visceral leishmaniasis/HIV-coinfected patients during and after pentamidine secondary prophylaxis in Ethiopia: a single-arm clinical trial. *Clin Infect Dis*. 2018;66:444–451.
166. Cota GF, de Sousa MR, de Assis TS, et al. Exploring prognosis in chronic relapsing visceral leishmaniasis among HIV-infected patients: circulating *Leishmania* DNA. *Acta Trop*. 2017;172:186–191.
167. Jarvis JN, Lockwood DN. Clinical aspects of visceral leishmaniasis in HIV infection. *Curr Opin Infect Dis*. 2013;26:1–9.
168. Aderie EM, Diro E, Zachariah R, et al. Does timing of antiretroviral treatment influence treatment outcomes of visceral leishmaniasis in Northwest Ethiopia? *Trans R Soc Trop Med Hyg*. 2017;111:107–116.
169. Zijlstra EE, Musa AM, Khalil EA, et al. Post-kala-azar dermal leishmaniasis. *Lancet Infect Dis*. 2003;3:87–98.
170. Musa AM, Khalil EA, Raheem MA, et al. The natural history of Sudanese post-kala-azar dermal leishmaniasis: clinical, immunological and prognostic features. *Ann Trop Med Parasitol*. 2002;96:765–772.
171. Zijlstra EE, Khalil EA, Kager PA, et al. Post-kala-azar dermal leishmaniasis in the Sudan: clinical presentation and differential diagnosis. *Br J Dermatol*. 2000;143:136–143.
172. Mondal D, Khan MG. Recent advances in post-kala-azar dermal leishmaniasis. *Curr Opin Infect Dis*. 2011;24:418–422.
173. Ganguly S, Saha P, Chatterjee M, et al. PKDL—a silent parasite pool for transmission of leishmaniasis in kala-azar endemic areas of Malda district, West Bengal, India. *PLoS Negl Trop Dis*. 2015;9:e0004138.
174. Ridolfo AL, Gervasoni C, Antinori S, et al. Post-kala-azar dermal leishmaniasis during highly active antiretroviral therapy in an AIDS patient infected with *Leishmania infantum*. *J Infect*. 2000;40:199–202.
175. Hasnain MG, Shomik MS, Ghosh P, et al. Post-kala-azar dermal leishmaniasis without previous history of visceral leishmaniasis. *Am J Trop Med Hyg*. 2016;95:1383–1385.
176. Ramesh V, Kaushal H, Mishra AK, et al. Clinico-epidemiological analysis of post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study. *BMC Public Health*. 2015;15:1092.
177. Das S, Mandal R, Rabidas VN, et al. Chronic arsenic exposure and risk of post kala-azar dermal leishmaniasis development in India: a retrospective cohort study. *PLoS Negl Trop Dis*. 2016;10:e0005060.
178. Zijlstra EE. The immunology of post-kala-azar dermal leishmaniasis (PKDL). *Parasit Vectors*. 2016;9:464.
179. Sultana A, Zakaria SM, Bhuiyan SI, et al. Spectrum of skin lesions of post-kala-azar dermal leishmaniasis in kala-azar endemic areas of Bangladesh. *Mymensingh Med J*. 2012;21:529–532.
180. Molina R, Ghosh D, Carrillo E, et al. Infectivity of post-kala-azar dermal leishmaniasis patients to sand flies: revisiting a proof of concept in the context of the kala-azar elimination program in the Indian subcontinent. *Clin Infect Dis*. 2017;65:150–153.
181. No JH. Visceral leishmaniasis: revisiting current treatments and approaches for future discoveries. *Acta Trop*. 2016;155:113–123.
182. Berman JD. U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. *Clin Infect Dis*. 1999;28:49–51.
183. Meyerhoff A. U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. *Clin Infect Dis*. 1999;28:42–48, discussion 49–51.
