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REVIEW ARTICLE

Cutaneous Leishmaniasis: Recent Developments in Diagnosis and Management

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Abstract This review focuses on recent developments in the diagnosis, treatment, management, and strategies for the prevention and control of cutaneous leishmaniasis (CL) caused by both Old and New World *Leishmania* species. CL is caused by the vector-borne protozoan parasite *Leishmania* and is transmitted via infected female sandflies. The disease is endemic in more than 98 countries and an estimated 350 million people are at risk. The overall prevalence is 12 million cases and the annual incidence is 2–2.5 million. The World Health Organization considers CL a severely neglected disease and a category 1 emerging and uncontrolled disease. The management of CL differs from region to region and is primarily based on local experience-based evidence. Most CL patients can be treated with topical treatments, but some *Leishmania* species can cause mucocutaneous involvement requiring a systemic therapeutic approach. Moreover, *Leishmania* species can vary in their sensitivity to available therapeutic options. This makes species determination critical for the choice of treatment and the clinical outcome of CL. Identification of the infecting parasite used to be laborious, but now the *Leishmania* species can be identified relatively easy with new DNA techniques that enable a more rational therapy choice. Current treatment guidelines for CL are based on poorly designed and reported trials. There is a lack of evidence for potentially beneficial treatments, a desperate need for large well-conducted studies, and standardization of future trials. Moreover, intensified research programs to improve vector control, diagnostics, and the therapeutic arsenal to contain further incidence and morbidity are needed.

Key Points

Cutaneous leishmaniasis is an emerging uncontrolled and neglected infection affecting millions yearly.

With modern molecular techniques, *Leishmania* species determination is increasingly common and critical for the choice of treatment and the clinical outcome.

Current cutaneous leishmaniasis management is for a considerable part non-evidence based; therefore, intensified research programs to improve vector control, diagnostics, and the therapeutic arsenal are needed.

1 Introduction

Leishmaniasis is caused by vector-borne protozoan parasites of the genus *Leishmania* and transmitted via infected

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female sandflies (*Phlebotomus* and *Lutzomyia*). The disease is endemic in more than 98 countries and an estimated 350 million people are at risk. The overall prevalence is 12 million cases and the annual incidence is 2–2.5 million cases. In most countries, the incidence numbers are probably underestimated because cases are not recognized and reporting is not mandatory [1].

Depending on the infecting species, an infection with *Leishmania* parasites can give rise to three clinical manifestations. The first is localized cutaneous leishmaniasis (CL) with single to multiple skin ulcers, satellite lesions, or nodular lymphangitis. The second is CL with mucosal involvement (MCL) and the third is systemic visceral leishmaniasis (VL) with involvement of internal organs, such as the liver, spleen, and bone marrow, which is lethal if not appropriately treated [2].

CL is worldwide the most prevalent clinical form of leishmaniasis, and 90 % of all CL cases occur in only seven countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria [3]. According to the Eurocentric world view, *Leishmania* parasites are divided into Old World species: *L. (L.) major*, *L. infantum*, and *L. (L.) tropica* (prevalent around the Mediterranean basin, the Middle East, the horn of Africa, and the Indian subcontinent), and New World species, such as *L. (L.) amazonensis*, *L. (L.) chagasi, L. mexicana L*, *L. (V.) naiffi*, *L. (V.) braziliensis*, and *L. (V.) guyanensis* (endemic in Middle and South America). Whereas most Old World species cause self-limiting ulcers in most cases, New World species cause a syndrome called American tegumentary leishmaniasis comprising CL plus a variety of other manifestations, such as MCL and the much rarer diffuse and disseminated cutaneous leishmaniasis (DCL) [4].

Apart from the variety of species-driven clinical manifestations, *Leishmania* species vary in sensitivity to available therapies [5]. This makes species determination critical for the clinical outcome of leishmaniasis. In contrast to many other infectious diseases, identification of the infecting *Leishmania* parasite used to be laborious. *Leishmania* parasites can now be identified relatively easy with new DNA techniques.

