

The Relationship between Leishmaniasis and AIDS: the Second 10 Years

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INTRODUCTION

Updated statistics on the human immunodeficiency virus (HIV)/AIDS situation in December 2006 have shown that an estimated 39.5 million people are living with HIV/AIDS, among whom approximately 95% reside in developing countries ([http://www.unaids.org/en/HIV_data/epi2006/default.asp]). Of 4.3 million new HIV infections in 2006, 2.8 million (65%) occurred in sub-Saharan Africa, which continues to be the most severely affected part of the world. In this region, HIV has had a marked negative impact, reducing adult life expectancy by 50% in several countries. Although lack of access to treatment remains a major challenge, the number of people receiving highly active antiretroviral therapy (HAART) in sub-Saharan Africa recently passed 1 million for the first time. Weakness within the health systems has been identified as a key obstacle to expanding treatment, care, and preventive services; in addition, poor adherence to long-term therapy contributes to the expansion of AIDS.

As recognized in the resolution of the 60th World Health Assembly (http://ftp.who.int/gb/ebwha/pdf_files/WHA60/A60_10-en.pdf), leishmaniasis is one of the most neglected tropical diseases, with more than 12 million people worldwide currently infected and 2 million new cases reported each year; 350 million people are considered at risk, and the number of new cases is increasing. The disease affects the poorest populations in 88 countries, particularly developing countries (12). Two basic clinical forms, namely, cutaneous leishmaniasis (CL), a disfiguring and stigmatizing disease, and visceral leishmaniasis (VL) or kala-azar, which is fatal without treatment, are recognized. VL is prevalent in 70 countries, with the largest focus in South Asia, accounting for an estimated 300,000 cases in 2006. East Africa has the second largest focus of VL, with approximately 30,000 cases per year, while Brazil is third, with 4,000 reported cases in 2006. There is an alarming occurrence of new foci and an increase in the incidence of VL in east Africa (8, 214, 278). The true incidence of VL is underestimated because surveillance systems are lacking and misdiagnosis, especially with malaria, is common; failure to diagnose the disease leads to an increased case fatality rate.

CL is present in at least 82 countries, with an estimated annual incidence of 1.5 million cases worldwide. Several clinical forms are possible, depending on the *Leishmania* species involved, including localized cutaneous leishmaniasis (LCL), which often heals without treatment; diffuse cutaneous leishmaniasis (DCL), which is very difficult to treat; and finally, mucocutaneous leishmaniasis (MCL), which is the most severe form, as it produces disfiguring lesions and mutilation of the face (204).

The first case of leishmaniasis associated with HIV infection was reported in 1985, while the number of cases has subse-

quently increased rapidly in southern Europe. Thirty-five countries have since reported cases of coinfection. After the introduction of HAART, the number of coinfecting cases in European countries where the disease is endemic fell sharply. However, the problem has expanded to other major foci of leishmaniasis in the world due to increased overlap of the two diseases.

The HIV/AIDS pandemic has modified the natural history of leishmaniasis (9). HIV infection increases the risk of developing VL by 100 to 2,320 times in areas of endemicity, reduces the likelihood of a therapeutic response, and greatly increases the probability of relapse (107, 141, 189, 221). At the same time, VL promotes the clinical progression of HIV disease and the development of AIDS-defining conditions. Both diseases exert a synergistic detrimental effect on the cellular immune response because they target similar immune cells (177, 260).

Leishmania-HIV coinfection is currently reported for 2 to 9% of all VL cases in given countries of endemicity (267), but this proportion is likely to increase dramatically. In selected populations, such as in Humera, Ethiopia, the rate of coinfection in VL patients is 15 to 30% (143). In general, it is accepted that the reported global incidence of coinfection is underestimated, partly because VL occurs among neglected populations and because it is not on the CDC list of opportunistic infections, so it is rarely reported in AIDS notification systems. The introduction of HAART has totally changed the course of HIV/AIDS infections and the outcome of associated opportunistic infections. However, access to HAART in developing countries remains inadequate. In addition, there are many unresolved questions related to the management of *Leishmania*-HIV-coinfecting patients that justify this review.