184. Sundar S, Jha TK, Thakur CP, et al. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis*. 2003;37:800–804.
185. Goswami RP, Goswami RP, Das S, et al. Short-course treatment regimen of Indian visceral leishmaniasis with an Indian liposomal amphotericin B preparation (Fungisome). *Am J Trop Med Hyg*. 2016;94:93–98.
186. Pandey K, Pal B, Siddiqui NA, et al. Efficacy and safety of liposomal amphotericin B for visceral leishmaniasis in children and adolescents at a tertiary care center in Bihar, India. *Am J Trop Med Hyg*. 2017;97:1498–1502.
187. Rahman R, Goyal V, Haque R, et al. Safety and efficacy of short course combination regimens with AmBisome, miltefosine and paromomycin for the treatment of visceral leishmaniasis (VL) in Bangladesh. *PLoS Negl Trop Dis*. 2017;11:e0005635.
188. Copeland NK, Aronson NE. Leishmaniasis: treatment updates and clinical practice guidelines review. *Curr Opin Infect Dis*. 2015;28:426–437.
189. Tamiru A, Tigabu B, Yifru S, et al. Safety and efficacy of liposomal amphotericin B for treatment of complicated visceral leishmaniasis in patients without HIV, North-West Ethiopia. *BMC Infect Dis*. 2016;16:548.
190. Romero GA, Costa DL, Costa CH, et al. Efficacy and safety of available treatments for visceral leishmaniasis in Brazil: a multicenter, randomized, open label trial. *PLoS Negl Trop Dis*. 2017;11:e0005706.
191. Yardley V, Croft SL. A comparison of the activities of three amphotericin B lipid formulations against experimental visceral and cutaneous leishmaniasis. *Int J Antimicrob Agents*. 2000;13:243–248.
192. Sundar S. Drug resistance in Indian visceral leishmaniasis. *Trop Med Int Health*. 2001;6:849–854.
193. Mueller YK, Kolaczinski JH, Koech T, et al. Clinical epidemiology, diagnosis and treatment of visceral leishmaniasis in the Pokot endemic area of Uganda and Kenya. *Am J Trop Med Hyg*. 2014;90:33–39.
194. World Health Organization. Control of visceral leishmaniasis in Somalia: achievements in a challenging scenario, 2013-2015. *Wkly Epidemiol Rec*. 2017;92:566–572.
195. Bermudez H, Rojas E, Garcia L, et al. Generic sodium stibogluconate is as safe and effective as branded meglumine antimoniate, for the treatment of tegumentary leishmaniasis in Isiboro Secure Park, Bolivia. *Ann Trop Med Parasitol*. 2006;100:591–600.
196. Veeken H, Ritmeijer K, Seaman J, et al. A randomized comparison of branded sodium stibogluconate and generic sodium stibogluconate for the treatment of visceral leishmaniasis under field conditions in Sudan. *Trop Med Int Health*. 2000;5:312–317.
197. Rijal S, Chappuis F, Singh R, et al. Sodium stibogluconate cardiotoxicity and safety of generics. *Trans R Soc Trop Med Hyg*. 2003;97:597–598.
198. Sundar S, Sinha PR, Agrawal NK, et al. A cluster of cases of severe cardiotoxicity among kala-azar patients treated with a high-osmolarity lot of sodium antimony gluconate. *Am J Trop Med Hyg*. 1998;59:139–143.
199. Gasser RA Jr, Magill AJ, Oster CN, et al. Pancreatitis induced by pentavalent antimonial agents during treatment of leishmaniasis. *Clin Infect Dis*. 1994;18:83–90.
200. Winship KA. Toxicity of antimony and its compounds. *Adverse Drug React Acute Poisoning Rev*. 1987;6:67–90.
201. Mueller M, Balasegaram M, Koummuki Y, et al. A comparison of liposomal amphotericin B with sodium stibogluconate for the treatment of visceral leishmaniasis in pregnancy in Sudan. *J Antimicrob Chemother*. 2006;58:811–815.
202. Bhattacharya SK, Sinha PK, Sundar S, et al. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. *J Infect Dis*. 2007;196:591–598.