Two recent Cochrane reviews on the current treatment for Old and New World CL conclude that most clinical treatment trials have been designed and reported poorly, resulting in a lack of evidence for potentially beneficial treatments [6, 7]. This can in part be attributed to the lack of financial incentive for pharmaceutical companies to invest in the development of drugs for a disease that is believed to primarily affect people that lack financial resources. Moreover, drug trials for CL are challenging because the disease mainly occurs in remote areas; as a result, proper follow-up is problematic and many studies have been affected by a considerable number of loss to follow-up events. There is a desperate need for large well-conducted studies that evaluate long-term effects of current therapies, and it is suggested that an international platform should be created to improve the quality and standardization of future trials to inform clinical practice.

On World Health Day 2014, the World Health Organization (WHO) highlighted the serious and increasing threat of vector-borne diseases, including leishmaniasis, with the slogan "Small bite, big threat" [8]. As a category 1 emerging and uncontrolled disease, leishmaniasis is considered a severely neglected disease and intensified research programs to improve vector control, diagnostics, and the therapeutic arsenal to contain further incidence and morbidity are needed. In this review, we focus on recent developments in the diagnosis, treatment, prevention, and strategies for the management and control of CL caused by both Old and New World species.

We performed a literature search for articles in PubMed published between 2012/01/01 and 2014/10/31 and filtered on the mesh terms humans and cutaneous leishmaniasis or cutaneous leishmania. The following publication languages were included: English, French, Spanish, and Portuguese. The search was narrowed down by using the following items: prevention or control or therapy/narrow[filter] or diagnosis/broad[filter] or clinical trial[ptyp] or classical article[ptyp] or comparative study[ptyp] or clinical trial, phase i[ptyp] or clinical trial, phase ii[ptyp] or clinical trial, phase iii[ptyp] or clinical trial, phase iv[ptyp] or controlled clinical trial[ptyp] or evaluation studies[ptyp] or guideline[ptyp] or multicenter study[ptyp] or review[ptyp] or practice guideline[ptyp] or randomized controlled trial[ptyp] or systematic[sb] or validation studies[ptyp].

2 Laboratory Diagnosis

The diagnosis of CL is based on clinical features (supported by epidemiologic data) and laboratory testing. Numerous diagnostic methods have been described with a huge variation in diagnostic accuracy, including direct parasitologic examination (microscopy, histopathology, and parasite culture) and/or indirect testing with serology and molecular diagnostics [9]. The selection of the diagnostic test employed often depends on the available infrastructure and resources of the diagnostic facility and not on diagnostic accuracy. Here, we selected only general employed diagnostic methodologies for discussion.

2.1 Direct Microscopy, Histopathology, and Culture

Parasitologic diagnosis is still considered the gold standard in leishmaniasis diagnosis because of its high specificity. This is typically undertaken by histopathologic examination

of fixed tissue or parasite in vitro culture from material from suspected lesions. Microscopical diagnosis of CL is performed by the direct identification of amastigotes in Giemsa-stained lesion smears of biopsies, scrapings, or impression smears. Amastigotes appear as round or oval bodies, about 2–4 μm in diameter, with characteristic nuclei and kinetoplasts (Fig. 1). The material from the ulcer margin usually has the highest yield. A comparative study between widely used scraping smears and fine needle aspiration cytology found a significant difference between the two methods in favor of fine needle aspiration in the detection of amastigotes and microgranuloma, slide background, and patient comfort [10]. A simplified collection method is the press-imprint-smear (PIS). When compared with histopathology for the diagnosis of CL, PIS was positive in 85.3 % in study cases suspected of CL, and histopathology was only positive in 44 %. PIS is considered a rapid and relatively sensitive method for the diagnosis of CL [11].

