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REVIEW ARTICLE

Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments

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Abstract

This review is an update of an earlier narrative review published in 2015 on developments in the clinical management of cutaneous leishmaniasis (CL) including diagnosis, treatment, prevention and control measurements. CL is a vector-borne infection caused by the protozoan parasite *Leishmania*. The vector is the female sandfly. Globally, CL affects 12 million cases and annually 2 million new cases occur. CL is endemic in almost 100 countries and the total risk population is approximately 350 million people. WHO lists CL an emerging and uncontrolled disease and a neglected tropical disease. Local experience-based evidence remains the mainstay for the management of CL. Whereas intralesional therapeutic options are the first treatment option for most CL patients, those with mucocutaneous and disseminated involvement require a systemic therapeutic approach. Moreover, different *Leishmania* species can vary in their treatment outcomes. Therefore, species determination is critical for optimal CL clinical management. New DNA techniques allow for relatively easy *Leishmania* species determination, yet they are not easily implemented in resource-limited settings. There is a desperate need for novel, less toxic, and less painful treatment options, especially for children with CL. Yet, the large and well conducted studies required to provide the necessary evidence are lacking. To further control and potentially eliminate CL, we urgently need to improve vector control, and diagnostics, and we require efficient and safe vaccines. Alas, since CL primarily affects poor people, biotechnical companies dedicate little investment into the research programs that could lead to diagnostic, pharmaceutical, and vaccine innovations.

Key Points

WHO acknowledges cutaneous leishmaniasis is an emerging, uncontrolled, and neglected infection affecting millions yearly.

Leishmania species determination based on molecular diagnostics is key in the clinical management of cutaneous leishmaniasis, but is unavailable in many low- and middle-income endemic settings.

The required evidence for novel, less toxic, and less painful cutaneous leishmaniasis management is currently lacking.

To contain and ultimately eliminate cutaneous leishmaniasis, we need comprehensive research programs including vector control, diagnostics, and vaccines.

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1 Introduction

Leishmaniasis is a vector-borne infection caused by the protozoan parasite of the genus *Leishmania*. The vectors are female sandflies (*Phlebotomus* and *Lutzomyia*). The World Health Organization (WHO) has designated leishmaniasis a neglected tropical disease (NTD), thus emphasizing its considerable impact not only on health, but on societies at large with a high economic burden [1]. Leishmaniasis is endemic in almost 100 countries and the estimated total risk population is approximately 350 million people. Each year, an estimated 2 million new cases occur and the overall prevalence is 12 million cases. In most countries, the incidence numbers are probably underestimated since cases are not recognized and reporting is not mandatory [2, 3]. This is the case in many sub-Saharan African countries, for example, where burden estimations are lacking, and as a result essential control measures cannot be effected [4].

Depending on the *Leishmania* species, the disease can cause three main clinical manifestations: (1) localized cutaneous leishmaniasis (CL) characterized by cutaneous ulcers, sometimes accompanied by satellite lesions and/or nodular lymphangitis; (2) muco-cutaneous leishmaniasis (MCL) involving mucosa, and underlying connective tissues such as cartilage structures in combination with CL disease; and (3) visceral leishmaniasis (VL) affecting internal organs, like liver, spleen, and bone marrow. VL can be lethal, just like MCL, albeit the latter less frequently [5]. American tegumentary leishmaniasis (ATL) can be considered as a fourth syndrome caused by New World *Leishmania* species, containing CL and MCL manifestations for the most part, but also much rarer forms such as diffuse and disseminated CL [6].

With 600,000 to 1 million new cases annually worldwide, CL is the most prevalent clinical leishmanial manifestation. Moreover, only eight countries contribute to 90% of cases: Afghanistan, Algeria, Brazil, Iran, Pakistan, Peru, Saudi Arabia, and Syria [7]. Conflicts, such as in Syria recently, caused CL outbreaks due to healthcare disruption, and potential human to human transmission due to massive overcrowding [8]. Outbreaks occurred not only in the actual war zones, but also among refugees sheltered in safe countries such as Turkey, Jordan, and Lebanon [9].

Based on the European world view, *Leishmania* parasites are divided into two dominant groups: (1) Old World species found in the Mediterranean basin, the Middle East, the horn of Africa and the Indian subcontinent, such as *L. (L.) major, L. infantum,* and *L. (L.) tropica*; and (2) the New World species that consists of species found in Middle and South America, such as *L. (L.) amazonensis*, *L. (L.) chagasi*, *L. mexicana L*, *L. (Viannia.) naiffi*, *L. (V.) braziliensis*, and *L. (V.) guyanensis*. Old World species predominantly cause self-limiting ulcers, whereas New World species can be severely destructive and even cause death, mostly in relation to MCL disease.

Not all *Leishmania* species are susceptible to the currently available array of therapeutic options [10]. Therefore, species determination is key for the clinical outcome of patients with CL or MCL caused by an unknown species. This is often the case in ill returning travelers (e.g., backpackers) who visited multiple leishmaniasis endemic regions where a range of species with varying susceptibility patterns reside. Based on the successful molecular *Leishmania* species determination, novel parasite species-driven disease manifestations have been unveiled in different regions of the world [11]. For example, *L. donovani* complex, generally associated with VL, is now also associated with a CL disease presentation [12]. Overlap of VL and CL clinical presentations were described earlier for *L. infantum* [13].

The two recently updated Cochrane reviews on the treatment for Old [14], and New World CL [15] once again demonstrate that the sparse number of clinical trials available are often poorly designed and/or reported upon. As a result, we lack the evidence for potentially beneficial treatments. In part, this can be explained by the absence of pharmaceutical industry interest in the development of novel antileishmanial therapeutics, since it is widely believed that the return on investment will be risky, as the disease mainly affects people in low- and middle-income countries that lack financial resources. Conducting drug trials for CL can be challenging because CL is mainly endemic in remote areas. As a result, patient follow-up is often difficult, and many studies are severely affected by a large amount of loss to follow up data. To inform clinical practice, we urgently need large trials that can produce the necessary data on the long-term effects of current and promising experimental therapies. The establishment of an international platform has been suggested to improve the quality and standardization of future trials.

With the slogan "Small bite, big threat", WHO launched its initiative in 2014 to fight the emerging threat of vector-borne diseases, including leishmaniasis [16]. WHO considers leishmaniasis an emerging, uncontrolled, and severely neglected disease. Containment of further incidence and morbidity, and more research programs for the improvement of vector control, novel diagnostics, and the extension of the current therapeutic arsenal are required. Here, we will focus on current developments in the diagnosis, treatment, prevention, and strategies for the management and control of CL caused by Old and New World species.