203. Ostyn B, Hasker E, Dorlo TP, et al. Failure of miltefosine treatment for visceral leishmaniasis in children and men in South-East Asia. *PLoS ONE*. 2014;9:e100220.
204. Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. *Ther Clin Risk Manag*. 2007;3:733–740.
205. Dorlo TP, Rijal S, Ostyn B, et al. Failure of miltefosine in visceral leishmaniasis is associated with low drug exposure. *J Infect Dis*. 2014;210:146–153.
206. Deep DK, Singh R, Bhandari V, et al. Increased miltefosine tolerance in clinical isolates of *Leishmania donovani* is associated with reduced drug accumulation, increased infectivity and resistance to oxidative stress. *PLoS Negl Trop Dis*. 2017;11:e0005641.
207. Mondelaers A, Sanchez-Canete MP, Hendrickx S, et al. Genomic and molecular characterization of miltefosine resistance in *Leishmania infantum* strains with either natural or acquired resistance through experimental selection of intracellular amastigotes. *PLoS ONE*. 2016;11:e0154101.
208. Sundar S, Jha TK, Thakur CP, et al. Injectable paromomycin for Visceral leishmaniasis in India. *N Engl J Med*. 2007;356:2571–2581.
209. Jamil KM, Haque R, Rahman R, et al. Effectiveness study of paromomycin IM injection (PMIM) for the treatment of visceral leishmaniasis (VL) in Bangladesh. *PLoS Negl Trop Dis*. 2015;9:e0004118.
210. Musa A, Khalil E, Hailu A, et al. Sodium stibogluconate (SSG) and paromomycin combination compared to SSG for visceral leishmaniasis in East Africa: a randomised controlled trial. *PLoS Negl Trop Dis*. 2012;6:e1674.
211. Musa AM, Younis B, Fadlalla A, et al. Paromomycin for the treatment of visceral leishmaniasis in Sudan: a randomized, open-label, dose-finding study. *PLoS Negl Trop Dis*. 2010;4:e855.
212. Melaku Y, Collin SM, Keus K, et al. Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. *Am J Trop Med Hyg*. 2007;77:89–94.
213. Kimutai R, Musa AM, Njoroge S, et al. Safety and effectiveness of sodium stibogluconate and paromomycin combination for the treatment of visceral leishmaniasis in Eastern Africa: results from a pharmacovigilance programme. *Clin Drug Investig*. 2017;37:259–272.
214. Wasunna M, Njenga S, Balasegaram M, et al. Efficacy and safety of AmBisome in combination with sodium stibogluconate or miltefosine and miltefosine monotherapy for African visceral leishmaniasis: phase II randomized trial. *PLoS Negl Trop Dis*. 2016;10:e0004880.
215. Sundar S, Sinha PK, Rai M, et al. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet*. 2011;377:477–486.
216. Monge-Maillo B, Lopez-Velez R. Treatment options for visceral leishmaniasis and HIV coinfection. *AIDS Rev*. 2016;18:32–43.
217. Cota GF, de Sousa MR, Fereguetti TO, et al. Efficacy of anti-*Leishmania* therapy in visceral leishmaniasis among HIV infected patients: a systematic review with indirect comparison. *PLoS Negl Trop Dis*. 2013;7:e2195.
218. Costa S, Machado M, Cavadas C, et al. Antileishmanial activity of antiretroviral drugs combined with miltefosine. *Parasitol Res*. 2016;115:3881–3887.
219. van Griensven J, Diro E, Lopez-Velez R, et al. HIV-1 protease inhibitors for treatment of visceral leishmaniasis in HIV-co-infected individuals. *Lancet Infect Dis*. 2013;13:251–259.
220. Lopez-Velez R, Videla S, Marquez M, et al. Amphotericin B lipid complex versus no treatment in the secondary prophylaxis of visceral leishmaniasis in HIV-infected patients. *J Antimicrob Chemother*. 2004;53:540–543.
