Expert Review of Anti-infective Therapy

ISSN: 1478-7210 (Print) 1744-8336 (Online) Journal homepage: informahealthcare.com/journals/ierz20

Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis

Hiro Goto & José Angelo Lauletta Lindoso

To cite this article: Hiro Goto & José Angelo Lauletta Lindoso (2010) Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis, Expert Review of Anti-infective Therapy, 8:4, 419-433, DOI: 10.1586/eri.10.19

To link to this article: https://doi.org/10.1586/eri.10.19

Published online: 10 Jan 2014.

EXPERT REVIEWS

Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis

Expert Rev. Anti Infect. Ther. 8(4), 419–433 (2010)

Hiro Goto† and José Angelo Lauletta Lindoso

†*Author for correspondence*
Laboratório de Soroepidemiologia do Instituto de Medicina Tropical da Universidade de São Paulo, Avenida Dr Enéas de Carvalho Aguiar nº 470, Prédio II, 4º andar, CEP 05403-000, São Paulo, SP, Brazil
Tel.: +55 113 061 7023
Fax: +55 113 061 8270
hgoto@usp.br
hgoto@pq.cnpq.br

Tegumentary leishmaniasis, comprising the cutaneous and mucocutaneous forms, is caused by at least 13 dermotropic species of protozoa of the genus *Leishmania*, most of which are prevalent in the New World. Although diseases in the Old and New Worlds share similar characteristics, the ultimate manifestations and severity are quite different, with more severe forms associated with mucosal lesions observed in the New World. For the diagnosis and treatment of leishmaniasis, differences based on clinical features, usefulness/sensitivity of diagnostic methods and therapeutic responses are mainly emphasized. We present a critical review of the diagnostic methods, their contribution and the necessity for their improvement/development, particularly in molecular diagnosis aimed at detection and species identification, as well as serodiagnosis. In addition to a review of the drugs currently utilized, we describe differences in their effectiveness in Old and New World leishmaniasis. HIV/*Leishmania* coinfection is also presented in the context of diagnosis and treatment.

Keywords: amphotericin B • coinfection • ELISA • HIV • human • *Leishmania* • PCR • pentamidine • pentavalent antimonials • serodiagnosis

Leishmaniasis is a disease caused by different species of protozoa of the genus *Leishmania* that are transmitted by Phlebotomine sandflies. *Leishmania* are injected into the vertebrate host as a promastigote (the elongated form with an external flagellum), which is phagocytosed by different phagocytic cells in the host. Within cells of the mononuclear phagocyte system (its habitat), promastigotes differentiate into amastigotes (the round form without an external flagellum) and then proliferate, establishing the infection.

Leishmaniasis is considered as an emergent and re-emergent disease, and there has been a worrisome increase in its incidence, mostly in the last two decades, in certain parts of the world due to the migration of people from rural to urban areas seeking work opportunities, migration as a consequence of war, disturbances in microenvironments due to climate change and human intervention, deterioration of socioeconomic conditions, the presence of HIV/*Leishmania* coinfection, and so on [1]. Leishmaniasis is prevalent in tropical and subtropical areas, but due to the increase in international travel, it also appears to be an important disease in people living in nonendemic areas [2].

Leishmaniasis encompasses visceral and tegumentary forms, including cutaneous and mucocutaneous forms. Tegumentary leishmaniasis is prevalent in 82 countries, and its incidence is estimated to be 1.5 million cases per year. Most (90%) of the cases are reported in Africa (mainly in Morocco, Ethiopia and Tunisia), the Middle East (mainly in Afghanistan, Pakistan, Iran, Iraq, Syria and Saudi Arabia) and Latin America (mainly in Brazil, Bolivia, Colombia, Ecuador, Peru and Venezuela) [1,3].

Dermotropic strains of *Leishmania* belong to the order Kinetoplastida, family Trypanosomatidae, genus *Leishmania*, and subgenus *Leishmania* or *Viannia*, which include approximately 20 species that differ in their geographical distribution. In Asia, Africa and Europe, tegumentary leishmaniasis is caused by *Leishmania* (*Leishmania*) *major, Leishmania* (*Leishmania*) *tropica, Leishmania* (*Leishmania*) *aethiopica* and some zimodemes from *Leishmania infantum*. In the New World, mainly Latin America, the species involved are numerous and are of the subgenus *Leishmania* and *Viannia*. *Leishmania* (*Viannia*) *braziliensis* is the most prevalent species, followed by *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania* (*Viannia*)

 10.1586/ERI.10.19 ISSN 1478-7210

guyanensis, and *Leishmania* (*Viannia*) *panamensis*, although other species such as *Leishmania* (*Leishmania*) *mexicana*, *Leishmania* (*Leishmania*) *pifanoi*, *Leishmania* (*Leishmania*) *venezuelensis, Leishmania* (*Viannia*) *peruviana, Leishmania* (*Leishmania*) *shawi* and *Leishmania* (*Viannia*) *lainsoni* are present mostly in the Amazon region and in Central America. In the Old World, *Leishmania* (*Leishmania*) *donovani*, a viscerotropic species, may determine a cutaneous disease during or after visceral leishmaniasis and is known as post-kala-azar dermal leishmaniasis (PKDL) [3–5].

Diagnosis of leishmaniasis

Diagnosis of leishmaniasis is based on criteria that consider epidemiological data, clinical features and laboratory test results.

Epidemiological criteria

The epidemiological link among individuals in an endemic area is evident, but it constitutes important information for the diagnosis of travelers living in nonendemic areas who have spent some time in areas endemic for leishmaniasis. Tegumentary leishmaniasis is one of the dermatological syndromes diagnosed in travelers. In a retrospective study employing data from the GeoSentinel Surveillance Network, comprising a 10-year period from 1997 to 2006, skin-related diagnoses were reported for 4594 patients, and tegumentary leishmaniasis was diagnosed in 3.3%. The travel destination of these patients was Latin America, and in 15% the travel duration had been less than 2 weeks [6]. In another two studies carried out in Europe, some patients with cutaneous leishmaniasis had traveled to endemic countries in the Old World and others to the New World [7,8].

Clinical criteria

The clinical features of tegumentary leishmaniasis are diverse, depending on the *Leishmania* species involved and host factors, including immune status. However, the initial evolution of the lesion is similar at the site of the insect bite. After an incubation period of 2 weeks to 3 months, a small, itchy erythematous papule or nodule appears, sometimes preceded or accompanied by draining lymph node enlargement. This initial lesion may cure spontaneously or evolve, usually after some months, to patent disease with different clinical features [9,10]. The correlation of clinical features and species of *Leishmania* is currently not straightforward because, in some areas, many different species coexist and, furthermore, species identification is not usually performed for clinical specimens due to the complexity of the laboratory procedures as well as their accessibility.

In general terms, species prevalent in the Old World determine limited clinical manifestations compared with New World species. *L.* (*L.*) *major*, *L.* (*L.*) *tropica* and *L.* (*L.*) *aethiopica*, species that are prevalent in the Old World, determine the characteristic ulcerative lesion of localized cutaneous leishmaniasis (LCL) in 86–98% of cases that usually cure spontaneously after a period ranging from 3 months to 2 years [11]. In some cases, *L.* (*L.*) *tropica* infection evolves to a relapsing lesion known as leishmaniasis recidiva cutis or leishmaniasis recidivans [11], or mucosal leishmaniasis (ML) [12], and *L.* (*L.*) *aethiopica* infection to diffuse cutaneous leishmaniasis (DCL) [11]. In addition, *L.* (*L.*) *donovani* may determine PKDL [13]. The species present in the New World determine more varied clinical manifestations, including the aggressive and destructive ML. In the following sections, the features of the different forms are described, with an emphasis on manifestations caused by the New World species. We also highlight manifestations observed in HIV/*Leishmania* coinfection, which can present unusual forms. A detailed description of the clinical manifestations of Old World cutaneous leishmaniasis can be found in a recently published comprehensive review by Akilov *et al.* [11].

Localized cutaneous leishmaniasis

Localized cutaneous leishmaniasis (**Figure 1A**) is the most prevalent form of the disease and is most commonly caused by dermotropic *Leishmania* species [7]. The lesions appear on an exposed area of the body surface, varying in number from one to ten. The established lesion is a round, painless ulcer that is well delimited with a central crust that is sometimes hemorrhagic. It may cure spontaneously, leaving a hypopigmented, smooth, thin scar. Depending on the host–parasite balance and other undefined factors, some cases evolve to other forms of the disease.

Leishmaniasis recidiva cutis

Leishmaniasis recidiva cutis (**Figure 1B**) is known in the Old World to be associated with *L.* (*L.*) *tropica* infection, the occurrence of which is rare in the New World. Characteristic papular and vesicular lesions appear after clinical cure in or around the scar of the healed sore after a variable period of time from months to years. Most of the identified parasites in the New World were of the subgenus *Viannia* [14], but *L.* (*L.*) *amazonensis* in Brazil [15] and *L.* (*V.*) *panamensis* in Ecuador [16] were also observed.

Disseminated leishmaniasis

Disseminated leishmaniasis (DL) (**Figure 1C**) is characterized by the presence of multiple (10–300) pleomorphic lesions, mainly acneiform and papular, in two or more noncontiguous areas of the body [17]. In 29% of cases, a mucosal lesion is found. In an area in the northeast of Brazil, the frequency of this condition has increased from 0.2 to 1.9% among tegumentary leishmaniasis cases in two decades. In these cases, *L.* (*V.*) *braziliensis* was the only species encountered [18,19].

Diffuse cutaneous leishmaniasis

Diffuse cutaneous leishmaniasis is a true anergic form of tegumentary leishmaniasis and is characterized by the presence of nodular lesions that do not ulcerate (**Figure 1D & 1E**) [20]. It is a rare condition. It has been reported in South America, Central America and Ethiopia. The lesions are rich in parasites and the species involved are *L.* (*L.*) *mexicana,* and *L.* (*L.*) *amazonensis* in the New World and *L.* (*L.*) *aethiopica* in the Old World.