Parasite culture in tubes containing Novy-MacNeal-Nicolle medium from suspected lesions is difficult, requires significant technical expertise, is prone to contamination, and is time consuming [12]. The sensitivity of culture tends to be low and highly variable [13]. Recently developed mini- and micro-culture technologies have the advantage of being less costly because of the smaller volume of culture medium required, easier to use, and more sensitive, even when the parasite burden is low [12]. A disadvantage of micro-culture is that this technology does not allow for further species determination. In a recent study, the performance of micro-culture was assessed on 273 subjects who fulfilled the criteria for CL. Sensitivity and specificity for micro-culture were 98.4 % (95 % CI = 96.1–99.1 %) and 100.0 %, respectively [14]. Additional advantages of this method are simplicity and the fact that diagnostic samples are retrieved by a needle-free method.

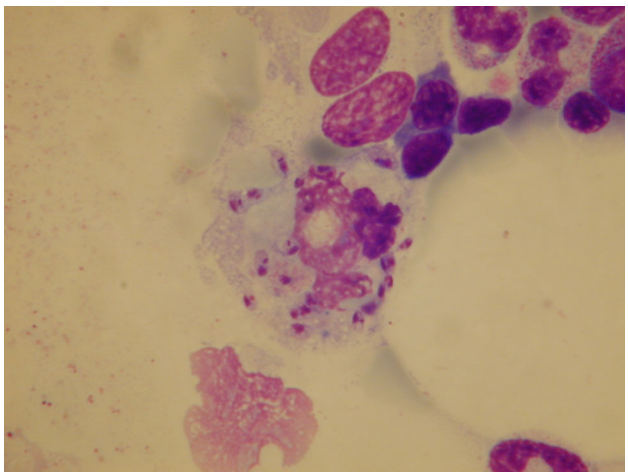

Fig. 1 Leismaniasis amastigotes. In the skin, amastigotes appear as intracellular round or oval bodies, about 2–4 μm in diameter, with characteristic nuclei and kinetoplasts

2.2 Imunologic Diagnostic Methods

Current serologic tests for CL are mainly based on formats such as indirect fluorescent antibody, enzyme-linked immunosorbent assay (ELISA), western blot, lateral flow assay, and direct agglutination test. However, these formats are not widely employed for the diagnosis of CL, because of the poor humoral response provoked by the infection and the consequential low sensitivity [9, 15].

Furthermore, most currently available serologic tests are preliminary based on either a total parasite lysate or whole promastigote yielding in aspecific reactions. Recent developments suggest that incorporation of specific purified antigen preparations or recombinant *Leishmania* antigens for serologic diagnosis would increase the operational characteristics of these tests. This is following the success of the rK39 antigen for the sero-diagnosis of VL [16]. Heat shock proteins (HSPs), and in particular HSP83, have appeared as potential candidates. An ELISA based on recombinant HSP83 has shown good performance in the diagnosis of CL, next to ML and VL, compared with an ELISA-based crude *L. major* antigen in terms of sensitivity and specificity. Furthermore, using a chemiluminescent ELISA to measure levels of anti-α-galactosyl antibodies in human sera, it was found that individuals infected with either *L. tropica* or *L. major* had significantly elevated levels (up to 9-fold higher) of anti-α-Gal IgG compared with healthy control individuals [17]. In addition, the assay had higher sensitivity than microscopy analysis. Interestingly, up to 2 years following confirmed CL cure, individuals had 28-fold higher levels of anti-α-Gal IgG compared with healthy volunteers. Monitoring levels of anti-α-Gal antibodies is proposed as both a diagnostic tool and as a biomarker of a cure of Old World CL [17].

An innovative diagnostic test that is currently under evaluation is the CL Detect™ Rapid Test, which is a qualitative membrane-based immunoassay for the detection of all clinically relevant species of the genus *Leishmania* that cause CL in skin samples. The test is currently being evaluated (http://clinicaltrials.gov/show/NCT01865032).