221. Ramesh V, Singh R, Avishek K, et al. Decline in clinical efficacy of oral miltefosine in treatment of post kala-azar dermal leishmaniasis (PKDL) in India. *PLoS Negl Trop Dis*. 2015;9:e0004093.

222. el Hassan AM, Ghalib HW, Zijlstra EE, et al. Post kala-azar dermal leishmaniasis in the Sudan: clinical features, pathology and treatment. *Trans R Soc Trop Med Hyg*. 1992;86:245–248.
223. Ghosh S, Das NK, Mukherjee S, et al. Inadequacy of 12-week miltefosine treatment for Indian post-kala-azar dermal leishmaniasis. *Am J Trop Med Hyg*. 2015;93:767–769.
224. Thakur CP, Narain S, Kumar N, et al. Amphotericin B is superior to sodium antimony gluconate in the treatment of Indian post-kala-azar dermal leishmaniasis. *Ann Trop Med Parasitol*. 1997;91:611–616.
225. Musa AM, Khalil EA, Mahgoub FA, et al. Efficacy of liposomal amphotericin B (AmBisome) in the treatment of persistent post-kala-azar dermal leishmaniasis (PKDL). *Ann Trop Med Parasitol*. 2005;99:563–569.
226. Basher A, Maruf S, Nath P, et al. Case report: treatment of widespread nodular post kala-azar dermal leishmaniasis with extended-dose liposomal amphotericin B in Bangladesh: a series of four cases. *Am J Trop Med Hyg*. 2017;97:1111–1115.
227. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis*. 2004;27:305–318.
228. Karimkhani C, Wanga V, Coffeng LE, et al. Global burden of cutaneous leishmaniasis: a cross-sectional analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis*. 2016;16:584–591.
229. Bailey F, Mondragon-Shem K, Hotez P, et al. A new perspective on cutaneous leishmaniasis—implications for global prevalence and burden of disease estimates. *PLoS Negl Trop Dis*. 2017;11:e0005739.
230. World Health Organization. Leishmaniasis in high-burden countries: an epidemiological update based on data reported in 2014. *Wkly Epidemiol Rec*. 2016;91:287–296.
231. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1545–1602.
232. Stahlman S, Williams VF, Taubman SB. Incident diagnoses of leishmaniasis, active and reserve components, U.S. Armed Forces, 2001-2016. *MSMR*. 2017;24:2–7.
233. Magill AJ. Cutaneous leishmaniasis in the returning traveler. *Infect Dis Clin North Am*. 2005;19:241–266, x–xi.
234. Lederman ER, Weld LH, Elyazar IR, et al. Dermatologic conditions of the ill returned traveler: an analysis from the GeoSentinel Surveillance Network. *Int J Infect Dis*. 2008;12:593–602.
235. Bienzle U, Ebert F, Dietrich M. Cutaneous leishmaniasis in Eastern Saudi Arabia. Epidemiological and clinical features in a nonimmune population living in an endemic area. *Tropenmed Parasitol*. 1978;29:188–193.
236. van Thiel PP, Leenstra T, de Vries HJ, et al. Cutaneous leishmaniasis (*Leishmania major* infection) in Dutch troops deployed in northern Afghanistan: epidemiology, clinical aspects, and treatment. *Am J Trop Med Hyg*. 2010;83:1295–1300.
237. Willard RJ, Jeffcoat AM, Benson PM, et al. Cutaneous leishmaniasis in soldiers from Fort Campbell, Kentucky returning from Operation Iraqi Freedom highlights diagnostic and therapeutic options. *J Am Acad Dermatol*. 2005;52:977–987.
238. Grimaldi G Jr, Tesh RB, McMahon-Pratt D. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. *Am J Trop Med Hyg*. 1989;41:687–725.
239. Wright NA, Davis LE, Aftergut KS, et al. Cutaneous leishmaniasis in Texas: a northern spread of endemic areas. *J Am Acad Dermatol*. 2008;58:650–652.