Mucocutaneous leishmaniasis

Also known as 'espundia', mucocutaneous leishmaniasis occurs years after the onset of cutaneous leishmaniasis and is characterized by the destruction of oral–nasal and pharyngeal cavities,

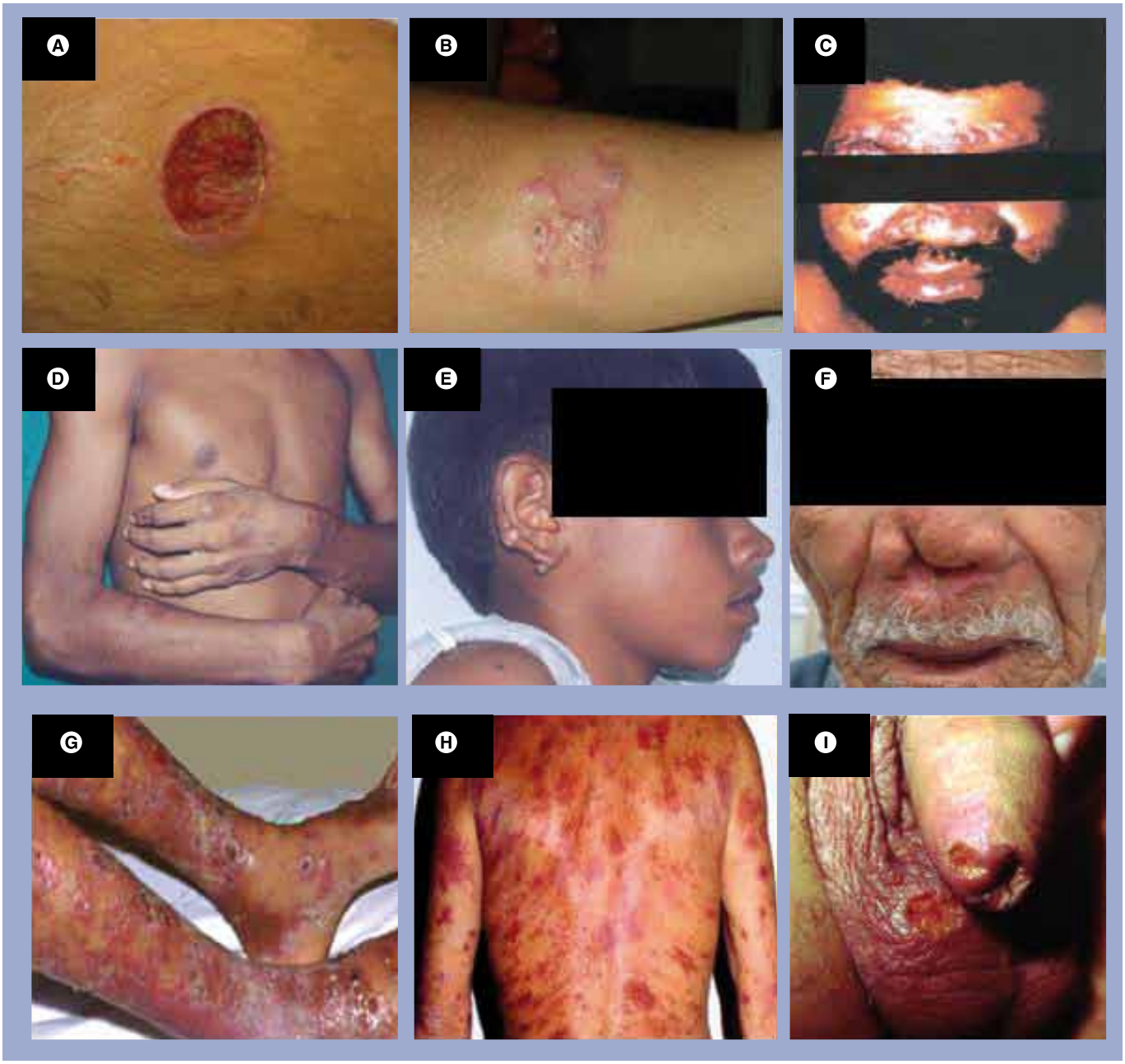

Figure 1. Clinical forms of tegumentary leishmaniasis. (A) Localized cutaneous leishmaniasis presenting single ulcer on the leg (reprinted with permission from Luiza K Oyafuso, Instituto de Infectologia Emilio Ribas de São Paulo, Brazil). **(B)** Leishmaniasis recidiva cutis presenting papules and vesicles around the healed lesion of cutaneous leishmaniasis on the leg (reprinted with permission from Jackson ML Costa, Universidade Federal do Maranhão, Brazil). **(C)** Disseminated cutaneous leishmaniasis presenting numerous ulcers on the face (reprinted with permission from Jackson ML Costa). **(D)** Diffuse cutaneous leishmaniasis presenting infiltrated nodules on the arms and thorax (reprinted with permission from Fernando T Silveira, Universidade Federal do Pará-Brazil, Brazil). **(E)** Diffuse cutaneous leishmaniasis presenting infiltrated nodules on the ear (reprinted with permission from Fernando T Silveira). **(F)** Mucosal leishmaniasis with destructive lesion in the nose (reprinted with permission from Luiza K Oyafuso). **(G)** Atypical cutaneous leishmaniasis in a HIV-infected patient presenting multiple ulcers on the legs and feet. **(H)** Atypical cutaneous leishmaniasis in HIV-infected patient presenting erythematous plaques on the back, and **(I)** atypical cutaneous leishmaniasis in a HIV-infected patient presenting ulcers on the scrotum and penis. Images G, H and I reproduced from [28] with permission from Wiley-Blackwell.

potentially evolving to disfiguring lesions (Figure 1F). The initial symptoms are mild, with nasal inflammation and stuffiness, but ulceration and perforation of the septum may slowly ensue. The lesion may extend to the face, soft palate, pharynx or larynx. The mucosal lesion may be accompanied by a cutaneous lesion. *L.* (*V.*) *braziliensis* is present in the majority of cases, but other species are also found: *L.* (*V.*) *panamensis*, *L.* (*V.*) *guyanensis*, *L.* (*L.*) *amazonensis* and *L.* (*L.*) *major* [17]. The frequency of ML

varies according to the geographical location. In Brazil, it varies from 0.4% in the south [21] to 1.4% in the central region [22], and to 2.7% in the northeast [23]. In Andean countries, ML reaches an average of 7.1% [24]; Bolivia exhibits a high frequency of 20% [25], Ecuador a medium frequency of 7.7% [26], Colombia a low frequency of 2.3% and Venezuela a very low frequency of 0.4% [24].

Tegumentary leishmaniasis in HIV-infected patients

HIV/*Leishmania* coinfection has been reported in 34 countries. In the Old World, there are reports of PKDL in HIV-infected patients [27]. In the New World, the manifestations can be similar to those found in nonimmunosuppressed patients with no signs of aggravation, but they can be quite unusual. We recently reported a series of cases of tegumentary leishmaniasis in HIV-positive patients from Brazil [28], where we found a wide variety of lesions including papules, nodules, plaques (Figure 1H) and diverse ulcerations (Figure 1G). Furthermore, we observed different forms of mucosal lesions, such as widespread, diffuse infiltration of the mucosal surface of the palate. Interestingly, genital lesions (Figure 1I) were present in 27% of patients. Of note, in HIV-infected patients, tegumentary leishmaniasis may present as immune reconstitution inflammatory syndrome with the appearance of new, disseminated lesions or worsening of pre-existing ones, associated with a recovery of $CD4^+$ T-cell counts and decreased virus loads upon antiretroviral treatment [29].

Differential diagnosis

Differential diagnosis should be made for sporotrichosis, cutaneous *Mycobacterium* infection, venous stasis or traumatic ulcers, sickle-cell anemia-related ulcers, blastomycosis, sarcoidosis, syphilis, Kaposi's sarcoma, leprosy, chromoblastomycosis, squamous cell carcinoma, basal cell carcinoma, B-cell cutaneous lymphoma, seborrheic keratosis, pyoderma gangrenosum, pyogenic skin infections including ecthyma and idiopathic midline granuloma [17,30].

Laboratory tests for diagnosis

In all cases, it is desirable to have the diagnosis of leishmaniasis confirmed by the finding of the etiological agent or its antigen or molecule in the sample obtained from the lesion. When these approaches fail, immunological tests are used to provide indirect parameters for the diagnosis.

Parasitological diagnosis

A search for amastigotes can be performed using light microscopy to directly examine the biopsy specimen, scraping or impression smears subjected to Giemsa staining. Biopsy and aspirate samples can be further cultured in blood agar base, formerly known as Novy, McNeal and Nicolle medium, overlaid by liver infusion triptose or Schneider's liquid medium, or injected into a susceptible animal such as a hamster for parasite recovery. The sensitivity of the direct examination is low, at approximately 50–70% in the Old World [31,32] and even lower, at approximately 15–30%, in the New World where chronic cases and ML are frequent [33–34]. The detection level is higher, reaching 44–58% by culturing the samples and 38–52% by injection into hamsters [34,35]. When biopsy specimens were submitted for immunohistochemistry, *Leishmania* amastigotes or antigen were detected at higher proportions; 41.4 [34] and 88.5% [33] using an immunofluorescence technique and 58.6 [34] and 64.5% [33] using an immunoperoxidase technique. Excluding direct microscopic examination, other methods require a complex laboratory structure and technical skills, as well as longer periods of time to obtain the results.

These approaches to etiological agent detection have relatively low sensitivity and different methods do not identify the species of *Leishmania*. Therefore, recent efforts are aimed at developing assays to detect the parasite DNA.

Detection of Leishmania *DNA*

A variety of molecular approaches have been developed for the diagnosis of leishmaniasis (for a comprehensive review see [36]). A molecular approach for the diagnosis of leishmaniasis, based on the detection of *Leishmania* DNA, has two goals: detection of *Leishmania,* similar to other parasitological methods; and identification of the *Leishmania* species, which is not achieved by other methods, except when cultured promastigotes are analyzed using *Leishmania* species-specific monoclonal antibodies [37] or by isoenzyme profiling [38]. Among the laboratory methods employed to detect the etiological agent or its material, in reasonably equipped laboratories, PCR is considered to be a good method for use in the diagnosis. Some of the advantages of this method when compared with other parasitological methods are: the possibility of detecting *Leishmania* DNA, even with a low parasite load; specificity; the fast availability of results; the possibility of using different biological materials; and the possibility of detecting the DNA of amastigotes and promastigotes.

Different primer sequences specific for different targets in the DNA of *Leishmania* have been used to improve parasite detection. Some are nuclear DNA such as the SSU rRNA gene [39], multilocus microsatellite DNA [40], some repetitive sequences [41], the tubulin gene [42], the *gp63* gene locus [43] and internal transcribed spacer regions [44,45]. Others are extrachromosomal DNA such as the repetitive kinetoplast DNA (kDNA) [46]. The latter is considered as an attractive target for PCR due to the abundance of minicircles in the parasite.