We performed a literature search for articles in PubMed published between 01 January 2015 (end date of our previous search [17]) and 22 February 2022 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 epidemiological data) and laboratory testing. Numerous diagnostic methods have been described with a huge variation in diagnostic accuracy, including direct parasitological examination (microscopy, histopathology, and parasite culture), and/or indirect testing with serology and molecular diagnostics [18]. The selection of the diagnostic test employed often depends on the available infrastructure and resources of the diagnostic facility, and not so much on diagnostic accuracy. Here we selected generally employed diagnostic methodologies only, for discussion.

2.1 Direct Microscopy, Histopathology, and Culture

Direct parasitological diagnosis is still considered the gold standard in leishmaniasis diagnosis because of its high specificity, although there are concerns about its sensitivity [18]. Furthermore, this strategy requires expert health staff to collect the diagnostic specimen and to perform the parasitological tests. Furthermore, direct diagnosis can be time consuming (hours to weeks) depending on the methodology used and laboratory structure [19]. Direct parasitological diagnosis can be performed by histopathological analysis of formalin embedded tissue, or in vitro parasite culture from specimens from suspected lesions. With the help of light microscopy, amastigotes can be detected directly in lesional smears of biopsies, scrapings, or impression smears stained by Giemsa's method. Amastigotes are identified as round or oval bodies 2–4 µm in diameter, with typical nuclei and kinetoplasts (Fig. 1). With the aid of immunohistochemical staining (e.g., CD1a antibodies), amastigotes can be detected more easily [18]. Specimens from the ulcer base usually result in the highest yield. To detect amastigotes and microgranuloma, fine needle aspiration (FNA) cytology is superior to scraping smears, with more patient comfort [20]. A simplified collection method is the Press–Imprint–Smear (PIS). When compared with histopathology, 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 [21].

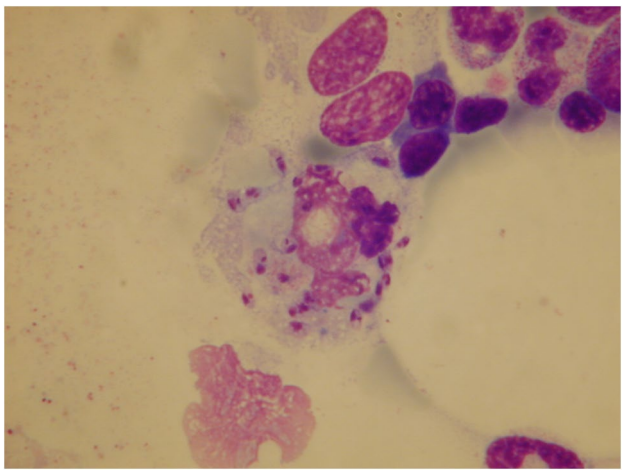

Fig. 1 Leishmaniasis amastigotes. Amastigotes appear in skin biopsies as round or oval structures with characteristic nuclei and kinetoplasts, about 2–4 µm in diameter

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 [22]. The sensitivity of culture tends to be low and highly variable [23]. Simplicity and (needle) pain-free sampling are additional advantages of culture. Mini-culture and micro-culture technologies are less costly since they require less culture medium; they also provide easier application and higher sensitivity, even in samples with a low parasite yield [22]. Yet, micro-culture does not allow additional species determination, which as described above is often required to prescribe proper treatment.

2.2 Indirect Serological Diagnostic Methods

Indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), western blot, and lateral flow assay are the mainstay formats used as serological CL tests. However, serology is not used widely to diagnose CL, since the humoral response provoked by the infection is poor and the sensitivity thus low [18, 24, 25]. In fact, recent guidelines do not recommend the use of serology for the diagnosis of CL.

Yet, there are still attempts made to improve the serodiagnosis of CL. A relative successful approach seems to be the use of crude antigens derived from amastigotes of local *Leishmania* strains [26]. In contrast, a systematic review by Zanetti et al. revealed that more specific (recombinant) antigens yielded better diagnosis results, that is, sensitivity (93.8–100%) and specificity (82.5–100%) for

ATL, compared with crude soluble antigen-based ELISAs [27]. More than 70 different antigens have been evaluated as ELISA diagnostic markers for (M)CL [28]. For example, an ELISA based on a recombinant conserved *Leishmania* hypothetical protein (which showed a high amino acid sequence homology between CL- as well as VL-causing *Leishmania* species) was found to be more specific and sensitive in detecting (M)CL patients than an ELISA based on *L. braziliensis* soluble antigens [29].

Given its complexity and infrastructural requirements, alternatives for ELISA and IFA are sought in the form of point of care (PoC) or rapid diagnostic tests (RDTs). Several RDTs have been developed for VL, mainly based on (variants of) a 39 amino acid sequence repeat of a kinesin-related protein of *L. donovani,* with relatively good diagnostic performance, except for East Africa [30]. In contrast, only few have been developed for CL. The CL Detect™ Rapid Test (a membrane-based qualitative immunoassay using polyclonal antibodies against amastigote peroxidoxin) is the most prominent example used for the detection of all clinically relevant *Leishmania* species that cause CL in skin samples. This RDT has been evaluated in Tunisia, Morocco [31], Afghanistan [32], Suriname [33], and Ethiopia [33], with variable diagnostic accuracy rates. The CL Detect™ Rapid Test was initially developed to detect *L. major* (which often comes at a relative high parasite density) peroxidoxin antigen. As a result, the discrepant lower sensitivities found in several studies (Afghanistan, Suriname, and Ethiopia) could either be caused by differences in non-*L. major* species involved, that may be less abundant, or have a lower expression of the peroxidoxin antigen, and/or produce a different variant of the antigen [34].

2.3 Leishmania Skin Test

The Leishmania intradermal skin test (LST) or Montenegro skin test (MST) is a marker of cellular immune response (a delay-type hypersensitivity response) and can be used for an indirect CL diagnosis because of its simple use and because of its high predictive value, being positive in more than 90% of CL cases with good sensitivity and specificity values [35, 36]. In addition, the LST can be useful in epidemiological studies to monitor exposure and immunity to *Leishmania* as well as in vaccine studies as a surrogate marker of immunity [37]. The test, comparable to the well-known Mantoux tuberculosis test, is based on the intradermal injection of *Leishmania* extracts (leishmanin) and results in a cutaneous reaction. If the skin reaction to LST is $\geq$ 5 mm, the test is considered positive, and if $<$ 5 mm, negative. Otherwise diagnosed CL patients with a negative LST have a predictive value for relapse or treatment failure, probably because of an inadequate T-cell mediated response [35, 38].