2.3 Leishmania Skin Test

The Leishmania intradermal skin test (LST) or Montenegro skin test (MST) is a marker of cellular immune response and occasionally used in CL diagnosis (e.g., in epidemiologic surveys and vaccine studies) because of its simple use and because of its high sensitivity of 86.4 % up to 100 % [18]. Delayed-type hypersensitivity skin reactions to LST ≥5 mm are considered positive and <5 mm

are considered negative. Patients with negative LST and diagnostic confirmation by other tests are more prone to relapse or treatment failure [18, 19]. The main disadvantages of the LST or MST are that it requires culture facilities to produce the MST antigen, that different antigen preparations impact test sensitivity, and that the test does not distinguish between past and present infections [20].

There is evidence that when LST data are supported with information on the production of antigen-specific interferon-γ (IFN-γ), this could better assist in determining whether a suspected case has been exposed to a *Leishmania* infection [21]. In contrast, it is reported that the LST is significantly more sensitive than IFN-γ levels in persons who have been cured of CL [22].

2.4 Nucleic Acid Amplification Tests

2.4.1 Polymerase Chain Reaction (PCR)

Many molecular diagnostic tests have been developed for the diagnosis of CL, as these are assumed to have better sensitivity and specificity than traditional diagnostic methods and allow the use of less invasive sampling for diagnosis [23, 24]. In particular PCR, either as a single test or in a nested format or as a quantitative assay, has been widely exploited. Numerous tests targeting many different gene sequences have been developed over the last decades, with the ribosomal DNA internal transcribed spacer 1 sequence [25–27], or sequences within the kinetoplast DNA of *Leishmania* genus as the main targets [28, 29]. Next to this, several other PCR-like assays, such as a high-tech fluorescence resonance energy transfer based on a real-time assay [23, 30], or assays based on HSP70 or tryparedoxin peroxidase gene targets [31, 32] amongst many others, are under evaluation.

As there are no defined general accepted protocols and almost each laboratory applies its own in-house method, a head-to-head comparison of the different PCR methods needs to be undertaken. In particular, studies addressing inter-laboratory comparisons are scarce and the initiative by Cruz and co-workers [24] who proposed a protocol for inter-laboratory comparisons of conventional and real-time PCR methods should be taken on.

2.4.2 Isothermal Platforms

PCR requires adequate infrastructure and technically skilled operators, making tests based on this platform less suitable for resource-restricted laboratories in disease-endemic countries. In an attempt to partly circumvent these requirements, isothermal diagnostic platforms have been developed in recent years. Nucleic acid sequence-based amplification, an isothermal reaction targeting parasite RNA, has been developed for leishmaniasis [33]. Oligochromatography for post-amplification analysis further circumvents the use of complex equipment while preserving appropriate diagnostic performance characteristics [21, 34, 35].

A further development in isothermal molecular diagnostics is loop-mediated isothermal reaction (LAMP), which is performed at 60 and 65 °C, uses only one enzyme (*Bst* DNA polymerase) for amplification, and is able to produce large amounts of DNA within 30–60 min. Importantly, the specificity of the reaction is high because it uses six primers and the end product can be visualized directly using simple detection methods [36].

The initial LAMP test for CL was a generic reverse transcriptase (RT-)LAMP, targeting the conserved region of the 18S ribosomal RNA gene. Amplification was visualized by the pre-amplification addition of fluorescent detection reagent and a simple ultraviolet lamp. By using a reverse-transcriptase step, the system detected infections between 10 and 100 parasites per mL and the sensitivity of RT-LAMP for CL patients was 98 % [36]. Different research groups further developed this technology for various applications [37–39].

The application of LAMP on boiled swab samples is an interesting advance to develop a simple and rapid (point-of-care) diagnostic method for CL [40, 41]. This approach has the potential advantages of using LAMP as a molecular diagnostic test in endemic regions where medical resources are limited.