In laboratories where molecular diagnosis is a routine procedure for diagnosis, the most commonly addressed targets are kDNA and SSU rRNA. In studies using kDNA as the target to assess the sensitivity of the PCR, the results for the detection rate both in the Old World and New World were found to be high, with values of 100% in cutaneous leishmaniasis [47] and 97.1% in ML [48].

An important approach in the diagnosis of tegumentary leishmaniasis is the characterization of the *Leishmania* species, which is not performed in other methods used to detect the etiological agent. A considerable future challenge is to discriminate the various species present in the New World and to obtain and test samples, preferably without the need for parasite growth in culture.

For the identification of species, repetitive and polymorphic sequences are either directly targeted or discriminated using restriction enzymes for targeted products. The former approach was used to target the glucose-6-phosphate dehydrogenase gene, the products of which discriminate between different species of *Leishmania* from the New World, distinguishing *L.* (*V.*) *braziliensis* from other species of the *Viannia* subgenus [49]. Similarly, when the isomerase mannose enzyme gene (*MPI*) is targeted, it is possible to differentiate *L.* (*V.*) *braziliensis* from *L.* (*V.*) *peruviana* [50]. The latter approach was applied for certain targets, such as kDNA, internal transcribed spacer, heat-shock protein (*Hsp*)70 gene [51] and glycoprotein of molecular mass 63 gene (*GP63*), using restriction enzymes to disclose polymorphisms; the resulting restriction fragment length polymorphism (RFLP) distinguishes various *Leishmania* species [52]. PCR-RFLP with *Hsp70* is particularly interesting, since various New World species can be distinguished and the parasite samples can be obtained directly from the lesion [51].

Real time-PCR (RT-PCR) using primers specific to the *Leishmania* genus or species has been evaluated more recently for the diagnosis of leishmaniasis. It mainly aims to approach its polymorphism to identify *Leishmania* species but also to measure parasite load in the lesion [53–55]. It is a very promising method and, although it requires the appropriate laboratory structure (expensive equipment and technical skill), the results are obtained much more quickly, with less likelihood of contamination, compared with conventional PCR.

There are several targets and different methodologies used to detect *Leishmania* and to distinguish the different species; however, PCR-based protocols must be standardized and optimized for use in different centers in order to achieve comparable and reliable results. To achieve this goal, some recommendations include the use of standardized extraction protocols, internal controls, a standard *Leishmania* strain control, replicate assays and participation in an external quality control program.

Immunological test-based diagnosis

Anti-*Leishmania* delayed-type hypersensitivity, known as the Montenegro test or the Leishmanin skin test, and anti-*Leishmania* antibody assays are used as indirect parameters of *Leishmania* infection.

Montenegro or Leishmanin skin test

The Montenegro skin test reveals *Leishmania* infection, and therefore it is used in epidemiological studies to determine infection prevalence. However, the test does not distinguish between present and past infection, and thus its importance as a diagnostic tool is questionable for people living in endemic areas. The test has demonstrated positive results in patients more than 19 months after treatment [56–58], and positive results were also observed in 75% of noninfected individuals with no disease manifestation in the past, and living in an area endemic for leishmaniasis [56]. This test may be useful, however, for the diagnosis of travelers living in nonendemic areas. For the Montenegro skin test, 0.1 ml of *Leishmania* antigen (the *Leishmania* species utilized and the preparation vary in different countries and laboratories) is injected into the forearm. When the local induration is 5 mm or more after 48–72 h, the result is considered positive. Positivity is detected after 4 months of the appearance of lesions. Patients with LCL, ML and DL present with positive results. Positivity in LCL patients varies and is approximately 82–89% [56–60]. In ML [58] and DL [19] patients' positivity is usually 100%. DCL patients provide a negative test [61]. In 11% of patients with PKDL and concomitant visceral leishmaniasis, the Montenegro test is positive, and in those without concomitant visceral leishmaniasis, 37% are positive [13].

Serological diagnosis

More commonly used assays for serodiagnosis in leishmaniasis are the indirect immunofluorescence assay (IIFA) and ELISA. Serodiagnosis is not a routine procedure for the diagnosis of cutaneous leishmaniasis in the Old World due to the variable or low sensitivity of the tests and cross-reactivity with other infections [62,63]. Some studies have shown a sensitivity of 60% using ELISA [64]. However, in a recent study in Turkey, more promising results were obtained, with sensitivity reaching 88% by ELISA [65], demonstrating its potential as a complementary approach for diagnosis.

In the New World, initial studies with a large sample size from the North and Northeast of Brazil also reported a low sensitivity of 27.7% using the IIFA and 66.9% by ELISA [66] for cutaneous leishmaniasis samples. Higher sensitivities of 56.7% for IIFA and 93.3% for ELISA were obtained for mucocutaneous leishmaniasis patients [67]. More recent results demonstrate the better performance of assays, even though the sensitivity remains low depending on the antigen preparation used. Using the *L. major* total antigen that is widely available in Brazil for IIFA and is provided by Bio-Manguinhos/FIOCRUZ (Rio de Janeiro, Brazil), the sensitivity reaches 75.4%, whereas the use of 'in house' antigen preparations with *L. braziliensis* and *L. major*-like species provides a sensitivity of 81.5 and 95.4%, respectively. The performance of ELISA using antigen preparations with the latter species showed a sensitivity of 95.7 and 78.7%, respectively [68]. Although the antibody response has, overall, been considered to be gender-specific, the results may suggest species-related variation in the results. This issue was addressed in a study that utilized samples from cutaneous leishmaniasis patients infected with *L.* (*V.*) *guyanensis* and *L.*(*V.*) *braziliensis*, carefully paired according to age, gender and time of disease evolution and using *L.* (*L.*) *amazonensis* preparations as antigen. Although the sensitivity was only slightly lower for *L.* (*V.*) *guyanensis*-infected patient samples than for the *L.* (*V.*) *braziliensis* samples, showing a sensitivity by IIFA of 79.6% and 71.7%, respectively, and by ELISA of 98.2 and 85.0%, respectively, their titers were quite different. For both assays, the titers of *L.* (*V.*) *guyanensis* samples were significantly lower than those of the *L.* (*V.*) *braziliensis* samples [69]. Using another type of assay, the direct agglutination test (DAT) using lyophilized promastigotes, a test that can be performed in less-equipped laboratories, the species specificity of the reaction was

more striking. In patients infected with *L. aethiopica*, using the same species of the parasite, the sensitivity reached 90.5% and the specificity 91.8%; with non-homologous antigen, a sensitivity lower than 20% was observed [70]. This certain species specificity of the results of the serological tests may explain the low sensitivity observed in some studies using samples from areas in which many different species are prevalent, for example, the Amazon region in northern Brazil. On the other hand, this suggests that serodiagnosis can be improved.

In order to develop assays using specific species, one impediment is the culture of some species of *Leishmania,* such as *L.* (*V.*) *braziliensis,* which is difficult to grow and maintain in culture. An alternative may be the use of recombinant antigens, which, besides parasite growth-independent production, have advantages such as a more standardized and uniform production. Some of these antigens, *L. major* Hsp60 [71] and *L. braziliensis* Hsp70 [72], were cloned and the products tested using cutaneous leishmaniasis and mucocutaneous samples from Colombia with promising results. *L.* (*L.*) *infantum* Hsp83 [73] was also tested using a limited number of cutaneous and mucocutaneous samples and showed 100% reactivity, interestingly without any cross-reactivity with Chagas' disease samples. Considering those data showing a certain species specificity of the antibody reactivity, the development of assays using combined recombinant antigens should be contemplated.

In tegumentary leishmaniasis, the anti-*Leishmania* antibody level does not remain high after treatment [69], and therefore positive results generally indicate current infection. Hence, there is room for the use of immunological tests for the diagnosis of ongoing infection, and such tests deserve research and development.

Considering that HIV/*Leishmania* is becoming an important medical problem, the performance of immunological tests must be evaluated. Data from HIV/*Leishmania*-infected individuals, based on observations in the Mediterranean area, showed a relatively low sensitivity [74]. However, in coinfected patients in Brazil, the sensitivity was not low, showing 77% positivity in serology [28]. This proportion of positivity may be related to the endemicity of leishmaniasis in Brazil, and the fact that anti-*Leishmania* antibody-producing memory cells may be preserved, even in the presence of immunosuppression due to HIV infection.

Considering the high prevalence of leishmaniasis in poor areas and in developing countries, tests characterized by easy application in the field and in modestly equipped laboratories must be developed. Immunochromatography-based rapid tests and DAT are in this category. They have been used for the diagnosis of visceral leishmaniasis with variable performance [75], but it may not be possible to use the rapid tests in particular, due to the lower antibody titer in tegumentary leishmaniasis. DAT revealed species specificity-related performance [70] and therefore it may be impractical in areas where different species prevail unless reactivity to different species were to be tested at the same time.

Treatment

Drug treatment for leishmaniasis has been available since the beginning of the 20th Century, but only a few drugs have been developed for use. Although we observed differences between various *Leishmania* species, susceptibility to drugs, and disease manifestations between Old World and New World leishmaniasis, the same drugs are used for treatment. Although the WHO has provided a recommendation for the treatment of leishmaniasis, different therapeutic guidelines exist in different countries and regions of the world that suggest the complexity of the therapeutic approach in leishmaniasis. Since, in addition, we face an increase in the resistance to available drugs in some regions, a global discussion is needed to improve the use of available drugs and to further novel drug development.

Antimonials are the most commonly prescribed treatment, although other drugs have been used with varying success and other therapeutic modalities have been used as a topical treatment. *Leishmania* species, clinical presentation, extensiveness and the existence of nodular lymphangitis or comorbidities, such as HIV infection, influence the choice of therapy [76]. Other factors that influence the choice of therapy may be earlier therapeutic failures, local availability of the drug and localization of the lesions. Although nonfatal, cutaneous leishmaniasis is treated to accelerate cure, to reduce scar formation, especially at cosmetic sites, and to prevent parasite dissemination (i.e., ML) or relapse. The aim of chemotherapy is clinical healing of lesions and elimination of the parasite by destroying them or improving the host's ability to heal the lesion [77]. Below, we discuss the major drugs available to treat tegumentary leishmaniasis, the problems related to resistance and the responses of different *Leishmania* species to treatment.