The LST has been available for over a century, but despite its clinical value, its use has greatly declined in the last decade due to unavailability of standardised antigen. As yet, there is no production under good manufacturing practices conditions. The immune response against *Leishmania* is cell mediated, and depends on interferon (IFN)-γ induced activation of macrophages. Potentially, leishmanin can be used in a yet to be developed in vitro IFN-γ release assay (IGRA) with similar prognostic value as the LST test, but with the advantages of improved standardization and patient comfort [37].

2.4 Nucleic Acid Amplification Tests

2.4.1 Polymerase Chain Reaction (PCR)

Molecular approaches, and in particular PCR, have revolutionized CL diagnostics since they have superior test characteristics (both in sensitivity and specificity) in comparison with the previously mentioned traditional diagnostic methods. Moreover, molecular tests can be used in combination with less invasive sampling [39]. The main PCR sequence targets used for diagnostic assays in the recent past are the ribosomal DNA internal transcribed spacer 1 sequence [40–42], or sequences within the kinetoplast DNA (kDNA) of *Leishmania* genus [43, 44]. Head-to-head comparisons of different PCR tests are lacking, and there are no defined general accepted protocols, since almost every laboratory applies its own in-house method [39]. However, a recent systematic review and meta-analysis clearly confirmed that PCR is the most accurate diagnostic approach for CL, but the number of studies (n = 13) that could be included for this review was limited [45]. An important observation from this study was that simple smear samples are sufficient to get a reliable PCR result, whereas older non-molecular techniques would require more invasive sampling methods such as needle aspirates or skin biopsies. Furthermore, it is noteworthy that quantified PCR (qPCR) is suggested to monitor treatment responses in CL patients and may even be used to predict treatment failures [46].

2.4.2 Isothermal Platforms

A disadvantage of PCR diagnostics is the requirement for advanced laboratory infrastructure and technicians. This makes PCR-based platforms in general less suitable for disease endemic countries, that often have to rely on resource-restricted laboratories. In an attempt to simplify PCR requirements, isothermal diagnostic platforms have been recently developed and evaluated. Where PCR platforms require strictly controlled and varying temperature cycles, isothermal tests can be performed under a fixed temperature.

Loop-mediated isothermal reaction (LAMP) is an example of an isothermal molecular method that is performed

at 60 °C 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. LAMP specificity is high because it uses six primers and the end product can be visualized directly using simple detection methods, such as naked eye reading (turbidity) and lateral flow strips (Fig. 2) [47, 48]. LAMP is therefore widely adopted in CL diagnostics.

The initial reverse transcriptase (RT-)LAMP test for CL targeted the conserved region of the 18S ribosomal RNA (rRNA) gene. The pre-amplification addition of a fluorescent detection reagent and a simple UV lamp visualized the amplification when the target was present in the samples of patients with lesions containing between 10 and 100 parasites per mL with 98% sensitivity [47]. This platform was further developed into a LAMP test detecting in principal all CL- and VL-causing *Leishmania* species based on primers specific for the 18S ribosomal DNA and the conserved region of mini circle kinetoplast DNA (kDNA). RT-LAMP was marketed through a collaboration between Eiken Company (Japan) and the Foundation for Innovative New Diagnostics (Switzerland) [49]. Many research groups have now taken this platform or modified versions using different targets, like cysteine proteinase b gene, to further it for various applications [50–52]. A systematic review and meta-analysis on LAMP assays reported a sensitivity ranging from 83 to 99% and specificity from 31 to 94% for LAMP, compared with microscopy, and a sensitivity ranging from 80 to 99% and specificity from 91 to 98% compared with various PCR platforms [48]. The application of LAMP on swab samples opens up the possibility to develop a simple and rapid PoC molecular diagnostic method for CL in resource-limited settings [53, 54].

Recombinase polymerase amplification (RPA) is a second isothermal technology that has the potential for application in resource-limited settings, because of its low infrastructural demands. The RPA process employs three essential enzymes: a recombinase, a single-stranded DNA-binding protein, and strand-displacing polymerase. The RPA reaction can be performed at 37–42 °C in, for example, a water bath or heat block. An interesting feature of the RPA reaction is that it can detect RNA as well as DNA, without the need for a separate step to produce cDNA [55]. RPA uses standard PCR detection methods, such as real-time fluorescent detection, gel electrophoresis, and lateral flow strip detection. RPA assays have now also been developed for CL, but a systematic review of its diagnostic performance is not yet available. Most RPA for CL exploit kDNA as a target, often in combination with a lateral flow (LF) stick read-out [56–58]. It is also possible to design your own primers and probes by using software from the RPA manufacturer and using the TriTryp database [59, 60]. So far, observed diagnostic sensitivity and specificity of employed RPA for CL are slightly lower compared with (q)PCR or LAMP [58, 60]. However, a recent evaluation of an RPA lateral flow test for CL in a clinic in the Peruvian Amazon basin found a good 91.2% sensitivity (confidence interval [CI] 86.5–94.4), and a 93% (CI 88.6–95.8) positive predictive value compared with kDNA-PCR. It highly surpassed microscopy as a diagnostic method, strongly suggesting the possibility to enhance the performance of RPA [57].