240. McHugh CP, Melby PC, LaFon SG. Leishmaniasis in Texas: epidemiology and clinical aspects of human cases. *Am J Trop Med Hyg*. 1996;55:547–555.
241. Clarke CF, Bradley KK, Wright JH, et al. Case report: emergence of autochthonous cutaneous leishmaniasis in northeastern Texas and southeastern Oklahoma. *Am J Trop Med Hyg*. 2013;88:157–161.
242. Maia-Elkhoury AN, Yadon E, Idali Saboya Diaz Z, et al. Exploring spatial and temporal distribution of cutaneous leishmaniasis in the Americas, 2001-2011. *PLoS Negl Trop Dis*. 2016;10:e0005086.
243. Ridley DS. The pathogenesis of cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg*. 1979;73:150–160.
244. Kumar R, Engwerda C. Vaccines to prevent leishmaniasis. *Clin Transl Immunology*. 2014;3:e13.
245. Zaph C, Uzonna J, Beverley SM, et al. Central memory T cells mediate long-term immunity to *Leishmania major* in the absence of persistent parasites. *Nat Med*. 2004;10:1104–1110.
246. Stager S, Rafati S. CD8(+) T cells in leishmania infections: friends or foes? *Front Immunol*. 2012;3:5.
247. Novais FO, Carvalho LP, Passos S, et al. Genomic profiling of human *Leishmania braziliensis* lesions identifies transcriptional modules associated with cutaneous immunopathology. *J Invest Dermatol*. 2015;135:94–101.
248. Bacellar O, Faria D, Nascimento M, et al. Interleukin 17 production among patients with American cutaneous leishmaniasis. *J Infect Dis*. 2009;200:75–78.
249. Christensen SM, Dillon LA, Carvalho LP, et al. Meta-transcriptome profiling of the human-*Leishmania braziliensis* cutaneous lesion. *PLoS Negl Trop Dis*. 2016;10:e0004992.
250. Grybchuk D, Akopyants NS, Kostygov AY, et al. Viral discovery and diversity in trypanosomatid protozoa with a focus on relatives of the human parasite *Leishmania*. *Proc Natl Acad Sci USA*. 2018;115:E506–E515.
251. Ives A, Ronet C, Prevel F, et al. Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. *Science*. 2011;331:775–778.
252. Kariyawasam R, Grewal J, Lau R, et al. Influence of *Leishmania* RNA virus 1 on proinflammatory biomarker expression in a human macrophage model of American tegumentary leishmaniasis. *J Infect Dis*. 2017;216:877–886.
253. Adaui V, Lye LF, Akopyants NS, et al. Association of the endobiont double-stranded RNA virus LRV1 with treatment failure for human leishmaniasis caused by *Leishmania braziliensis* in Peru and Bolivia. *J Infect Dis*. 2016;213:112–121.
254. Bourreau E, Ginouves M, Prevot G, et al. Presence of *Leishmania* RNA virus 1 in *Leishmania guyanensis* increases the risk of first-line treatment failure and symptomatic relapse. *J Infect Dis*. 2016;213:105–111.
255. Cota GF, de Sousa MR, Fereguetti TO, et al. The cure rate after placebo or no therapy in American cutaneous leishmaniasis: a systematic review and meta-analysis. *PLoS ONE*. 2016;11:e0149697.
256. Dowlati Y. Cutaneous leishmaniasis: clinical aspect. *Clin Dermatol*. 1996;14:425–431.
257. Reithinger R, Dujardin JC, Louzir H, et al. Cutaneous leishmaniasis. *Lancet Infect Dis*. 2007;7:581–596.
258. Convit J, Pinardi ME, Rondon AJ. Diffuse cutaneous leishmaniasis: a disease due to an immunological defect of the host. *Trans R Soc Trop Med Hyg*. 1972;66:603–610.
259. Bryceson AD. Diffuse cutaneous leishmaniasis in Ethiopia. 3. Immunological studies. IV. Pathogenesis of diffuse cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg*. 1970;64:380–393.