First-line drugs

Pentavalent antimonials (Sb^v) are the first-line drugs used to treat tegumentary leishmaniasis caused by different species (Table 1). Unfortunately, an increase in treatment failure has been documented in several regions of the world. This drug is available as one of two formulations: meglumine antimoniate and sodium stibogluconate. The mechanism of action is not known, but the drug inhibits the activity of the glycolytic and fatty acid oxidative pathways in amastigotes [77]. The major side effects are arthralgy and myalgy, however, severe side effects related to cardiotoxocity or renal failure can occur, mainly in older patients. Use of this drug is not indicated during pregnancy. The efficacy of Sb^v varies according to the geographic region, species of *Leishmania* and clinical presentation. In the New World, the efficacy of antimonials for the treatment of cutaneous leishmaniasis has been variable. In Bolivia, treatment failure was observed in 7% of patients [78], in 16% in Brazil [79], and in 39% of patients in Colombia [80]. In the Old World, failure of this drug is approximately 13%, and this drug is considered as satisfactory for the treatment of cutaneous leishmaniasis. In ML, the cure rate ranges from 30 to 90% with antimonials, depending on the country in which the study was carried out and the dosage used [81–83].

Amphotericin B has been used to treat leishmaniasis and, in some parts of the world, it is the drug of choice. This drug acts on ergosterol present in the *Leishmania* membrane. By increasing the permeability of the cell membrane, it promotes an ion

Table 1. Recommended doses of first-line drugs to treat tegumentary leishmaniasis according to the WHO and the Ministry of Health of Brazil.

Clinical form	Pentavalent antimonial		Amphothericin B		Pentamidine	
	WHO	*Brazil*	*WHO*	*Brazil*	*WHO*	*Brazil*
Localized cutaneous leishmaniasis	20 mg/kg/day for 20 days	10–20 mg/kg/day (15 mg/kg/day) for 20 days	NA	1 mg/kg/day Total dose: 1–1.5 g	4 mg/kg/day	4mg/kg/day Maximum total dose: 2.0 g
Disseminated cutaneous leishmaniasis	20 mg/kg/day for 20 days	10–20 mg/kg/day for 20 days	NA	1 mg/kg/day Total dose: 1–1.5 g	NA	4 mg/kg/day Maximum total dose: 2.0 g
Diffuse cutaneous leishmaniasis	20 mg/kg/day for 20 days	20 mg/kg/day for 20 days	NA	1 mg/kg/day Total dose: 1–1.5 g	NA	NA
Mucosal leishmaniasis	NA	20 mg/kg/day for 30 days	NA	1 mg/kg/day Total dose: 2.5–3 g	NA	4 mg/kg/day Maximum total dose: 2.0 g

NA: Not available.

influx into the parasite, both promastigotes and amastigotes, leading to their death [84]. Four drug formulations are available: amphotericin B deoxycholate, liposomal amphotericin, cholesterol dispersion amphotericin and lipid complex amphotericin. All formulations share a similar efficacy, however, differences have been observed regarding the side effects of the formulations, with more intense side effects associated with amphotericin B deoxycolate, which can induce renal injury.

Pentamidine has been used with success to treat cutaneous leishmaniasis or ML in some regions of the New World. This drug interferes with *Leishmania* DNA synthesis, modifying the morphology of the kinetoplast, and promotes fragmentation of the mitochondrial membrane, killing the parasite. This drug was shown to present the same efficacy as antimonials [85]. Hypoglycemia and hyperglycemia are the main adverse effects of pentamidine.

Alternative drugs

Miltefosine was originally used to treat cancer. It is a phosphorylcholine ester of hexadecanol, a membrane-active alkylphospholipid. It is contraindicated in women with child-bearing potential because of its teratogenic effects in animal studies [77]. Miltefosine was effective in curing patients with visceral leishmaniasis in India; however, few studies on the treatment of tegumentary leishmaniasis have been reported. In Colombia, a cure rate of 89–100% was observed for cutaneous leishmaniasis and was dependent on the dose used [86]; however, in general, the results in the New World are poor [87].

Azoles, which were initially designed to treat fungal infection, have been used to treat tegumentary leishmaniasis [88]. Some reports demonstrate the efficacy of fluconazole, ketoconazole, and itraconazole to treat leishmaniasis. These drugs inhibit 14-α-demethylation of lanosterol to ergosterol in cell wall synthesis and promote membrane permeability of *Leishmania*. A cure rate of between 55 and 79% was observed in the Old World using these drugs.

Paromomycin acts on *Leishmania* both *in vitro* and *in vivo*. This drug has been used for parentheral and local application in the treatment of tegumentary leishmaniasis in both the New and Old Worlds [89]. A meta-analysis involving 14 randomized controlled trials including 1221 patients revealed that topical paramomycin associated with methylbenzethonium chloride was similar to intralesional pentavalent antimony in its efficacy in treating the Old World cutaneous leishmaniasis. However, the response to topical paramomycin associated with methylbenzethonium chloride was worse than parenteral pentavalent antimony in treating cutaneous leishmaniasis in the New World. Similar efficacy to Sb^V in the treatment of leishmaniasis in the New World was observed when parenteral paramomycin was used to treat New World LCL [90].

Azithromycin presents activity against *L. major in vitro* and *in vivo*, but its mechanism of action is not yet known. Reports from the New and Old World show divergent results, with cure rates of 85% [91] and 27.6% [92], respectively.

Allopurinol alone or in association with antimonials has been used to treat leishmaniasis both in the New and Old World and has presented discordant results. The drug was not effective when used alone [93]; however, in association with a low dose of antimonials, treatment achieved similar results to those obtained using full-dose antimonials [94].

Dapsone and rifampicin are also used to treat leishmaniasis. Using dapsone, a cure rate of 82% was observed in the Old World [95], but a very poor response was observed in the New World [96]. Rifampicin alone or in combination with other drugs has been used to treat leishmaniasis with divergent results, from a cure rate of 70–80% [97,98] to almost no response. The poor response is seemingly related to the parasite species *L.* (*L.*) *aethiopica* and *L.* (*V.*) *braziliensis* [99,100].

Some drugs, such as paramomycin, ketoconazole, antimonials, azithromycin and imiquimod, have been used topically to treat cutaneous leishmaniasis, mainly in the Old World. This practice is not a routine procedure in the New World, probably because of the risk of progression to mucosal involvement. More promising results were obtained when paramomycin was used in association with methylbenzethonium chloride.

Some physical modalities have been used to treat cutaneous leishmaniasis, mainly in the Old World. *Leishmania* promastigotes are thermosensitive, and heat and cold treatments have been

applied for the treatment of cutaneous leishmaniasis. Infrared heat and ultraviolet light have been used with success. Cryosurgery using CO_2 or liquid N_2 has also demonstrated success, however, the efficacy of this remains questionable. In a large-scale study, Al-Gindan *et al.* [101] obtained a cure rate of 27% [76]. Another physical treatment employed is photodynamic therapy (PDT) using porphyrin precursors as sensitizers to treat cutaneous leishmaniasis [102]. An advantage of PDT is the cosmetic result, and this treatment may prevent the development of drug resistance. Limitations of this method include the need for specific equipment, application only to cutaneous lesions and impediment to use when *Leishmania* species that develop mucosal lesions are involved. There are some reports on cutaneous leishmaniasis treatment in the Old World utilizing PDT, but few reports in the New World. Recently, we used PDT on *L.* (*L.*) *amazonensis* promastigotes *in vitro* and observed *Leishmania* death using a high concentration of the porphyrin precursor methylene blue. Furthermore, in patients with cutaneous leishmaniasis, PDT was used in association with antimonial treatment and wound healing was observed to occur in half the time of that achieved for patients receiving only antimonial injection [Lindoso JAL, Pers. Comm.]. In fact, it is necessary to evaluate the topical or physical treatment of leishmaniasis, mainly in the New World.

New targets for the development of drugs against *Leishmania* have been studied but are still in the experimental phase. Some of these drugs include the sirtuin family of NAD-dependent deacetylases [103], topoisomerase [104], protease inhibitors [105], and inhibitors of the mevalonate pathway such as terpene nerolidol [106]. In addition, the antiestrogen tamoxifen has shown activity against *Leishmania amazonensis* and *Leishmania chagasi* both *in vitro* and *in vivo* in experimental models [107,108]. Using proteomics and transcriptomic tools, some other targets have been identified as candidate drug targets for *Leishmania* [109].

Immunotherapy/immunomodulation

Immunotherapy is based on the belief that the patient has a defective immune response against the parasite, and thus is an attempt to increase the specific immune response. For this reason, immunotherapy has been introduced as an alternative therapy in specific clinical situations. *Leishmania* antigen alone or in combination with other antigens, such as BCG, has been used to treat tegumentary leishmaniasis with partial success. Convit *et al.* [110] and Mayrink *et al.* [111] revisited immunotherapy and reported the treatment of LCL and ML using vaccines based on whole promastigote preparations, obtaining cure rates ranging from 76 to 94%. Recombinant *Leishmania* antigen has been identified as a candidate for immunotherapy. Leish-111f, formulated in monophosphoryl lipid A with a squalene oil emulsion [112], was used to treat refractory ML with promising results [113]. Immunotherapy associated with antimonials could be an alternative for the treatment of leishmaniasis, including HIV–*Leishmania* coinfection, PKDL and chronic refractory tegumentary leishmaniasis [114]. Furthermore, drug toxicity and the emergence of resistance could be dramatically reduced if the present long-term monotherapy was supplemented with immunotherapy. Although this modality of treatment is increasing, clinical trials are necessary to demonstrate its benefit in some clinical situations. However, analysis of the immunopathogenesis of New World cutaneous leishmaniasis and ML has revealed that lesion development is more dependent on the immune inflammatory process. Disease manifestation and severity in all forms but DCL are due to hypersensitivity rather than immunosuppression. The observation that there is no reduction in the time for healing of the lesion with the early introduction of treatment suggests that the pathogenesis is related more to the inflammatory process rather than to the amount of the parasite in the lesion [18]. Some data show an increased production of inflammatory modulators, such as TNF-α and IFN-γ, in ML [115], which is more evident when compared with cutaneous leishmaniasis [116]. For this reason, the use of immunomodulators associated with some drugs seems appropriate and it has been tested in some patients with promising results. Pentoxifylline, an inhibitor of TNF-α, has been used in association with antimonials to treat mucosal and cutaneous leishmaniasis, and a reduction in healing time has been observed [117,118]. Another drug, imiquimod, is considered an activator of Toll-like receptor 7 and a mediator of cytokine production (IFN-α, TNF-α, IL-1 and IL-12) that may directly activate macrophages, enhancing the local immune response. Imiquimod has been used in combination with a systemic antimonial in the treatment of cutaneous leishmaniasis and presented a cure rate of 90% in patients with cutaneous leishmaniasis refractory to pentavalent antimonial treatment [119]. It has been shown that it is also more effective in the initial treatment of cutaneous leishmaniasis [120]. In a recent clinical trial in Peru, Miranda-Verastegui *et al.* showed that this combination was better than placebo plus pentavalent antimony [121].