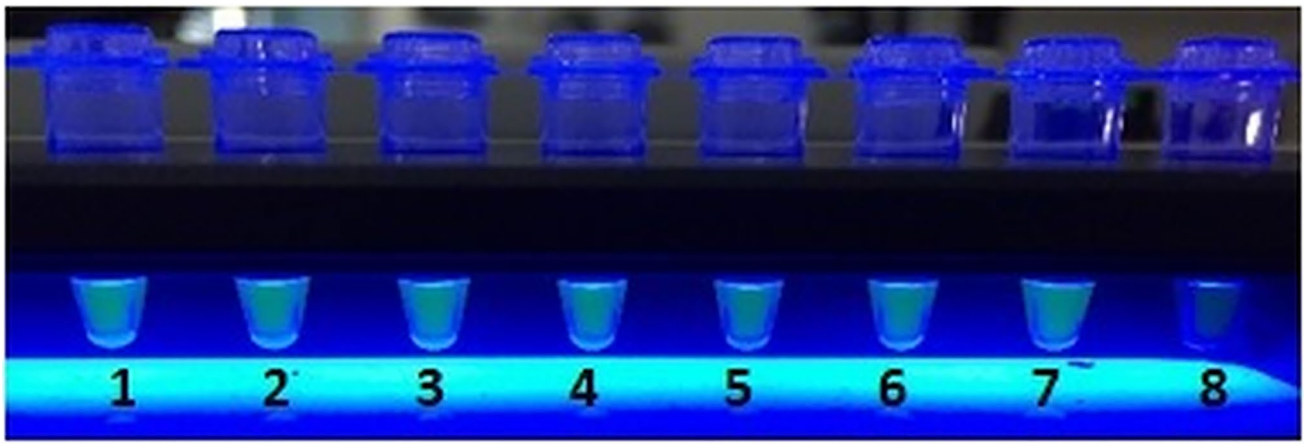

Fig. 2 Loop-mediated isothermal reaction (LAMP) molecular diagnostics. Many molecular diagnostic techniques require adequate infrastructure and technically skilled operators, making these tests less suitable for resource-restricted laboratories in disease endemic countries. LAMP is an isothermal diagnostic platform that partly circumvents these requirements. The picture shows visualization of LAMP results under UV light. Positive samples (1–7) show turbidity, whereas the negative sample (8) has remained transparent

2.4.3 Sampling for Molecular Biology

Rapid and affordable CL tests will have to rely on non-invasive sampling. To further the evaluation of the clinical accuracy of the most optimal molecular platform, unification of the sample source, the method of DNA recovery, and the type of molecular test is required [61]. Adams and co-workers found that a lesional swab processed with Qiagen® DNA extraction was the most efficient, sensitive, and specific recovery method for *Leishmania* DNA, when compared with aspirate samples, which were also more painful and more complicated to obtain [61]. This approach has now been further validated in several other studies that support the use of cotton swabs for the non-invasive collection of diagnostic material for subsequent PCR analysis [62–65]. Although rubbing over the lesion with cotton swabs potentially yields a low parasitic load, several studies have indicated that the choice of the subsequent nucleic acid extraction and amplification method influences the results obtained with cotton swab collection [61, 63]. Moreover, the minimally invasive cotton swab collection method could be useful for children or in cases where the cosmetic outcome is of importance, such as in patients with facial lesions [65].

To collect specimens for PCR tests, FTA cards (Whatman filter paper cards) have also been successfully used. Apart from being minimally invasive for patients, FTA cards are easy to handle for medical personnel and easily transported under dry conditions, whereas for cotton swabs, a storage buffer might be needed for transportation [66, 67]. Several studies have recently confirmed good results with the FTA card sampling method [68, 69].

Another attractive non-invasive DNA sampling method is the use of tape strips to obtain tissue from the surface of ulcerative or closed skin lesions of CL patients. DNA recovered from infected skin was about 10 times higher than that obtained from healthy skin, and molecular analysis by PCR after conventional DNA isolation with a kit resulted in 100% sensitivity [70].

2.5 Species Determination

Leishmania species cannot be distinguished from each other by light microscopy. Thus, other strain identification methods have been developed [10, 71]. The first attempts for strain identification were performed with isoenzyme analysis (or multi locus enzyme electrophoreses, MLEE) and led to the construction of phylogenetic classifications [72]. MLEE was even capable of the differentiation of anthroponotic variants from zoonotic variants within a single species [73]. The MLEE technique uses the variation in the electrophoretic mobility of Leishmanial enzymes, and strains are classified along so-called zymodemes. Currently, MLEE is only performed in a few reference laboratories, because it requires large amounts of cultured promastigotes, making it a costly and time consuming procedure [74].

Another culture-dependent method is the use of monoclonal antibodies to identify *Leishmania* species. The method depends on parasite isolation and subsequent culturing of promastigotes. The antibodies are mostly used for specification of New World *Leishmania* species and to a much lesser extend the Old World species [75].

Given the technical difficulties with large-scale culturing of parasites, alternative typing methods are being developed, and in particular based on genetic characteristics of the parasite. Molecular methods offer robust species typing. Van der Auwera and DuJardin have provided a state of the art overview of the currently deployed and available *Leishmania* species typing assays, with a focus on methods that are applicable globally rather than validated locally [76]. Furthermore, this review outlines the consensus taxonomy for the genus *Leishmania*, including debatable species attributions.

Nowadays, species discrimination methods use PCR (to amplify the DNA required for typing) in combination with restriction fragment length polymorphism (RFLP) analysis or sequencing. Currently, the mini-exon gene, crucial in the trans-splicing process of nuclear mRNA, is an excellent genotyping marker that is present as 100–200 tandem repeated copies per nuclear genome. Each mini-exon repeat consists of three major parts [77]. As a result of the variability in sizes of the amplification products, preliminary discrimination between the major complexes (i.e., Old World *Leishmania*, New World *Leishmania*, and New World *Viannia* complexes) is possible [77, 78]. WHO reference strains of *Leishmania* and cultured isolates from patients were used to validate the mini-exon PCR-RFLP genotyping scheme [77, 78]. The mini-exon method is now widely accepted as a high resolution, sensitive, and specific tool that can help in the clinical management of CL [10, 79–81].

Alternative gene targets for typing have been identified and are extensively reviewed by Van der Auwera and Dujardin, such as cytochrome B (encoded on the kDNA maxi circles), glycoprotein 63, cysteine proteinase B, Heat Shock Protein 70 and glucose-6-phosphate dehydrogenase [76]. The choice of target and method will strongly depend on the species and question (epidemiology, treatment etc.) addressed.

3 Species-Based Clinical Management

Most CL patients are identified as probable cases, solely based on patient history and physical examination, since they are found in endemic regions lacking the resources of laboratory confirmation tests. Apart from a typical clinical presentation, a probable case can be identified based on a

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

number of findings such as younger age, typical professions such as mining, farming, military and hunting, not using bed nets, residing in deforested, rural, sub-urbane sites (Fig. 3), living close to pets and cattle, a history of travelling such as tourism or labor migration (where immunologically naïve cases enter a CL endemic area), and the season of exposure (rainy seasons and El Niño are associated with CL) [82–86]. Typical CL cutaneous lesions are ulcers covered with hemorrhagic crusts on unexposed skin of the extremities and face, accompanied by satellite lesions and/or lymphangitis (Fig. 4). In MCL, the ear nose and throat tract, especially the mucosal tissue and underlying structures such as cartilage bones are affected.