2.4.3 Sampling for Molecular Biology

Non-invasive sampling for *Leishmania* detection is essential for quick and affordable diagnosis. However, a significant variation in clinical accuracy of molecular diagnostic methods for CL is commonly observed, depending on the sample source, the method of DNA recovery, and the molecular test, and only a few attempts have been made to compare these variables [42]. Adams and co-workers evaluated two swab and aspirate samples from lesions of patients with suspected CL alongside standard diagnosis by microscopic detection of amastigotes or culture of parasites from lesion material. Swab sampling, which is painless and simple to perform, combined with Qiagen® DNA extraction proved the most efficient, sensitive, and specific recovery method for *Leishmania* DNA [42].

Flinders Technology Associates (FTA®) cards (Whatman filter paper cards) have also been successfully used to collect samples, including DNA specimens for PCR analysis. This method is minimally invasive for patients, easy to handle for medical personnel, and can easily be transported for analysis [43, 44].

Recently, other body samples, such as conjunctival swabs, are being considered as a source for CL tests but will require further research [45, 46].

2.5 Species Determination

Under a light microscope, all *Leishmania* species are morphologically undistinguishable from each other, yet species or strain identification is very relevant for patient management (see next paragraph) [5, 47]. For several decades, isoenzyme analysis of *Leishmania* has been used for strain typing and this has allowed the construction of phylogenetic classifications [48], and even the differentiation between antroponotic and zoonotic variants within a single species [49]. This methodology is based on variation in the electrophoretic mobility of enzymes isolated from *Leishmania* parasites. Strains are consigned to various zymodemes. This highly specialized method is performed in a few reference laboratories only, because it is costly, time consuming, and requires large quantities of cultured promastigotes [50]. Therefore, alternative typing methods are being developed, in particular, based on genetic characteristics of the parasite. PCR-based methods in combination with restriction fragment length polymorphism analysis or sequencing enable correct species discrimination. Over the last decade, several gene targets have been identified for this purpose. A widely employed target is the mini-exon gene, which is involved in the trans-splicing process of nuclear messenger RNA, and is present as 100–200 tandem repeated copies per nuclear genome. Each repeat consists of three major parts that make the mini-exon an excellent genotyping marker [51]. Marfurt and coworkers [51, 52] have developed a widely used typing technology comprising a PCR assay amplifying all the mini-exon sequences in a single reaction using universal primers, allowing preliminary discrimination between the major complexes (i.e., Old World *Leishmania*, New World *Leishmania*, and New World *Viannia* complexes) as a result of the variability in sizes of the amplification products. The mini-exon PCR-RFLP (Restriction Fragment Length Polymorphism) genotyping scheme was validated with cultured WHO reference strains of *Leishmania* and cultured isolates from patients [51, 52]. This methodology is now widely employed as a high-resolution, sensitive, and specific tool that can identify all clinically relevant *Leishmania* species [5, 53–55].

Alternative candidate genes for typing are HSP70, hexokinase, and phosphoglucomutase genes for several Old World species [50] and HSP70 gene regions for New World species [56, 57]. HSP70-based species identification tools are potentially globally applicable in different clinical and sampling contexts, and they could become the reference method for identification of *Leishmania* species in clinical specimens [58].

3 Species-Based Clinical Management

In many low-resource settings, CL is diagnosed without laboratory confirmation tests, and a probable CL case has to be identified based solely on the patient history and physical examination. Risk factors for CL to be addressed during history taking are: young age, professions involving farming, hunting, military, and mining activities (Fig. 2), denying the use of bed nets, exposure in rural, sub-urban, or deforested sites (Fig. 3), presence of pets and cattle, travel (immunologically naïve cases entering a CL-endemic area such as tourists and labor migrants are more often affected), and the season of exposure (rainy seasons and El Niño are associated with CL) [59–63]. Physical signs suspected for CL are crustaceous ulcerative lesions on unexposed areas such as the extremities and face, the presence of satellite lesions (Fig. 4), and/or lymphangitis (Fig. 5). In the case of MCL, the involvement of mucosal tissue in the ear, nose, and throat tract should be excluded.