The combination of antimonials and immunomodulators could be an alternative treatment for patients refractory to antimonial treatment. A better evaluation using new clinical trials is required to define the use of these drugs in clinical routine.

Efficacy of treatment

The identification of the factors associated with chemotherapy failure would allow better clinical management of patients. The efficacy of treatment depends on factors related to the host and to the parasite.

Host factors

The immune status of leishmaniasis patients has long been known to affect drug efficacy. This has proven to be of particular importance in relation to pentavalent antimonial treatment of DCL and HIV/*Leishmania* coinfection in the visceral form of leishmaniasis, where there is a deficiency in the specific T-cell-mediated immune response, leading to exacerbation of the infection. Using experimental models, the anti-leishmanial activities of antimonials and pentamidine have been shown to be T-cell dependent, whereas those of amphotericin B and miltefosine are T-cell independent [77]. Furthermore, we observed an increase in relapse in HIV-infected patients after treatment with antimonials [28].

Parasite factors

We have observed a clear difference between the responses of Old and New World *Leishmania* species. The Old World species *L. tropica* and *L. major,* but not *L. aethiopica,* are susceptible to both systemic and local treatment, while those from the New World are only susceptible to systemic treatment. This is due to the variety of *Leishmania* species that cause tegumentary leishmaniasis in America and in the Caribbean, with different clinical presentations. The influence of *Leishmania* species is clear in Latin America. In Peru, patients infected with *L.* (*V.*) *guyanensis* were found to be much more responsive to Sb^V therapy than patients infected with *L.* (*V.*) *braziliensis* [119]. On the other hand, in Brazil, failure of antimonial therapy was higher in patients infected with *L.* (*V.*) *guyanensis* (73.7%) than in those infected with *L.* (*V.*) *braziliensis* (49.2%) [82]. This difference could be explained by the differences between the strains of *Leishmania,* even though the species were the same. In addition, differences in the dose or treatment regimen cannot be disregarded.

The efficacy of drugs for the treatment of leishmaniasis is often a consequence of differences in the sensitivity of *Leishmania* species to the drugs, as well as the immune status of the patient or the pharmacokinetic properties of the drug.

From the data available in the literature, it is very difficult to extract conclusions regarding the best drugs or combinations of drugs for the treatment of both Old and New World leishmaniasis, owing to the differences between and the scientific accuracy of the studies. Recently, a Spanish group published a meta-analysis on interventions for tegumentary leishmaniasis in the Old [122] and New [123] Worlds, based on various databases. Although it is difficult to draw any clear conclusion, the analysis shows more frequent use and efficacy of oral and topical treatment in Old World leishmaniasis and systemic treatment, mainly antimonials alone or in combination with other drugs in the New World, confirmed by another meta-analysis performed by a Brazilian group. The authors included 12 articles that met inclusion criteria with 1150 patients, and concluded that pentavalent antimonials were the drugs most involved, with a cure rate of 76.6%. When compared with pentavalent antimonials, pentamidine had a similar cure rate; however, other drugs, such as paramomycin and imidazole, had an inferior response. It is possible to conclude that pentavalent antimonials are the drugs of choice in the treatment of cutaneous leishmaniasis and pentamidine is a good alternative drug to treat it [85]. Most important from these analyses is their conclusion that we reproduce here: 'There is a desperate need for large well-conducted studies that evaluate long-term effects of current therapies. We suggest the creation of an international platform to improve quality and standardization of future trials in order to inform clinical practice' [122,123].

Expert commentary & five-year view

Tegumentary leishmaniasis constitutes a serious public health problem in different parts of the world with a significant increase in its incidence that has mostly occurred in the last two decades. It affects areas considered endemic for leishmaniasis but it is also becoming an important disease in travelers living in nonendemic areas who have visited endemic areas. In addition, the recent finding that HIV/*Leishmania* coinfection is increasing is another concern of ours. For this challenge, healthcare systems should be prepared, and research and development improved on present and upcoming diagnostic methods, treatment procedures and novel drugs.

For appropriate diagnosis and treatment of tegumentary leishmaniasis, knowledge on disease manifestation, diagnostic approaches and their availability, and treatment options should be extended broadly to professionals in endemic and nonendemic areas.

These achievements would come from the solid and increasing knowledge of usual disease manifestations, but also of unusual manifestations that may occur in immunosuppressed individuals. Examples of these are the manifestations seen in individuals infected with HIV or in undernourished people in a war environment or refugee camp. Regarding disease manifestation, with the availability of conventional or new methods to identify *Leishmania* species from patient samples, studies correlating disease manifestation, evolution and therapeutic response to parasite species are needed. These studies will bring considerable benefits for the treatment of patients and will ensure the proper follow up of patients. In the coming 5 years, studies investigating these areas should have high priority. From these studies, new insight concerning *Leishmania* species-related pathogenic mechanisms, with consequent improvements in the treatment approaches, should hopefully appear.

A crucial point in the laboratory diagnosis is etiological agent detection and *Leishmania* species identification. In this field, molecular diagnostic procedures are more promising but are still complex and expensive procedures only feasible in places with well-structured laboratories.

For the detection of *Leishmania* in the diagnosis of leishmaniasis without species identification, approaches using material from the lesion, using anti-*Leishmania* antibodies and sensitive dye for viewing with the naked eye, would be ideal for use in the field. This is an area to focus on in coming years.

Studies on *Leishmania* species identification and correlation with clinical parameters should be sought with available methods but alternative methods feasible in more modest laboratories would be desirable. For this purpose, we may consider an indirect approach analyzing antibody production that seems to have some species specificity. Therefore, anti-*Leishmania* antibody detection should be revisited for diagnostic purposes. Furthermore, better analysis of a response that seems to be species specific and the development of approaches to test serum reactivity to different species in the same sample would improve the diagnostic potential of serological tests.

An initial approach at which to aim treatment is an accurate diagnosis without delay, but then the selection of a better drug or procedure is also important. For treatment, first-line drugs are pentavalent antimonials, amphotericin B and pentamidine. These drugs are used without considering *Leishmania* species differences, susceptibility to drugs in some regions and disease manifestations.

Old World leishmaniasis shows better therapeutic responses, except when caused by *L. aethiopica*. In the Old World, topical treatment is an alternative and products of this type should

be developed. In the New World, topical treatment should only be considered as a complementary measure owing to the risk of developing severe forms of mucosal or disseminated diseases. If this combination allows reductions of drug for systemic use it would be helpful. The development of new drugs is imperative, since those currently available are effective but have considerable side effects with inherent toxicity. In addition, inefficacy is observed in some geographical regions. Treatment guidelines and protocols have to be re-evaluated on a global basis considering the huge differences between Old and New World leishmaniasis.

Diagnosis and treatment are important questions concerning patients' healthcare. However, prevention is desirable, and in the case of tegumentary leishmaniasis, a vector-borne disease that has a silvatic mammalian reservoir, a vaccine would be almost the only choice and the dream.

Acknowledgements

The authors thank Fernando T Silveira, Jackson ML Costa and Luiza K Oyafuso for kindly providing and authorizing the publication of patients' lesion pictures.

Financial & competing interests disclosure

This work was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Pesquisa, Financiadora de Estudos e Projetos and Laboratório de Investigação Médica 38, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Tegumentary leishmaniasis, comprising the cutaneous and mucocutaneous forms, is increasing in endemic areas, but also among travelers living in nonendemic areas who visit endemic areas.
- *Leishmania* species of the Old World (three species) mostly determine localized cutaneous leishmaniasis that usually cures spontaneously. Species of the New World (at least ten species) determine varied clinical manifestations, including the aggressive and destructive mucosal leishmaniasis.
- The more widely used method for the ultimate diagnosis of leishmaniasis is the direct examination of Giemsa-stained material from the lesion using light microscopy, albeit its low sensitivity: 50–70% (Old World); 15–30% (New World).
- PCR for etiological diagnosis reaches sensitivity close to 100% using the most widely used targets, *Leishmania* kinetoplast DNA or SSU rRNA. However, standardized and optimized protocols are needed to achieve comparable and reliable results in different centers.
- Identification of the species of *Leishmania* by analyzing their polymorphisms and their correlation with disease would provide new insights into the *Leishmania* species-related pathogenic mechanisms, with consequent improvement in the care and treatment of patients.
- The Montenegro test (a delayed-type hypersensitivity test) is not indicated for diagnosis in endemic areas because it does not distinguish present and past infection, but it can be useful for the diagnosis of travelers living in nonendemic areas.
- Serodiagnosis is not a routine procedure for diagnosis due to low sensitivity in the Old World, but is routine in some centers in the New World. Some studies show better sensitivity and others show that the sensitivity is related to the *Leishmania* species specificity of the reaction. Thus, serodiagnosis deserves better evaluation, with the development of recombinant antigen-based tests.
- Drugs for the treatment of leishmaniasis are indistinctively used to treat Old and New World leishmaniasis. Old World leishmaniasis shows better therapeutic response. Treatment guidelines and protocols must be re-evaluated on a global basis considering these differences.
- The development of novel drugs is imperative, since although the available ones are effective, they cause considerable side effects.
- Tegumentary leishmaniasis in HIV-infected individuals is quite unusual in its clinical presentations and frequent relapses upon therapy are observed.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp. Immunol. Microbiol. Infect. Dis.* 27, 305–318 (2004).

2 Schwartz E, Hatz C, Blum J. New world cutaneous leishmaniasis in travelers. *Lancet Infect. Dis.* 6, 342–349 (2006).

3 Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. *Lancet* 366, 1561–1577 (2005).

4 Shaw JJ, Lainson R. Ecology and epidemiology: New World. In: *The Leishmaniases in Biology and Medicine* (*Volume I*). Peters W, Killick-Kendrick R (Eds). Academic Press, London, UK, 291–363 (1987).