Direct diagnostic tests (such as light microscopy) can help to establish a more definite diagnosis and aid clinical management. As discussed above, microscopy does not allow for species determination, and as such cannot guide the choice of therapy [87]. In many endemic regions, one species dominates the CL epidemic, and first-line treatment can be based on (trial and error-based) local experience. The situation can be more complicated when multiple *Leishmania* species prevail in the same region, each requiring different therapeutic options. This is the case throughout Middle and South America where the geographical spread of species causing both CL and MCL overlap in large areas [88–90]. CL and MCL require a different clinical approach (see next paragraph), thus the identification of the responsible species is key for the choice of treatment and follow-up [91, 92].

Cheap, reliable, easy to perform, and rapid species-specific diagnostic tests are urgently needed to guide clinical management and prevent unnecessary treatment failure and late complications. PoC near patient rapid diagnostics are especially helpful in low resource endemic settings since they allow for timely treatment (preferably during the first clinic visit), and a reduction of return visits to prevent loss to follow-up. In non-endemic settings, clinical management based on CL species identification is of importance when dealing with returning travelers with CL, especially when multiple endemic regions with a variety of causative species have been visited [10, 84]. Lastly, in clinical trials where well defined cases are crucial for the quality of generated data, species determination is also necessary.

3.1 Clinical Dilemmas: Co-Infections and Unusual Presentations

Asymptomatic infection is the most common outcome after *Leishmania* inoculation [93]. This is based on epidemiological studies where individuals with a positive Montenegro skin test proved resistant and capable of controlling the infection without evidence of tissue damage. The mechanisms responsible for controlling the parasite are not fully understood, but might aid the development of effective vaccines.

Diffuse CL and disseminated CL are rare presentations that are often missed initially since they mimic other generalized skin infections such as leprosy and fungal infections [94]. Diffuse CL is caused by anergy to *Leishmania* and a lack of T-cell mediated immunity. As a result, the response to the LST is negative or poor, and the number of parasites found in lesions/smears is massive. Diffuse CL is characterized by nodules, papules, plaques, and erythema and resembles lepromatous leprosy (in fact, lepromatous leprosy is also characterized by the absence of T-mediated immunity against *Mycobacterium leprae*). Mucocutaneous involvement is rarely seen. The response to therapy is poor in diffuse CL, and relapses occur frequently.

Disseminated CL is characterized by a strong T-cell hypersensitivity immune response, a strong positive LST reaction, and scanty parasites in lesions. In disseminated CL, necrotic ulcers can be found (yet not in diffuse CL), and the response to therapy is in general good (Fig. 4). Mucosal involvement is frequent seen. The dichotomy in the immunological responses of diffuse CL on the one hand and disseminated CL and MCL on the other hand has led to the proposition that leishmaniasis is a spectrum disease comparable with leprosy [95].

Many CL endemic regions may have geographical overlap with other highly prevalent infections such as helminthiasis, leprosy, trypanosomiasis, and fungal infections. Diagnostic problems may occur when concurrent infections cause similar lesions (e.g., CL and leprosy, Fig. 5), when different pathogens are present in the same lesions (e.g., *Leishmania* and *Sporothrix schenckii*), or when similarities between phylogenetically close pathogens affect accuracy of diagnostic tests (e.g., serology for leishmaniasis and Chagas' disease) [96]. This may lead to diagnostic errors and delays,