260. Bryceson AD. Diffuse cutaneous leishmaniasis in Ethiopia. I. The clinical and histological features of the disease. *Trans R Soc Trop Med Hyg*. 1969;63:708–737.
261. Convit J, Castellanos PL, Ulrich M, et al. Immunotherapy of localized, intermediate, and diffuse forms of American cutaneous leishmaniasis. *J Infect Dis*. 1989;160:104–115.
262. Sharifi I, Fekri AR, Aflatoonian MR, et al. Leishmaniasis recidivans among school children in Bam, South-east Iran, 1994-2006. *Int J Dermatol*. 2010;49:557–561.
263. Turetz ML, Machado PR, Ko AI, et al. Disseminated leishmaniasis: a new and emerging form of leishmaniasis observed in northeastern Brazil. *J Infect Dis*. 2002;186:1829–1834.
264. Carvalho EM, Barral A, Costa JM, et al. Clinical and immunopathological aspects of disseminated cutaneous leishmaniasis. *Acta Trop*. 1994;56:315–325.
265. Silva J, Queiroz A, Moura I, et al. Dynamics of American tegumentary leishmaniasis in a highly endemic region for *Leishmania* (*Viannia*) *braziliensis* infection in northeast Brazil. *PLoS Negl Trop Dis*. 2017;11:e0006015.
266. Scorza BM, Carvalho EM, Wilson ME. Cutaneous manifestations of human and murine leishmaniasis. *Int J Mol Sci*. 2017;18.
267. Velez ID, Jimenez A, Vasquez D, et al. Disseminated cutaneous leishmaniasis in Colombia: report of 27 cases. *Case Rep Dermatol*. 2015;7:275–286.
268. Machado PR, Rosa ME, Guimaraes LH, et al. Treatment of disseminated leishmaniasis with liposomal amphotericin B. *Clin Infect Dis*. 2015;61:945–949.
269. Queiroz A, Sousa R, Heine C, et al. Association between an emerging disseminated form of leishmaniasis and *Leishmania* (*Viannia*) *braziliensis* strain polymorphisms. *J Clin Microbiol*. 2012;50:4028–4034.
270. Golino A, Duncan JM, Zeluff B, et al. Leishmaniasis in a heart transplant patient. *J Heart Lung Transplant*. 1992;11(4 Pt 1):820–823.
271. Zijlstra EE. PKDL and other dermal lesions in HIV co-infected patients with leishmaniasis: review of clinical presentation in relation to immune responses. *PLoS Negl Trop Dis*. 2014;8:e3258.
272. Marfurt J, Nasereddin A, Niederwieser I, et al. Identification and differentiation of *Leishmania* species in clinical samples by PCR amplification of the miniexon sequence and subsequent restriction fragment length polymorphism analysis. *J Clin Microbiol*. 2003;41:3147–3153.
273. Suarez M, Valencia BM, Jara M, et al. Quantification of *Leishmania* (*Viannia*) kinetoplast DNA in ulcers of cutaneous leishmaniasis reveals inter-site and inter-sampling variability in parasite load. *PLoS Negl Trop Dis*. 2015;9:e0003936.
274. Weigle KA, de Davalos M, Heredia P, et al. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. *Am J Trop Med Hyg*. 1987;36:489–496.
275. Morizot G, Kendjo E, Mouri O, et al. Travelers with cutaneous leishmaniasis cured without systemic therapy. *Clin Infect Dis*. 2013;57:370–380.
276. Monge-Maillo B, Lopez-Velez R. Therapeutic options for Old World cutaneous leishmaniasis and New World cutaneous and mucocutaneous leishmaniasis. *Drugs*. 2013;73:1889–1920.
277. Heras-Mosteiro J, Monge-Maillo B, Pinart M, et al. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2017;(12):CD005067.
278. Gonzalez U, Pinart M, Rengifo-Pardo M, et al. Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2009;(2):CD004834.