5 Ashford RW, Bettini S. Ecology and epidemiology: Old World. In: *The Leishmaniases in Biology and Medicine* (*Volume I*). Peters W, Killick-Kendrick R (Eds). Academic Press, London, UK, 365–424 (1987).

6 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.* 12, 593–602 (2008).

•• **Presents data from the GeoSentinel Surveillance Network clinics around the world in the 10-year period between 1997 and 2006 that give the dimension of tegumentary leishmaniasis in travelers.**

7 Scarisbrick JJ, Chiodini PL, Watson J *et al.* Clinical features and diagnosis of 42 travellers with cutaneous leishmaniasis. *Travel Med. Infect. Dis.* 4, 14–21 (2006).

8 El Hajj L, Thellier M, Carriere J, Bricaire F, Danis M, Caumes E. Localized cutaneous

leishmaniasis imported into Paris: a review of 39 cases. *Int. J. Dermatol.* 43, 120–125 (2004).

9 Walton BC. American cutaneous and mucocutaneous leishmaniasis. In: *The Leishmaniases in Biology and Medicine* (*Volume II*). Peters W, Killick-Kendrick R (Eds). Academic Press, London, UK, 637–664 (1987).

10 Machado P, Araujo C, Da Silva AT *et al.* Failure of early treatment of cutaneous leishmaniasis in preventing the development of an ulcer. *Clin. Infect. Dis.* 34, E69–E73 (2002).

•• **Original data show that early treatment does not prevent the development of an ulcer and suggests the importance of the inflammatory process rather than the parasite load in ulcer formation.**

11 Akilov OE, Khachemoune A, Hasan T. Clinical manifestations and classification of Old World cutaneous leishmaniasis. *Int. J. Dermatol.* 46, 132–142 (2007).

• **Comprehensive and detailed description of clinical manifestations of Old World cutaneous leishmaniasis.**

12 Kharfi M, Fazaa B, Chaker E, Kamoun MR. [Mucosal localization of leishmaniasis in Tunisia: 5 cases]. *Ann. Dermatol. Venereol.* 130, 27–30 (2003).

13 Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Post-kala-azar dermal leishmaniasis. *Lancet Infect. Dis.* 3, 87–98 (2003).

• **Comprehensive review on post-kala-azar dermal leishmaniasis.**

14 Oliveira-Neto MP, Mattos M, Souza CS, Fernandes O, Pirmez C. Leishmaniasis recidiva cutis in New World cutaneous leishmaniasis. *Int. J. Dermatol.* 37, 846–849 (1998).

15 Bittencourt AL, Costa JM, Carvalho EM, Barral A. Leishmaniasis recidiva cutis in American cutaneous leishmaniasis. *Int. J. Dermatol.* 32, 802–805 (1993).

16 Calvopina M, Uezato H, Gomez EA, Korenaga M, Nonaka S, Hashiguchi Y. Leishmaniasis recidiva cutis due to *Leishmania* (*Viannia*) *panamensis* in subtropical Ecuador: isoenzymatic characterization. *Int. J. Dermatol.* 45, 116–120 (2006).

17 Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. *Lancet Infect. Dis.* 7, 581–596 (2007).

18 Turetz ML, Machado PR, Ko AI *et al.* Disseminated leishmaniasis: a new and emerging form of leishmaniasis observed in northeastern Brazil. *J. Infect. Dis.* 186, 1829–1834 (2002).

• **Parameters and characteristics of disseminated leishmaniasis are presented based on 42 cases.**

19 Costa JM, Marsden PD, Llanos-Cuentas EA *et al.* Disseminated cutaneous leishmaniasis in a field clinic in Bahia, Brazil: a report of eight cases. *J. Trop. Med. Hyg.* 89, 319–323 (1986).

20 Barral A, Costa JM, Bittencourt AL, Barral-Netto M, Carvalho EM. Polar and subpolar diffuse cutaneous leishmaniasis in Brazil: clinical and immunopathologic aspects. *Int. J. Dermatol.* 34(7), 474–479 (1995).

• **Initial description of disseminated leishmaniasis from Brazil with *Leishmania* species identification.**

21 de Castro EA, Luz E, Telles FQ *et al.* Eco-epidemiological survey of *Leishmania* (*Viannia*) *braziliensis* American cutaneous and mucocutaneous leishmaniasis in Ribeira Valley River, Parana State, Brazil. *Acta Trop.* 93, 141–149 (2005).

22 Gontijo CM, da Silva ES, de Fuccio MB *et al.* Epidemiological studies of an outbreak of cutaneous leishmaniasis in the Rio Jequitinhonha Valley, Minas Gerais, Brazil. *Acta Trop.* 81, 143–150 (2002).

23 Jones TC, Johnson WD Jr, Barretto AC *et al.* Epidemiology of American cutaneous leishmaniasis due to *Leishmania braziliensis braziliensis*. *J. Infect. Dis.* 156, 73–83 (1987).

24 Davies CR, Reithinger R, Campbell-Lendrum D, Feliciangeli D, Borges R, Rodriguez N. The epidemiology and control of leishmaniasis in Andean countries. *Cad. Saude Publica* 16, 925–950 (2000).

•• **Comprehensive review on comparative epidemiological, parasitological and clinical data on leishmaniasis in Venezuela, Colombia, Ecuador, Peru and Bolivia.**

25 Garcia AL, Parrado R, Rojas E, Delgado R, Dujardin JC, Reithinger R. Leishmaniases in Bolivia: comprehensive review and current status. *Am. J. Trop. Med. Hyg.* 80, 704–711 (2009).

26 Hashiguchi Y, Gomez Landires EA. A review of leishmaniasis in Ecuador. *Bull. Pan. Am. Health Organ.* 25, 64–76 (1991).

27 Puig L, Pradinaud R. *Leishmania* and HIV co-infection: dermatological manifestations. *Ann. Trop. Med. Parasitol.* 97(Suppl. 1), 107–114 (2003).

28 Lindoso JA, Barbosa RN, Posada-Vergara MP *et al.* Unusual manifestations of tegumentary leishmaniasis in AIDS patients from the New World. *Br. J. Dermatol.* 160, 311–318 (2009).

• **Describes unusual manifestations of tegumentary leishmaniasis in HIV-infected patients that may pose difficulties for diagnosis.**

29 Posada-Vergara MP, Lindoso JA, Tolezano JE, Pereira-Chioccola VL, Silva MV, Goto H. Tegumentary leishmaniasis as a manifestation of immune reconstitution inflammatory syndrome in 2 patients with AIDS. *J. Infect. Dis.* 192, 1819–1822 (2005).

•• **First report showing tegumentary leishmaniasis as a manifestation of immune reconstitution inflammatory syndrome in HIV-infected patients.**

30 David CV, Craft N. Cutaneous and mucocutaneous leishmaniasis. *Dermatol. Ther.* 22, 491–502 (2009).

31 Vega-Lopez F. Diagnosis of cutaneous leishmaniasis. *Curr. Opin. Infect. Dis.* 16, 97–101 (2003).

32 Al-Hucheimi SN, Sultan BA, Al-Dhalimi MA. A comparative study of the diagnosis of Old World cutaneous leishmaniasis in Iraq by polymerase chain reaction and microbiologic and histopathologic methods. *Int. J. Dermatol.* 48, 404–408 (2009).

33 Sotto MN, Yamashiro-Kanashiro EH, da Matta VL, de Brito T. Cutaneous leishmaniasis of the New World: diagnostic immunopathology and antigen pathways in skin and mucosa. *Acta Trop.* 46, 121–130 (1989).

34 Schubach A, Cuzzi-Maya T, Oliveira AV *et al.* Leishmanial antigens in the diagnosis of active lesions and ancient scars of American tegumentary leishmaniasis patients. *Mem. Inst. Oswaldo Cruz* 96, 987–996 (2001).

35 Weigle KA, de Davalos M, Heredia P, Molineros R, Saravia NG, D'Alessandro A. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. *Am. J. Trop. Med. Hyg.* 36, 489–496 (1987).

36 Reithinger R, Dujardin JC. Molecular diagnosis of leishmaniasis: current status and future applications. *J. Clin. Microbiol.* 45, 21–25 (2007).

• **Comprehensive review and perspectives on molecular techniques for diagnosis and identification of *Leishmania*.**

37 Grimaldi G, McMahon-Pratt D. Monoclonal antibodies for the identification of New World *Leishmania* species. *Mem. Inst. Oswaldo Cruz* 91, 37–42 (1996).

• **Review on the use of monoclonal antibodies for the identification of New World *Leishmania* species.**

38 Cupolillo E, Grimaldi G Jr, Momen H. Discrimination of *Leishmania* isolates using a limited set of enzymatic loci. *Ann. Trop. Med. Parasitol.* 89, 17–23 (1995).

• **Initial study that defined the isoenzyme analysis for identification of New World *Leishmania* species.**

39 van Eys GJ, Schoone GJ, Kroon NC, Ebeling SB. Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of *Leishmania* parasites. *Mol. Biochem. Parasitol.* 51, 133–142 (1992).

40 Russell R, Iribar MP, Lambson B *et al.* Intra and inter-specific microsatellite variation in the *Leishmania* subgenus *Viannia*. *Mol. Biochem. Parasitol.* 103, 71–77 (1999).

41 Piarroux R, Azaiez R, Lossi AM *et al.* Isolation and characterization of a repetitive DNA sequence from *Leishmania infantum*: development of a visceral leishmaniasis polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 49, 364–369 (1993).

42 Luis L, Ramirez A, Aguilar CM, Eresh S, Barker DC, Mendoza-Leon A. The genomic fingerprinting of the coding region of the β-tubulin gene in *Leishmania* identification. *Acta Trop.* 69, 193–204 (1998).

43 Victoir K, Banuls AL, Arevalo J *et al.* The *gp63* gene locus, a target for genetic characterization of *Leishmania* belonging to subgenus *Viannia*. *Parasitology* 117(Pt 1), 1–13 (1998).

44 Cupolillo E, Grimaldi Júnior G, Momen H, Beverley SM. Intergenic region typing (IRT): a rapid molecular approach to the characterization and evolution of *Leishmania*. *Mol. Biochem. Parasitol.* 73, 145–155 (1995).

45 Eisenberger CL, Jaffe CL. *Leishmania*: identification of Old World species using a permissively primed intergenic polymorphic-polymerase chain reaction. *Exp. Parasitol.* 91, 70–77 (1999).

46 de Bruijn MH, Barker DC. Diagnosis of New World leishmaniasis: specific detection of species of the *Leishmania braziliensis* complex by amplification of kinetoplast DNA. *Acta Trop.* 52, 45–58 (1992).