A more definite diagnosis to base further clinical management on can be made with non-specific diagnostic tests (e.g., light microscopy). These tests do not allow for species identification and *Leishmania* species vary in their sensitivity to available drugs [64]. If one species is dominating an endemic geographic region, the preferred treatment can be based on local (trial- and error-based) experience. However, geographically driven treatment is

Fig. 2 Gold mining in West Suriname. A gold digger (garimpeiros) at work in a highly leishmaniasis-endemic area, Benzdorp, district Sipaliwini, in Suriname. Miners are a well-known risk group for cutaneous leishmaniasis. Collection Ramdas (2010)

Fig. 3 Deforestation in Godo-olo, district Sipaliwini (Suriname). Human interference can cause leishmaniasis outbreaks owing to the disruption of the natural reservoir-vector transmission cycle of *Leismania* parasites and the introduction of an immune naïve labor force in the deforested area. Collection Ramdas (2009)

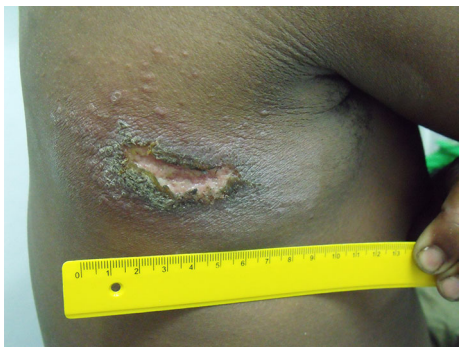

Fig. 4 Satellite lesions in cutaneous leishmaniasis. Local dissemination of *Leishmania* parasites from the primary lesion into the surrounding skin can give rise to satellite lesions. Collection Dr. Hu, Dermatological Service, Paramaribo, Suriname

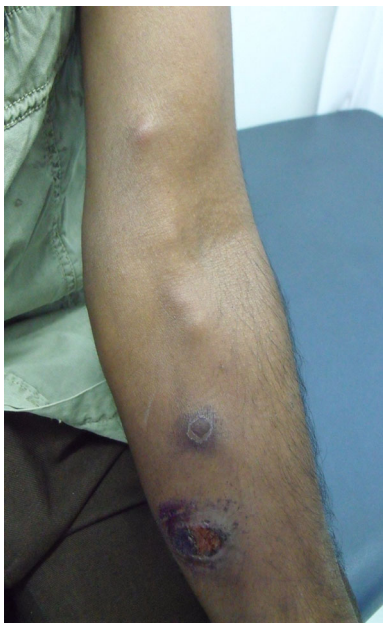

Fig. 5 Lymphangitis in cutaneous leishmaniasis. Loco-regional dissemination of *Leishmania* parasites via lymphatics can cause lymphangitis (also known as sporotrichoid dissemination, after the similar clinical picture seen in deep fungal infections). Collection Dr. Hu, Dermatological Service, Paramaribo, Suriname

inadequate in many endemic regions where multiple *Leishmania* species prevail, of which each require a different therapy. For example, in many settings in Middle and South America, species causing both CL and MCL are endemic in the same regions [65–67]. Because leishmaniasis manifestations require different management (see next paragraph), it is important to know which species is involved for the best treatment outcome, with the least side effects and late complications [68, 69].

For appropriate and effective clinical management, it is important that cheap and reliable species-specific diagnostic tests become available, especially in cases where treatment failure arises. In low-resource settings where healthcare infrastructure is sparse, rapid diagnostics are also critical to deliver timely treatment (preferably at the first consultation), thus preventing loss to follow-up due to required return visits. CL species-specific driven management is also important in non-endemic settings where travelers with CL are seen, especially if different endemic areas have been visited and the causative species is unclear [5, 61].