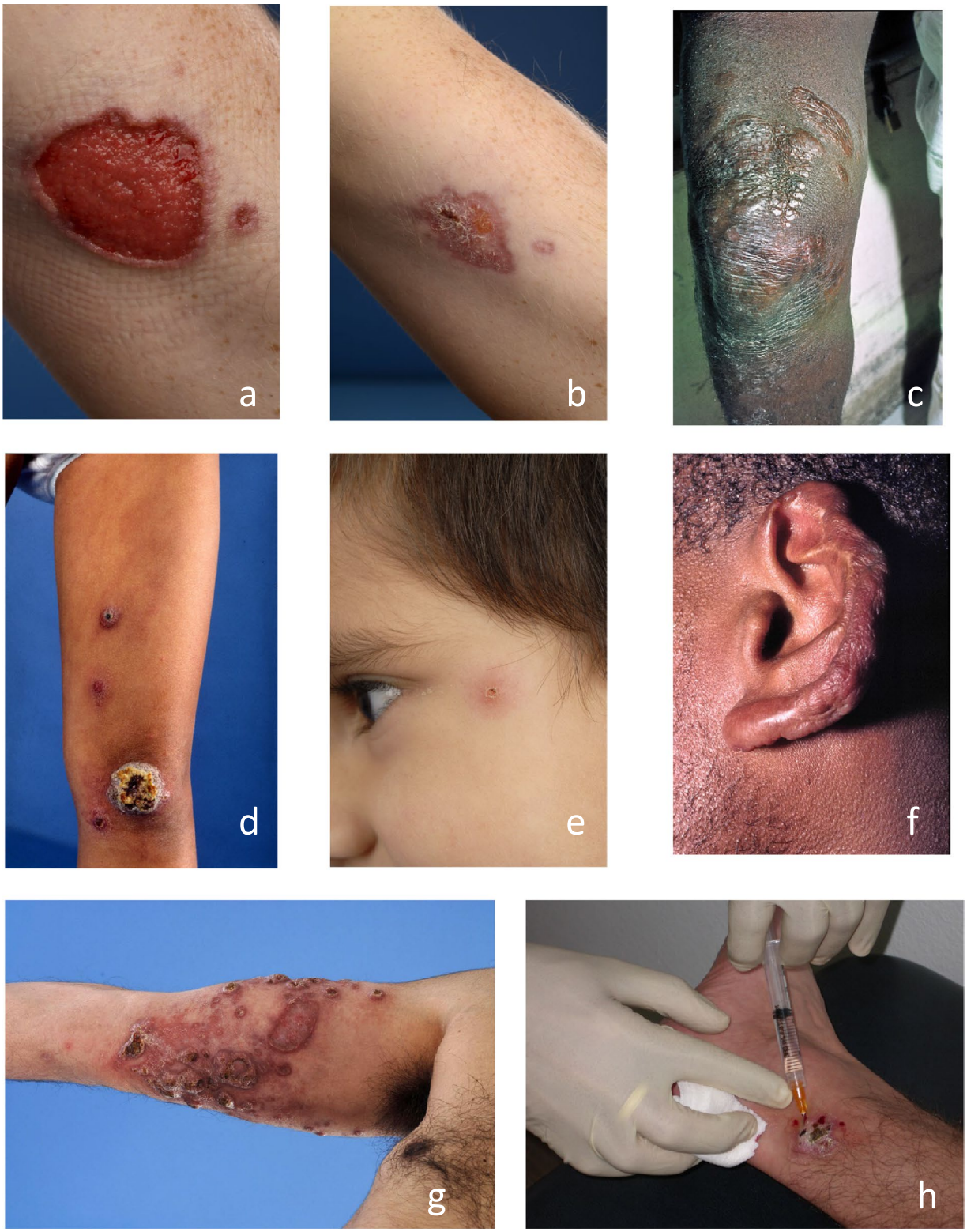

Fig. 4 Various manifestations of cutaneous leishmaniasis. **a** A cutaneous leishmaniasis ulcer with a satellite lesion on the lower arm. **b** Same patient as in a, healed after treatment with cryotherapy and intralesional antimonials. **c** Non-ulcerative cutaneous leishmaniasis lesion on the elbow. Multibacillary leprosy was considered but disproved in this patient. **d** Crusty cutaneous leishmaniasis ulcer with lymphangitis on the upper leg. **e** Typical cutaneous leishmaniasis lesion as often seen in children with *Leishmania infantum* contracted in North Africa. **f** Chiclero ulcer, typically affecting the ear with cartilage destruction. **g** Disseminated cutaneous lesions in a patient with mucocutaneous leishmaniasis caused *by L. guyanensis.* **h** Intralesional treatment of cutaneous leishmaniasis with antimonials. Photo credits: Department of Dermatology, Amsterdam UMC, Amsterdam, The Netherlands

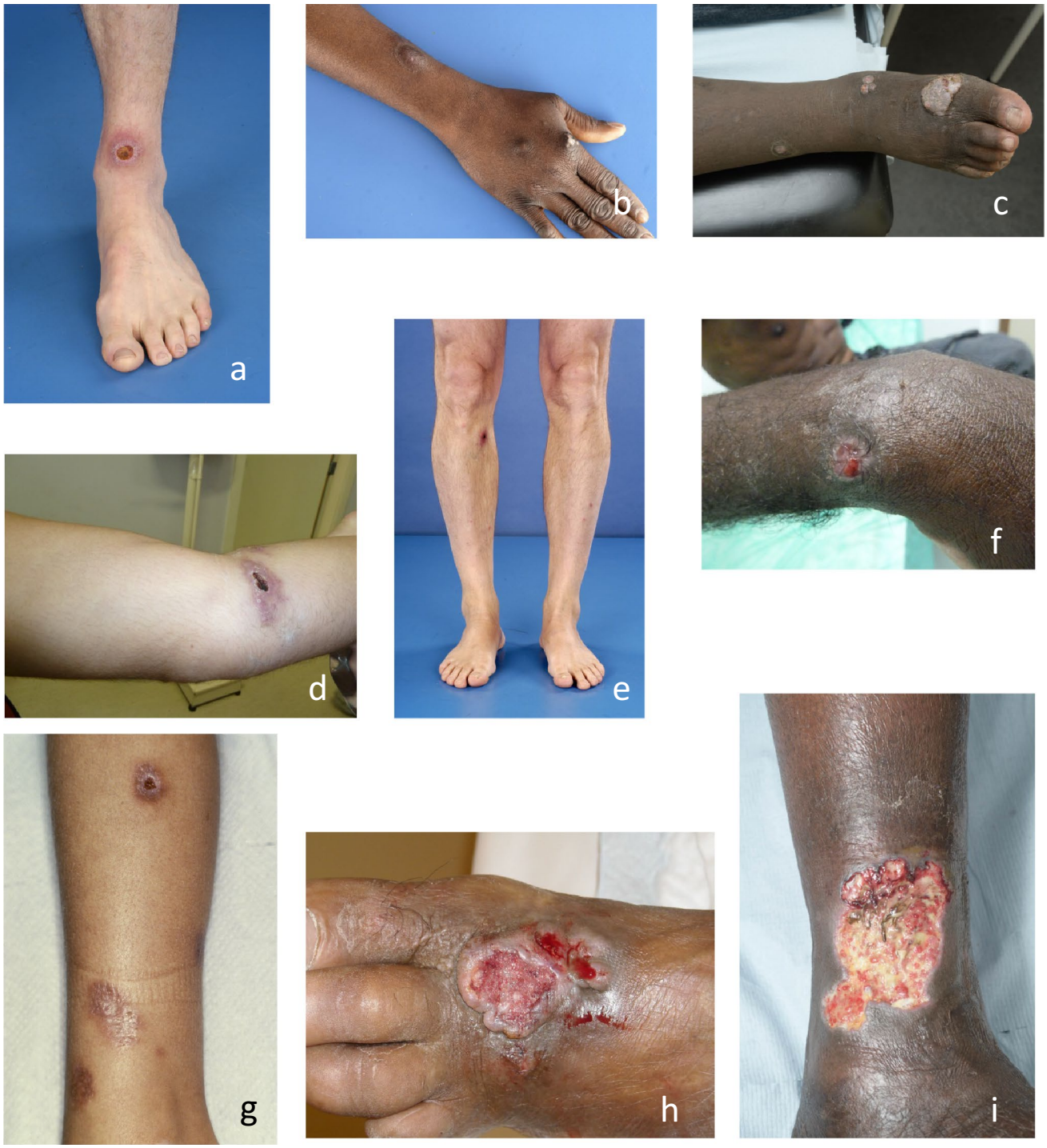

Fig. 5 Skin diseases that can mimic the clinical picture of cutaneous leishmaniasis. **a** Buruli ulcer caused by *Mycobacterium ulcerans* on the lower leg. **b** Atypical mycobacterial infection on the hand with lymphangitis caused by *M. marinum* infection. **c** Hyperkeratotic lesions caused by deep mycosis of the lower leg with lymphangitis in an immunosuppressed patient. **d** Ulcerative lesion caused by sporotrichosis with a satellite lesion on the arm. **e** Rickettsiosis on the lower leg with an eschar. **f** Ulcerative lesion in a patient with multibacillary leprosy. **g** Ulcerative lesions in a patient with necrobiosis lipoidica in a sporotrichoid dissimination pattern. **h** Squamous cell carcinoma on the dorsal foot. **i** Pyoderma gangrenosum ulcer with ragged margins and yellow necrosis on the lower leg. Photo credits: Department of Dermatology, Amsterdam UMC, Amsterdam, The Netherlands

and it can influence the effectiveness and safety of treatment. HIV co-infection in CL patients can increase the risk of CL recurrence and treatment failure [97]. Vice versa, CL can interfere with innate immunity, thus facilitating HIV progression [98].