•• **Sequencing of widely used kinetoplast DNA of *Leishmania* as a target for *Leishmania* detection.**

47 Nasereddin A, Bensoussan-Hermano E, Schonian G, Baneth G, Jaffe CL. Molecular diagnosis of old world cutaneous leishmaniasis and species identification by use of a reverse line blot hybridization assay. *J. Clin. Microbiol.* 46, 2848–2855 (2008).

48 Oliveira JG, Novais FO, de Oliveira CI *et al.* Polymerase chain reaction (PCR) is highly sensitive for diagnosis of mucosal leishmaniasis. *Acta Trop.* 94, 55–59 (2005).

49 Castilho TM, Shaw JJ, Floeter-Winter LM. New PCR assay using glucose-6-phosphate dehydrogenase for identification of *Leishmania* species. *J. Clin. Microbiol.* 41, 540–546 (2003).

• **One-step PCR that is able to distinguish *Leishmania (Viannia) braziliensis* from other *Leishmania (Viannia)* species.**

50 Zhang WW, Miranda-Verastegui C, Arevalo J *et al.* Development of a genetic assay to distinguish between *Leishmania viannia* species on the basis of isoenzyme differences. *Clin. Infect. Dis.* 42, 801–809 (2006).

• **PCR that can distinguish *L. (V.) braziliensis* from *Leishmania (Viannia) peruviana*.**

51 Garcia L, Kindt A, Bermudez H *et al.* Culture-independent species typing of neotropical *Leishmania* for clinical validation of a PCR-based assay targeting heat shock protein 70 genes. *J. Clin. Microbiol.* 42, 2294–2297 (2004).

•• **PCR-restriction fragment length polymorphism able to distinguish various *Leishmania* species and *Trypanosoma* in the New World.**

52 Marfurt J, Niederwieser I, Makia ND, Beck HP, Felger I. Diagnostic genotyping of Old and New World *Leishmania* species by PCR-RFLP. *Diagn. Microbiol. Infect. Dis.* 46, 115–124 (2003).

53 de Monbrison F, Mihoubi I, Picot S. Real-time PCR assay for the identification of cutaneous *Leishmania* parasite species in Constantine region of Algeria. *Acta Trop.* 102, 79–83 (2007).

54 Antinori S, Calattini S, Piolini R *et al.* Is real-time polymerase chain reaction (PCR) more useful than a conventional PCR for the clinical management of leishmaniasis? *Am. J. Trop. Med. Hyg.* 81, 46–51 (2009).

55 Castilho TM, Camargo LM, McMahon-Pratt D, Shaw JJ, Floeter-Winter LM. A real-time polymerase chain reaction assay for the identification and quantification of American *Leishmania* species on the basis of glucose-6-phosphate dehydrogenase. *Am. J. Trop. Med. Hyg.* 78, 122–132 (2008).

56 Sassi A, Louzir H, Ben Salah A, Mokni M, Ben Osman A, Dellagi K. Leishmanin skin test lymphoproliferative responses and cytokine production after symptomatic or asymptomatic *Leishmania major* infection in Tunisia. *Clin. Exp. Immunol.* 116, 127–132 (1999).

• **Strong data showing long-lived positive Leishmanin skin test after cure and also positivity in noninfected individuals in endemic areas.**

57 Reed SG. Diagnosis of leishmaniasis. *Clin. Dermatol.* 24, 471–478 (1996).

58 Shaw JJ, Lainson R. Leishmaniasis in Brazil: X. Some observations of intradermal reactions to different trypanosomatid antigens of patients suffering from cutaneous and mucocutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 69, 323–335 (1975).

59 Luz ZM, Silva AR, Silva Fde O, Caligiorne RB, Oliveira E, Rabello A. Lesion aspirate culture for the diagnosis and isolation of *Leishmania* spp. from patients with cutaneous leishmaniasis. *Mem. Inst. Oswaldo Cruz* 104, 62–66 (2009).

60 Reis Lde C, Brito ME, Almeida EL *et al.* Clinical, epidemiological and laboratory aspects of patients with American cutaneous leishmaniasis in the State of Pernambuco. *Rev. Soc. Bras. Med. Trop.* 41, 439–443 (2008).

61 Bryceson AD. Diffuse cutaneous leishmaniasis in Ethiopia. 3. Immunological studies. IV. Pathogenesis of diffuse cutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 64, 380–393 (1970).

62 Kar K. Serodiagnosis of leishmaniasis. *Crit. Rev. Microbiol.* 21, 123–152 (1995).

• **Review on serodiagnosis in the Old World.**

63 Edrissian GH, Darabian P. A comparison of enzyme-linked immunosorbent assay and indirect fluorescent antibody test in the sero-diagnosis of cutaneous and visceral leishmaniasis in Iran. *Trans. R. Soc. Trop. Med. Hyg.* 73, 289–292 (1979).

64 el Safi SH, Evans DA. A comparison of the direct agglutination test and enzyme-linked immunosorbent assay in the sero-diagnosis of leishmaniasis in the Sudan. *Trans. R. Soc. Trop. Med. Hyg.* 83, 334–337 (1989).

65 Zeyrek FY, Korkmaz M, Ozbel Y. Serodiagnosis of anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* in Sanliurfa Province, Turkey, where ACL is highly endemic. *Clin. Vaccine Immunol.* 14, 1409–1415 (2007).

66 Guimaraes MC, Celeste BJ, Franco EL. Diagnostic performance indices for immunofluorescent tests and enzyme immunoassays of leishmaniasis sera from northern and north-eastern Brazil. *Bull. World Health Organ.* 68, 39–43 (1990).

67 Guimaraes MC, Celeste BJ, Franco EL, Cuce LC, Belda W Jr. Evaluation of serological diagnostic indices for mucocutaneous leishmaniasis: immunofluorescence tests and enzyme-linked immunoassays for IgG, IgM and IgA antibodies. *Bull. World Health Organ.* 67, 643–648 (1989).

68 Barroso-Freitas AP, Passos SR, Mouta-Confort E *et al.* Accuracy of an ELISA and indirect immunofluorescence for the laboratory diagnosis of American tegumentary leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 103, 383–389 (2009).

69 Romero GA, de la Gloria Orge Orge M, de Farias Guerra MV, Paes MG, de Oliveira Macedo V, de Carvalho EM. Antibody response in patients with cutaneous leishmaniasis infected by *Leishmania* (*Viannia*) *braziliensis* or *Leishmania* (*Viannia*) *guyanensis* in Brazil. *Acta Trop.* 93, 49–56 (2005).

•• **Data clearly demonstrating that antibody reactivity varies considerably in individuals infected with different *Leishmania* species.**

70 Hailu A. The use of direct agglutination test (DAT) in serological diagnosis of Ethiopian cutaneous leishmaniasis. *Diagn. Microbiol. Infect. Dis.* 42, 251–256 (2002).

•• **Data clearly demonstrating that antibody reactivity varies considerably with the use of homologous or heterologous *Leishmania* species as antigen for direct agglutination test assay.**

71 Rey-Ladino JA, Joshi PB, Singh B, Gupta R, Reiner NE. *Leishmania major*: molecular cloning, sequencing, and expression of the heat shock protein 60 gene reveals unique carboxy terminal peptide sequences. *Exp. Parasitol.* 85, 249–263 (1997).

72 Amorim AG, Carrington M, Miles MA, Barker DC, de Almeida ML. Identification of the C-terminal region of 70 kDa heat shock protein from *Leishmania* (*Viannia*) *braziliensis* as a target for the humoral immune response. *Cell Stress Chaperones* 1, 177–187 (1996).

73 Celeste BJ, Angel SO, Castro LG, Gidlund M, Goto H. *Leishmania infantum* heat shock protein 83 for the serodiagnosis of tegumentary leishmaniasis. *Braz. J. Med. Biol. Res.* 37, 1591–1593 (2004).

74 Alvar J, Aparicio P, Aseffa A *et al.* The relationship between leishmaniasis and AIDS: the second 10 years. *Clin. Microbiol. Rev.* 21, 334–359 (2008).

• **Comprehensive review on HIV/*Leishmania* coinfection in the Mediterranean area, including performance of diagnostic tests.**

75 Boelaert M, El-Safi S, Hailu A *et al.* Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in East Africa and the Indian subcontinent. *Trans. R. Soc. Trop. Med. Hyg.* 102, 32–40 (2008).

• **Data showing the importance of a comparative study on performance of serological tests in different geographical regions.**

76 Mahajan VK, Sharma NL. Therapeutic options for cutaneous leishmaniasis. *J. Dermatolog. Treat.* 18, 97–104 (2007).

77 Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin. Microbiol. Rev.* 19, 111–126 (2006).

78 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.* 100, 591–600 (2006).

79 Oliveira-Neto MP, Schubach A, Mattos M, Goncalves-Costa SC, Pirmez C. A low-dose antimony treatment in 159 patients with American cutaneous leishmaniasis: extensive follow-up studies (up to 10 years). *Am. J. Trop. Med. Hyg.* 57, 651–655 (1997).

80 Palacios R, Osorio LE, Grajalew LF, Ochoa MT. Treatment failure in children in a randomized clinical trial with 10 and 20 days of meglumine antimonate for cutaneous leishmaniasis due to *Leishmania viannia* species. *Am. J. Trop. Med. Hyg.* 64, 187–193 (2001).

81 Franke ED, Wignall FS, Cruz ME *et al.* Efficacy and toxicity of sodium stibogluconate for mucosal leishmaniasis. *Ann. Intern. Med.* 113, 934–940 (1990).

82 Romero GA, Lessa HA, Orge MG, Macedo VO, Marsden PD. [Treatment of mucosal leishmaniasis with aminosidine sulfate: results of two year follow-up]. *Rev. Soc. Bras. Med. Trop.* 31, 511–516 (1998).

83 Amato VS, Tuon FF, Imamura R, Abegao de Camargo R, Duarte MI, Neto VA. Mucosal leishmaniasis: description of case management approaches and analysis of risk factors for treatment failure in a cohort of 140 patients in Brazil. *J. Eur. Acad. Dermatol. Venereol.* 23, 1026–1034 (2009).

84 Ellis M, Bernsen R, Ali-Zadeh H *et al.* A safety and feasibility study comparing intermittent high dose with daily standard dose liposomal amphotericin B for persistent neutropenic fever amphotericin B: spectrum and resistance. *J. Med. Microbiol.* 49(Suppl. 1), 7–10 (2009).