**EPIDEMIOLOGICAL INFORMATION ON
LEISHMANIA-HIV COINFECTION**

Southern Europe

A dedicated surveillance network, consisting of 13 reporting institutions (268) that increased to 16 institutions in 1998 (266), has been monitoring the situation of *Leishmania*-HIV coinfection in southern Europe since 1994. Periodic case reports showed that four countries, i.e., France, Italy, Portugal, and Spain, were mainly involved (9). The largest number of cases was consistently detected in Spain, which may be related to the reactivation of asymptomatic infections due to a greater geographical overlap between leishmaniasis and HIV infections compared with that for other southern European countries. However, it has also been demonstrated that newly acquired infections occur among intravenous drug users (IVDU) sharing *Leishmania*-infected syringes (11, 70, 186, 187), and at the time of these studies, Spain had five to seven times more

TABLE 1. Number of *Leishmania*-HIV coinfections recorded by the WHO for four countries in southern Europe

Period	No. of coinfections					Reference
	France	Italy	Portugal	Spain	Total	
January 1990–June 1998	259	229	117	835	1,440	266
January 1996–June 1998	132	85	88	412	717	266
January 1990–January 2001	318	335	159	1,099	1,911	83
January 2001–December 2006	29 ^a	42	98	130	299	WHO, unpublished data

^a From January 2001 to December 2005.

IVDU than the European average. However, the proportion of reactivated leishmaniasis cases in asymptomatic coinfecting patients compared with coinfecting cases due to contaminated syringes has not been established. Cumulative cases recorded in different year ranges (Table 1) show that an incidence peak ($n = 717$) occurred during January 1996 to June 1998, representing approximately 50% of the total number of cases ($n = 1,440$) reported during the period 1990–1998 (266). By early 2001, the cumulative number of cases recorded by the WHO was 1,911, of which 57% were from Spain (83). All case records were analyzed by a geographical information system, which showed that 75% of patients lived in coastal urban areas with relatively high population densities. The spatial pattern of coinfections mapped for the neighboring littorals of northeastern Spain and southern France suggested a progressive spread of cases from urban to rural areas (83).

A clear decrease in the incidence of *Leishmania*-HIV coinfection was observed by the end of the 1990s (79, 138, 225), which can be attributed to the routine use of HAART since 1997 in most of southern Europe. In the Leishmaniasis Reference Centers of Madrid, Montpellier, and Rome, which are members of the WHO network for monitoring *Leishmania*-HIV coinfection, an average of 35 cases (95% confidence interval [CI], 34 to 38), which represented the incidence peak, were diagnosed in 1997. Cases began to steadily decrease during the period 1998–2001, and thereafter a low-incidence plateau was shown through 2006 (average of 12 cases; 95% CI, 8 to 16) (Fig. 1), except in Portugal; the reasons for the lack of decline in this country are unknown. An additional 241 new cases of primary infections from Spain ($n = 95$), Portugal ($n = 64$), Italy ($n = 52$), and France ($n = 30$) were reported to

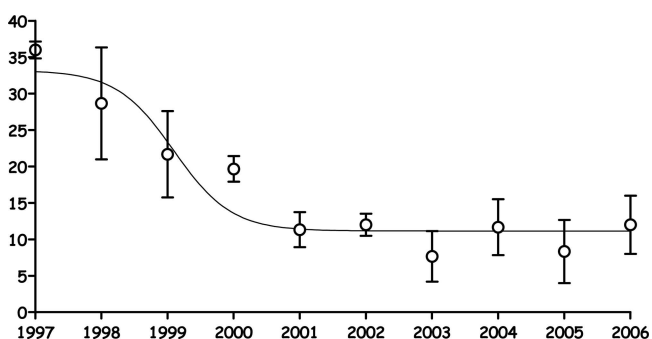


FIG. 1. Incidence trend of *Leishmania*-HIV coinfections recorded by three referral diagnosis centers, in France (1997–2005), Italy, and Spain (1997–2006). In these countries, HAART therapy for HIV treatment has been in routine use since 1997. Circles, means; bars, 95% confidence intervals.

WHO during the period 2001–2006; some persistent cases were also reported. Other countries, such as Switzerland, Germany, United Kingdom, and Greece, reported sporadic imported cases (6).

Coinfecting patients often have many episodes of active VL. More than 90% of these cases are confirmed by molecular methods to be relapses rather than reinfections (162). During 2001 to 2006, Spain and Portugal reported 95 primary infections versus 34 relapses (ratio of relapses to primary infections, 0.36) and 64 primary infections versus 21 relapses (ratio of relapses to primary infections, 0.33), respectively (WHO, unpublished data).