Species determination is also important for clinical trials because the outcome is affected by the species infecting the participants. A Cochrane review on the treatment of New World CL found that 4 out of 38 trials failed to mention the causative parasite [70]. Six trials mentioned the endemic nature of the parasite in the area and therefore assumed that the specific parasite strain was the species causing the development of the disease. Another two trials accepted that the parasites species were the same as in previous studies, and only 26 studies confirmed the causative species.

4 Treatment

In the majority of cases, CL is a self-healing disease. Nonetheless, nodular lymphangitis and MCL can lead to disabling and atrocious tissue destruction. Full recovery can take months to years, and this period can be characterized by function impairment, susceptibility to secondary infection, and the development of disfiguring permanent scars. Because little evidence-based data are available, most therapy options have to rely on expert opinions [5]. In many settings where species identification is unavailable,

the mode of therapy is mainly based on local expertise only. Most of the currently available therapeutic options are associated with significant toxicity and side effects. Therefore, a risk-benefit assessment must be made by an experienced clinician for each CL patient, and, in mild and indolent cases, a wait-and-see policy can sometimes be the best advisable option. Moreover, drug resistance is an emerging problem in the control of CL [64].

Several treatment options for CL are available. Pentavalent antimonals (sodium stibogluconate, Pentostam® or meglumine antimoniate, Glucantime®) remain the first-choice treatment for CL in most countries. Alternative treatment regimens include miltefosine, pentamidine isethionate, amphotericin B, antifungal agents (e.g., ketoconazole, fluconazole, itraconazol), paromomycine, granulocyte macrophage colony-stimulating factor, and heat therapy or cryotherapy [6, 70].

4.1 Treatment of Old World CL

In cases of a few (less than five) lesions, local therapy is preferred [5]. Local treatment combining intralesional antimony and cryotherapy proved more effective than antimony or cryotherapy alone, although as monotherapies, both also show high cure rates [71–73]. Heat therapy has also proven to be effective but requires special equipment [74, 75]. Systemic treatment can be considered for multiple lesions, disfiguring facial lesions, or lesions at sites that make topical treatment less desirable. Systemic (oral) miltefosine treatment is a promising option for patients with multiple or complicated Old World CL (*L. major*) lesions [76, 77].

4.2 Treatment of New World CL

Like for Old World CL, local treatment is also a good option in the case of a limited number of lesions caused by New World strains that do not cause MCL like *L. naiffi*, *L. chagasi*, and *L. mexicana* [78, 79]. When systemic treatment is needed for CL caused by *L. mexicana,* antimony is preferred because this strain shows resistance to miltefosine in in vitro and in vivo studies [80, 81]. *L. panamensis* or *L. amazonensis* rarely cause MCL, suggesting that there is no need for systemic treatment in closely monitored cases [82]. Therefore, in uncomplicated CL cases caused by the latter strains, combination local therapy of antimony and cryotherapy is advised instead of systemic therapy [5]. For single uncomplicated lesions caused by *L. guyanensis*, local therapy with antimony and cryotherapy can be considered, although MCL due to *L. guyanensis* is not as rare as formerly thought [83]. Systemic pentamidine is the treatment of choice for *L. guyanensis* lesions in Suriname and Guyana, but recent evidence from Manaus, Brazil, shows efficacy of only about 50–60 % [84]. As a general advice in the case of New World CL, extensive follow-up to exclude treatment failure including MCL manifestations is advisable [85, 86].

Because of the considerable risk of MCL, local therapy is not recommended for infections caused by *L. braziliensis*. Nevertheless, the dogma that *L. braziliensis* infection has to be treated systemically has been debated [70, 82]. Systemic antimony is considered the gold standard treatment for *L. braziliensis* infections. Miltefosine shows comparable results, although treatment success shows geographic variations, possibly related to differences in parasite strains [87–89]. Amphotericin B treatment shows at least equivalent results as antimony treatment but is considered an alternative treatment because of considerably more serious side effects and related costs [90–92].