Besides HIV infection, immunosuppression in general can lead to atypical clinical CL presentations [99]. Therefore, one should consider CL, especially in endemic regions, when granulomatous and tumorous skin diseases such as subcutaneous and deep mycosis, cutaneous lymphoma, pseudolymphoma, and basal and squamous cell carcinoma are deliberated. In a non-endemic setting, medical and travel history can help clinicians to consider CL in the differential diagnosis of complex and unusual appearing dermatoses.

4 Treatment

In most cases, CL is self-limiting. Nonetheless, irreversible disfiguring scar formation often occurs, and nodular lymphangitis and MCL can complicate the outcome with lasting disability and permanent unsightly destruction. The recovery period can be hindered by secondary bacterial infections and can take months to years, during which time patients have to deal with function impairment. In the absence of sound evidence, most therapy options are based on expert opinion [10]. Moreover, when species identification is not available, therapy options have to rely on local expertise. CL has been largely neglected for drug development because it affects poor people in poor regions of the world [100]. Most of the current drugs used to treat CL are decades old and have many limitations such significant toxicity and side effects [101]. Costs are another challenge in low- and middle-income endemic settings, especially with the systemic treatment options. A clinician with in-depth knowledge of the outcome of CL and the therapeutic options should make a tailored management plan in close discussion with the patient (shared decision making). A risk–benefit assessment of therapeutic options should be made, and in mild and indolent cases a wait-and-see policy can sometimes be the best option. Moreover, drug resistance is an emerging problem in the control of CL [87].

Several treatment options for CL are available. Pentavalent antimonials (sodium stibogluconate—Pentostam® or meglumine antimoniate—Glucantime®) remain the first treatment option for CL in most countries. Alternative treatment regimens include miltefosine, pentamidine isethionate, amphotericin B, antifungal agents (e.g., ketoconazole, fluconazole [102], itraconazole, paromomycin [103], granulocyte macrophage colony-stimulating factor [104], and heat therapy or cryotherapy [105–107]. Although attempts are being made to discover novel drugs against CL (e.g., via smart ex vivo compounds screening technology), promising therapeutic breakthroughs for CL are decades away from the clinic [108].

4.1 Treatment of Old World Cutaneous Leishmaniasis (CL)

Based on expert opinion, local therapy is preferred in patients with fewer than five lesions [10]. Compared with intralesional antimony or cryotherapy monotherapy, a combination of the two options proved more effective, even though differences were quite small [109–111]. Heat therapy is also effective [112–115], yet recently failed in over 90% of patients with CL caused by *L. tropica* [116]. The ratio behind both heat and cryotherapy is to kill parasites locally, and to induce an inflammatory response to indirectly eliminate parasites. Whereas liquid nitrogen is used for cryotherapy, heat therapy can be applied with different methods including infrared, ultrasound, exothermic crystallization thermotherapy, radiofrequency, and microwave. A major drawback of most local therapeutic options is the pain induced by the required intradermal injections. This makes local options especially challenging in children with CL.

Systemic treatment should be considered in patients with multiple lesions, potentially disfiguring facial involvement, or lesions at locations less fit for topical treatment. Miltefosine is an orally administered systemic treatment option that works well in patients with complicated Old World CL (*L. major*) lesions [117, 118]. Especially in children with Old World CL (caused by *L tropica* or *L major*), miltefosine seems a safe and effective alternative for painful local treatment options [119].

In a 2017 Cochrane review on therapeutic interventions for Old World CL, 40 new studies were identified since the previous update from 2008 [120]. Most studies were at unclear or high risk for most bias domains, and almost 40% lacked blinding. Only two additional comparisons could be added in the update: itraconazole cured more CL versus placebo; and there was no difference in the cured number of participants between paromomycin ointment versus vehicle arms. Both interventions caused mild side effects.

A number of experimental local treatments such as infiltration with hypertonic salt solutions [121], trichloroacetic acid [122], intralesional metronidazole [123], diphencyprone [124], carbon dioxide laser [125], intense pulsed light [126], photodynamic therapy [127], and systemic treatments such as cimetidine [128] in combination with antimoniate and chloroquine [129, 130] have been tried in small single-centered studies with varying results that need further evidence to conclude on their therapeutic value.

4.2 Treatment of American CL

In a 2019 Cochrane review on therapeutic interventions for American CL and mucocutaneous leishmaniasis (ACML), 37 new studies were identified since the previous update from 2009 [15]. In total, 75 studies were analyzed and the most assessed drugs were oral miltefosine and antimonials (particularly meglumine antimoniate). Most studies comprised participants with ulcerative CL lesions on the extremities, yet none included cases with diffuse cutaneous or disseminated CL. Most studies were hampered by unclear or a high risk of bias in at least one domain. Following are the most remarkable conclusions from the Cochrane review:

1. Intramuscular meglumine antimoniate given for 20 days to treat *L. braziliensis* and *L. panamensis* infections in ACML may increase the likelihood of complete cure.
2. Compared with placebo, at 6-month follow-up, oral miltefosine given for 28 days to treat *L. mexicana, L. panamensis,* and *L. braziliensis* infections in American cutaneous leishmaniasis (ACL) probably improves the likelihood of complete cure, with gastrointestinal side effects reported.
3. Comparing intramuscular meglumine antimoniate and miltefosine to treat *L. braziliensis, L. panamensis, L. guyanensis,* and *L. amazonensis* infections in ACML shows little to no difference to the likelihood of complete cure.

Based on expert opinion, local treatment can be considered in patients with a small number of lesions caused by American strains that are not associated with MCL like *L. naiffi*, *L. chagasi,* and *L. mexicana* [131, 132]. In patients with CL caused by *L. mexicana,* miltefosine treatment often fails due to antimicrobial resistance shown in both in vitro and in vivo studies; here, antimony (either locally or systemically administered) should be preferred [133, 134]. Provided CL patients affected by *L. panamensis* or *L. amazonensis* can be followed up closely, local treatment (e.g., combination local therapy of antimony and cryotherapy) could be considered, since these strains rarely cause MCL [10, 135, 136]. Although MCL due to *L. guyanensis* is not as rare as formerly thought, in patients with a few uncomplicated CL lesions, local therapy can also be considered [137]. Nonetheless, in Suriname and Brazil, systemic pentamidine is the preferred treatment option for *L. guyanensis* infections [138, 139], even though in Manaus, Brazil, efficacy proved lower with 50–60% success rates [140]. As a rule of thumb, strict follow-up is advisable in patients with American CL, to exclude treatment failure or progression towards MCL manifestations [141, 142]. The role of intralesional pentamidine has been studied in a small number of patients and requires additional evidence to prove its efficacy [143].