85 Tuon FF, Amato VS, Graf ME *et al.* Treatment of New World cutaneous leishmaniasis – a systematic review with a meta-analysis. *Int. J. Dermatol.* 47, 109–124 (2008).

• **Meta-analysis allowing an evaluation of the efficacy of drugs used in American tegumentary leishmaniasis.**

86 Soto J, Toledo J, Gutierrez P *et al.* Treatment of American cutaneous leishmaniasis with miltefosine, an oral agent. *Clin. Infect. Dis.* 33, E57–E61 (2001).

87 Soto J, Arana BA, Toledo J *et al.* Miltefosine for new world cutaneous leishmaniasis. *Clin. Infect. Dis.* 38, 1266–1272 (2004).

88 Khatami A, Firooz A, Gorouhi F, Dowlati Y. Treatment of acute Old World cutaneous leishmaniasis: a systematic review of the randomized controlled trials. *J. Am. Acad. Dermatol.* 57, 335 e331–e329 (2007).

89 Llanos-Cuentas A, Echevarria J, Seas C *et al.* Parenteral aminosidine is not effective for Peruvian mucocutaneous leishmaniasis. *Am. J. Trop. Med. Hyg.* 76, 1128–1131 (2007).

90 Kim DH, Chung HJ, Bleys J, Ghohestani RF. Is paromomycin an effective and safe treatment against cutaneous leishmaniasis? A meta-analysis of 14 randomized controlled trials. *PLoS Negl. Trop. Dis.* 3, e381 (2009).

91 Prata A, Silva-Vergara ML, Costa L *et al.* Efficacy of azithromycin in the treatment of cutaneous leishmaniasis. *Rev. Soc. Bras. Med. Trop.* 36, 65–69 (2003).

92 Layegh P, Yazdanpanah MJ, Vosugh EM, Pezeshkpoor F, Shakeri MT, Moghiman T. Efficacy of azithromycin versus systemic meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. *Am. J. Trop. Med. Hyg.* 77, 99–101 (2007).

93 Velez I, Agudelo S, Hendrickx E *et al.* Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis. A randomized, controlled trial. *Ann. Intern. Med.* 126, 232–236 (1997).

94 Llanos-Cuentas A, Echevarria J, Cruz M *et al.* Efficacy of sodium stibogluconate alone and in combination with allopurinol for treatment of mucocutaneous leishmaniasis. *Clin. Infect. Dis.* 25, 677–684 (1997).

95 Dogra J. A double-blind study on the efficacy of oral dapsone in cutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 85, 212–213 (1991).

96 Osorio LE, Palacios R, Chica ME, Ochoa MT. Treatment of cutaneous leishmaniasis in Colombia with dapsone. *Lancet* 351, 498–499 (1998).

97 Joshi RK, Nambiar PM. Dermal leishmaniasis and rifampicin. *Int. J. Dermatol.* 28, 612–614 (1989).

98 Kochar DK, Saini G, Kochar SK *et al.* A double blind, randomised placebo controlled trial of rifampicin with omeprazole in the treatment of human cutaneous leishmaniasis. *J. Vector Borne Dis.* 43, 161–167 (2006).

99 Bygbjerg IC, Knudsen L, Kieffer M. Failure of rifampin therapy to cure cutaneous leishmaniais. *Arch. Dermatol.* 116, 988 (1980).

100 Do Valle TZ, Oliveira Neto MP, Schubach A, Lagrange PH, Da Costa SC. New World tegumentar leishmaniasis: chemotherapeutic activity of rifampicin in humans and experimental murine model. *Pathol. Biol.* (*Paris*) 43, 618–621 (1995).

101 al-Gindan Y, Kubba R, el-Hassan AM, Omer AH, Kutty MK, Saeed MB. Dissemination in cutaneous leishmaniasis. 3. Lymph node involvement. *Int. J. Dermatol.* 28, 248–254 (1989).

102 van der Snoek EM, Robinson DJ, van Hellemond JJ, Neumann HA. A review of photodynamic therapy in cutaneous leishmaniasis. *J. Eur. Acad. Dermatol. Venereol.* 22, 918–922 (2008).

103 Kaur S, Shivange AV, Roy N. Structural analysis of trypanosomal sirtuin: an insight for selective drug design. *Mol. Divers.* 14(1), 169–178 (2010).

104 Das BB, Sen N, Dasgupta SB, Ganguly A, Das R, Majumder HK. Topoisomerase research of kinetoplastid parasite *Leishmania*, with special reference to development of therapeutics. *Indian J. Med. Res.* 123, 221–232 (2006).

105 Desai PV, Patny A, Gut J *et al.* Identification of novel parasitic cysteine protease inhibitors by use of virtual screening. 2. The available chemical directory. *J. Med. Chem.* 49, 1576–1584 (2006).

106 Arruda DC, D'Alexandri FL, Katzin AM, Uliana SR. Antileishmanial activity of the terpene nerolidol. *Antimicrob. Agents Chemother.* 49, 1679–1687 (2005).

107 Miguel DC, Yokoyama-Yasunaka JK, Uliana SR. Tamoxifen is effective in the treatment of *Leishmania amazonensis* infections in mice. *PLoS Negl. Trop. Dis.* 2, e249 (2008).

108 Miguel DC, Zauli-Nascimento RC, Yokoyama-Yasunaka JK, Katz S, Barbieri CL, Uliana SR. Tamoxifen as a potential antileishmanial agent: efficacy in the treatment of *Leishmania braziliensis* and *Leishmania chagasi* infections. *J. Antimicrob. Chemother.* 63, 365–368 (2009).

109 Kumari S, Kumar A, Samant M, Singh N, Dube A. Discovery of novel vaccine candidates and drug targets against visceral leishmaniasis using proteomics and transcriptomics. *Curr. Drug Targets* 9, 938–947 (2008).

110 Convit J, Ulrich M, Zerpa O *et al.* Immunotherapy of american cutaneous leishmaniasis in Venezuela during the period 1990–99. *Trans. R. Soc. Trop. Med. Hyg.* 97, 469–472 (2003).

- **Data on immunotherapy (combination of *Leishmania* antigen and bacillus Calmette–Guérin) given to more than 10,000 Venezuelan patients.**

111 Mayrink W, Magalhaes PA, Michalick MS *et al*. Immunotherapy as a treatment of American cutaneous leishmaniasis: preliminary studies in Brazil. *Parassitologia* 34, 159–165 (1992).

- **Outcomes of immunotherapy with *Leishmania* antigen was worse than that with antimonial treatment.**

112 Coler RN, Goto Y, Bogatzki L, Raman V, Reed SG. Leish-111f, a recombinant polyprotein vaccine that protects against visceral Leishmaniasis by elicitation of $CD4^+$ T cells. *Infect. Immun.* 75, 4648–4654 (2007).

113 Badaro R, Lobo I, Munos A *et al.* Immunotherapy for drug-refractory mucosal leishmaniasis. *J. Infect. Dis.* 194, 1151–1159 (2006).

114 Musa AM, Noazin S, Khalil EA, Modabber F. Immunological stimulation for the treatment of leishmaniasis: a modality worthy of serious consideration. *Trans. R. Soc. Trop. Med. Hyg.* 104(1), 1–2 (2009).

115 Bacellar O, Lessa H, Schriefer A *et al.* Up-regulation of Th1-type responses in mucosal leishmaniasis patients. *Infect. Immun.* 70, 6734–6740 (2002).

116 Carvalho LP, Passos S, Bacellar O *et al.* Differential immune regulation of activated T cells between cutaneous and mucosal leishmaniasis as a model for pathogenesis. *Parasite Immunol.* 29, 251–258 (2007).

- **Data strongly suggest the role of hypersensitivity in the pathogenesis and the more intense reactivity in mucosal leishmaniasis than in localized cutaneous leishmaniasis (LCL).**

117 Machado PR, Lessa H, Lessa M *et al.* Oral pentoxifylline combined with pentavalent antimony: a randomized trial for mucosal leishmaniasis. *Clin. Infect. Dis.* 44, 788–793 (2007).

- **Promising results with a immunomodulatory drug in combination with antimonials reinforce the hypersensitivity in the pathogenesis of New World leishmaniasis.**

118 Sadeghian G, Nilforoushzadeh MA. Effect of combination therapy with systemic glucantime and pentoxifylline in the treatment of cutaneous leishmaniasis. *Int. J. Dermatol.* 45, 819–821 (2006).

- **Promising results with an immunomodulatory drug in LCL patients in Iran.**

119 Arevalo I, Ward B, Miller R *et al.* Successful treatment of drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator. *Clin. Infect. Dis.* 33, 1847–1851 (2001).

120 Arevalo I, Tulliano G, Quispe A *et al.* Role of imiquimod and parenteral meglumine antimoniate in the initial treatment of cutaneous leishmaniasis. *Clin. Infect. Dis.* 44, 1549–1554 (2007).

121 Miranda-Verastegui C, Tulliano G, Gyorkos TW *et al.* First-line therapy for human cutaneous leishmaniasis in Peru using the TLR7 agonist imiquimod in combination with pentavalent antimony. *PLoS Negl. Trop. Dis.* 3(7), e491 (2009).

122 Gonzalez U, Pinart M, Reveiz L, Alvar J. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst. Rev.* CD005067 (2008).

•• **Comprehensive meta-analysis showing some conclusions on drugs used in the Old World but mainly highlights the need to improve the quality and standardization of future trials.**

123 Gonzalez U, Pinart M, Rengifo-Pardo M, Macaya A, Alvar J, Tweed JA. Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst. Rev.* CD004834 (2009).

•• **Comprehensive meta-analysis showing some conclusions on drugs used in the New World but mainly highlights the need to improve the quality and standardization of future trials.**

Affiliations

• Professor Hiro Goto
Laboratório de Soroepidemiologia do Instituto de Medicina Tropical da Universidade de São Paulo, Avenida Dr. Enéas de Carvalho Aguiar nº 470, Prédio II, 4º andar, CEP 05403-000, São Paulo, SP, Brazil
Tel.: +55 11 3061 7023
Fax: +55 11 3061 8270
hgoto@usp.br; hgoto@pq.cnpq.br
and
Department of Preventive Medicine, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

• José Angelo Lauletta Lindoso
Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, Laboratory of Soroepidemiology (LIM-38), Instituto de Infectologia Emílio Ribas, Secretaria de Estado da Saúde de São Paulo, São Paulo, Brazil