279. Gonzalez U, Pinart M, Reveiz L, et al. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst Rev*. 2008;(4):CD005067.
280. Wolf Nassif P, Perles de Mello TF, Navasconi TR, et al. Safety and efficacy of current alternatives in the topical treatment of cutaneous leishmaniasis: a systematic review. *Parasitology*. 2017;144:995–1004.
281. Kim DH, Chung HJ, Bleys J, et al. Is paromomycin an effective and safe treatment against cutaneous leishmaniasis? A meta-analysis of 14 randomized controlled trials. *PLoS Negl Trop Dis*. 2009;3:e381.
282. Shani-Adir A, Kamil S, Rozenman D, et al. *Leishmania tropica* in northern Israel: a clinical overview of an emerging focus. *J Am Acad Dermatol*. 2005;53:810–815.
283. Ben Salah A, Ben Messaoud N, Guedri E, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. *N Engl J Med*. 2013;368:524–532.
284. Ben Salah A, Buffet PA, Morizot G, et al. WR279,396, a third generation aminoglycoside ointment for the treatment of Leishmania major cutaneous leishmaniasis: a phase 2, randomized, double blind, placebo controlled study. *PLoS Negl Trop Dis*. 2009;3:e432.
285. Soto JM, Toledo JT, Gutierrez P, et al. Treatment of cutaneous leishmaniasis with a topical antileishmanial drug (WR279396): phase 2 pilot study. *Am J Trop Med Hyg*. 2002;66:147–151.
286. Cardona-Arias JA, Velez ID, Lopez-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: a meta-analysis of controlled clinical trials. *PLoS ONE*. 2015;10:e0122569.
287. Lopez-Carvajal L, Cardona-Arias JA, Zapata-Cardona MI, et al. Efficacy of cryotherapy for the treatment of cutaneous leishmaniasis: meta-analyses of clinical trials. *BMC Infect Dis*. 2016;16:360.
288. Asilian A, Sadeghinia A, Faghihi G, et al. Comparative study of the efficacy of combined cryotherapy and intralesional meglumine antimoniate (Glucantime) vs. cryotherapy and intralesional meglumine antimoniate (Glucantime) alone for the treatment of cutaneous leishmaniasis. *Int J Dermatol*. 2004;43:281–283.
289. el Darouti MA, al Rubaie SM. Cutaneous leishmaniasis. Treatment with combined cryotherapy and intralesional stibogluconate injection. *Int J Dermatol*. 1990;29:56–59.
290. Brito NC, Rabello A, Cota GF. Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: a systematic review. *PLoS ONE*. 2017;12:e0184777.
291. Mushtaq S, Dogra D, Dogra N. Clinical response with intralesional amphotericin B in the treatment of Old World cutaneous leishmaniasis: a preliminary report. *Dermatol Ther*. 2016;29:398–405.
292. Goyonlo VM, Vosoughi E, Kiafar B, et al. Efficacy of intralesional amphotericin B for the treatment of cutaneous leishmaniasis. *Indian J Dermatol*. 2014;59:631.
293. van der Snoek EM, Robinson DJ, van Hellemond JJ, et al. A review of photodynamic therapy in cutaneous leishmaniasis. *J Eur Acad Dermatol Venereol*. 2008;22:918–922.
294. Solomon M, Schwartz E, Pavlotsky F, et al. *Leishmania tropica* in children: a retrospective study. *J Am Acad Dermatol*. 2014;71:271–277.
295. Guery R, Henry B, Martin-Blondel G, et al. Liposomal amphotericin B in travelers with cutaneous and muco-cutaneous leishmaniasis: not a panacea. *PLoS Negl Trop Dis*. 2017;11:e0006094.
296. Wortmann G, Zapor M, Ressner R, et al. Liposomal amphotericin B for treatment of cutaneous leishmaniasis. *Am J Trop Med Hyg*. 2010;83:1028–1033.
297. Mohebali M, Fotouhi A, Hooshmand B, et al. Comparison of miltefosine and meglumine antimoniate