Because of the considerable risk of MCL seen with *L. braziliensis* infections, systemic antimony is the gold standard, and in general local therapy is not recommended. Yet again, when strict follow-up can be guaranteed, local treatment options for *L. braziliensis* infections can be considered in limited and uncomplicated cases [106, 135]. In some South American regions, miltefosine shows comparable treatment outcomes to antimony, whereas in other regions, miltefosine is inferior [144–147]. Treatment outcome variations are likely related to differences in susceptibility among local *L. braziliensis* strains. Although systemic amphotericin B is at least non-inferior to antimony to treat *L. braziliensis* MCL, costs and the severity of side effects make it the second-line treatment option [148–151]. Local amphotericin options currently do not seem to increase cure rates [152].

Because of the lack of trials evaluating the treatment of MCL caused by *L. panamensis, L. amazonensis,* and *L. guyanensis,* it is as advised to follow the *L. braziliensis* MCL treatment recommendations [10]. The combination of antimony and pentoxifylline is likely not more effective than antimony monotherapy in the treatment of CL caused by *L. braziliensis* [153]. Similarly, adding tamoxifen to antimony does not seem to improve cure rates [154].

5 Prevention

The old paradigm, prevention is better than cure, also applies to CL, not only for the patient but also for the community at large. Primary prevention consists of interventions against infected fly bites and should be offered to affected key populations such as farmers, hunters, military and mining laborers in CL endemic areas [82, 155, 156]. Here, an important intervention is sleeping under bed nets impregnated with insecticide and a maze that should be three times smaller than the bed nets used in the fight against malaria. To further reduce the chance of sand fly bites, bed nets can be impregnated with permethrin or other effective insect repellents [155]. This approach can be supplemented with indoor residual spraying [156], which has shown to be a (cost) effective measure for the prevention of CL [157]. However, emerging resistance of sand flies against insecticides is a concern in keeping these measures effective [158–160].

Intervention programs targeting reservoir-to-human transmission lack sound evidence on their effectiveness [161]. Although it is evident that the potential reservoir for zoonotic transmission is large and its full size not yet completely known, the exact contribution of mammalian species to disease transmission remains to be better established [162]. Canines are considered to be one of the main reservoirs and vaccination as well as culling strategies or the use

of insecticide-impregnated dog collars have been explored to reduce transmission, however with very variable success [155, 163].

A good understanding of community perceptions of the disease and healthcare-seeking behavior are essential to the further design of (cost-effective) preventive measures [85, 164, 165]. A recent review by Alidosti et al. revealed that providing educational programs to strengthen positive beliefs and understanding of the diseases and to correct negative beliefs and misunderstandings about CL will be useful to improve relevant behavioral changes towards prevention and control of CL [166]. Providing information on CL to key populations can improve the correct uptake of preventive measures, ameliorate risk behavior, and improve help-seeking behavior.

Early recognition of CL cases is of great importance, both for the individual patients, but also for public health purposes. In remote, sparsely populated, and underserved areas, mobile app-based care, and tele-dermatology applications can help in clinical management of suspected CL cases and surveillance [167].

Within the WHO NTD program, leishmaniasis is further integrated in the context of skin-related neglected tropical diseases (skin NTDs) [168]. Skin NTDs have in common that they are associated with stigmatization, discrimination, and socioeconomic problems. Moreover, skin NTDs often affect the same population. This offers opportunities such as integrated approaches to cover a number of skin NTDs. Integration is defined as a mode to optimize the use of limited resources, disability management, and community engagement, in addition to simplification of treatment pathways and the minimisation of stigma, through the implementation of activities to prevent two or more diseases simultaneously in the same community [167, 169]. Examples are the integration of screening programs, training of health workers and community volunteers, and the use of common laboratory and case management infrastructure.

5.1 Future Preventive Measures, Vaccination

The development (and delivery) of a vaccine against CL would ultimately be one of the most effective measures to control or even eliminate the disease [170]. It has been estimated that an effective vaccine against CL (70% efficacy, 10 years' protection) could prevent 41,000–141,000 cases of CL in Latin America for a smaller financial amount than needed to cover treatment of these cases [170, 171]. The road towards development of an effective vaccine against CL is difficult and complicated due to the complex interaction between the *Leishmania* parasites and the host immune system. However, naturally recovered CL infection mostly induces life-long immunity against the species that caused the primary infection, and this suggests that vaccination in theory should be possible [170, 172, 173]. This is further supported by the fact that 'leishmanization' (i.e., immunization of individuals with living parasites on an inconspicuous part of the body to protect against disfiguration lesions caused by a natural infection) indeed confers protection against CL [174]. The practice of leishmanization has nowadays been greatly abandoned because of safety issues and problems with standardization of the 'vaccine' [170, 174]. Still, the option of using a live attenuated vaccine, because of its ability to induce a protective Th1 response, is an attractive approach that is currently being explored [174]. Next to live attenuated vaccines, many experimental vaccines based on the use of (components of) dead parasites, recombinant proteins or DNA have been explored, but so far none have led to a licensed CL vaccine for use in humans [175]. The recent advances in vaccine development (fueled especially by the Covid-19 pandemic) will hopefully also be applied to NTDs in the near future, including leishmaniasis. Finally, deforestation, migration, and climate change, including global warming, may all contribute to spread of the vector and CL outside the currently known endemic areas [176].

6 Conclusions

Globally, CL affects millions. Most CL cases occur in low- to middle-income countries, which are often burdened by other ailments like malaria, tuberculosis, and HIV. At the same time, their governments have limited healthcare budgets and often have to rely on poor healthcare infrastructure. Because of lacking disease management and public health control interventions, CL is emerging and threatens to become an uncontrollable disease. Since it primarily affects poor people, pharmaceutical companies do not develop CL pipelines, because they fear their investments might not be returned. As a consequence, industry dedicates little investments and research into innovative diagnostics, treatments, and vaccines for CL. With the current molecular techniques, *Leishmania* species can be identified more easily, and species identification enables rational therapy management. Current CL treatment guidelines lack the required sound evidence and are mostly based on improperly designed and ill-conducted trials. Thus, there is an urgent need for large standardized and state-of-the-art trials that can evaluate potentially beneficial treatments, including less toxic drugs, and for children, painless modalities without injections. To further contain CL incidence and morbidity, we need intensified preventive research programs into improved vector control, vaccines, and diagnostics.

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