Because no evidence of specific treatment is available, the proposed treatment of MCL caused by *L. panamensis, L.amazonensis*, and *L. guyanensis* is as for MCL due to *L. braziliensis* [5]. Two small studies on the combination of antimony and pentoxifylline showed high cure rates of MCL and can be considered in the case of treatment failures in patients previously treated with a single modality [93, 94].

5 Prevention

Prevention is better than cure, both for the patient as well as the community at large. It is therefore an important tool in the control of CL. Primary prevention can be achieved by identifying risk groups and tackling known risk factors to prevent sandfly bites, such as farming, hunting, military, and mining activities without the protection of insecticides and clothing [59, 95].

In CL-endemic areas, it is also important to minimize the risk of sandfly bites during night rest. Because sandflies are very small, bed nets with a maze three times smaller as compared with bed nets for the prevention of malaria are required. Moreover, bed nets should be impregnated with permethrin or another effective insect repellent to further reduce the chance of sandfly bites.

Intervention programs focused on the natural reservoir of *Leishmania* have also been tried but with mixed efficiency. One study on vaccinating dogs with a prophylactic vaccine found a significant reduction in the amount of *Leishmania* cases in humans [95]. Impregnated dog collars and the treatment of dogs with insecticide drops have also shown a significant reduction in leishmaniasis disease burden. In contrast, in a large intervention campaign performed between 1988 till 1996 in Brazil, 150,000 *Leishmania* seropositive dogs were culled and over 1 million houses sprayed with insecticide, in an attempt to reduce the

transmission of VL [96]. Disappointingly, no evidence could be shown on the mortality and morbidity. All in all, sound evidence on the effectiveness of interventions targeted at reservoir-to-human transmission is lacking.

Education is in many cases a very cost-effective preventive measure [62]. Informing populations in CL-endemic areas leads to a better uptake of preventive measures, lower risk behavior, and earlier help-seeking behavior, diagnosis, and treatment. A cost-effectiveness study conducted in Argentina estimated the gain of combined prevention strategies for CL-endemic areas, including the implementation of insecticide-impregnated clothing and curtains plus early CL diagnosis training programs for healthcare workers [97]. With the insecticide intervention only, 220.71 disability-adjusted life-years (DALYs) per 100,000 inhabitants could be prevented at a cost of US$13,155.52 per DALY. The training program would not lower the prevalence of CL, but could prevent 170.63 DALYs per 100,000 inhabitants at a cost of only US$156.46 per DALY averted. Both strategies would cost less than three times the gross domestic product per capita and can thus be considered cost efficient according to the criteria of the WHO.

5.1 Future Preventive Measures, Vaccination

Like many parasitic infections, CL has the ability to induce little immune stimulation through the continuous variation of antigenic epitopes and immunosuppressive mechanisms [98]. These immune-evasive characteristics impose a serious challenge on the development of an effective CL vaccine [99]. A naturally recovered CL induces life-long immunity against the species that caused the primary infection. This suggests that vaccination should somehow be possible and this has led to significant research efforts in this field. However, to date there are no human vaccines available for use in vaccination programs.

6 Conclusions

CL is emerging as, and threatens to become an uncontrollable disease. Most CL patients live in low- to middle-income countries where governments are faced with limited healthcare budgets and a large burden caused by other ailments such as malaria, tuberculosis, and HIV. As a result, little research is dedicated to the diagnosis, management, and control of CL.

Current CL treatment guidelines are based on poorly designed and ill-reported trials. *Leishmania* species can now be identified relatively easily with modern molecular techniques enabling a more rational therapy choice. However, there is a lack of evidence for potentially beneficial treatments, and a desperate need for large well-conducted studies and the standardization of future trials. Moreover, pharmaceutical companies invest too little in the development of new treatment modalities for CL because they are afraid their expenses will not be returned by sufficient income in the future. Novel less toxic treatment modalities are required. Especially for children, therapeutic modalities that can be administered without painful injections are required. Intensified research programs to improve vector control and diagnostics, and provide efficient and safe vaccines to contain further incidence and morbidity of CL are urgently needed.

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