Epidemiological features and risk factors from cases reported during the period 1990–1998, representing the period of higher coinfection impact, and recently collated cases from the period 2001–2006, were analyzed and compared by WHO (266; WHO, unpublished data). The mean ages of patients were very similar in the two periods, i.e., 38.6 years (95% CI, 38.2 to 39.0 years) among 965 cases and 38.9 years (95% CI, 37.8 to 40.1 years) among 253 cases, respectively. Gender distributions were also similar, with male patients being highly prevalent in both periods (83.2% versus 88.5%, respectively). In contrast, the distributions by risk group for *Leishmania*-HIV coinfection showed significant differences between the earlier and more recent cases (Fig. 2). In both periods, three main risk factors, namely, IVDU and heterosexual and homosexual activities, accounted for about 94% of the analyzed cases. In the period 1990–1998, 781 cases that belonged to these risk groups were identified, among which IVDU accounted for 76% and heterosexual activity accounted for 12%, whereas among 95 cases analyzed during the period 2001–2006, the proportions of these groups were 67% and 23%, respectively (χ^2 test; $P = 0.01$). This difference may reflect the general trend of AIDS decline among IVDU paralleled by an increase among heterosexuals in Western Europe (<http://www.unaids.org>). The rates of homosexual coinfecting cases were similar in both periods (11% and 10%, respectively). With regard to immunological factors, coinfecting patients were severely immunosuppressed in both periods. In the period 1990–1998, 624 of 682 patients (91%) had <200 CD4⁺ lymphocytes/ μ l, while during the period 2001–2006 (an HAART era) a similar situation was evident in 113 of 128 patients (88%).

South Asia

South Asia is currently the main focus for VL in the world, accounting for 67% of the total leishmaniasis disease burden (117). In 2005, Bangladesh reported 7,000 cases, India reported 32,800, and Nepal reported 3,000, figures that are

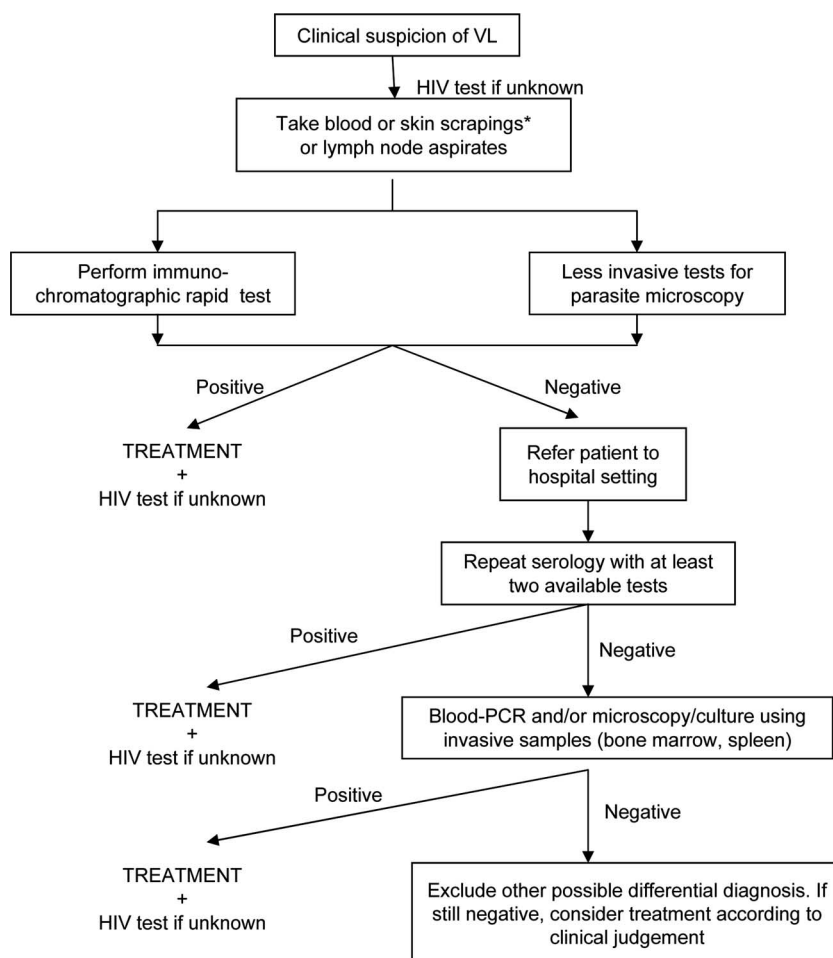


FIG. 2. Algorithm for the diagnosis of VL in HIV-infected patients.

known to be serious underestimations (34, 123, 230). In Bihar, India, a house-to-house survey demonstrated that only one in eight VL cases was counted in official government surveillance data (246). Additionally, there has been an upward trend in all three countries over the last several years, where 147 million people are estimated to be at risk of infection. More realistic estimates of annual incidence suggest approximately 250,000 cases in India, 40,000 in Bangladesh, and 3,000 in Nepal, which represents half of the VL burden in the world (41).

Interestingly, VL is concentrated in only 94 districts in all three countries and in four states in the northeast in India (Bihar, Uttar Pradesh, West Bengal, and Jharkhand). The state of Bihar accounts for >90% of cases and contributes to the spread of the disease into neighboring areas of Nepal. The reemergence of the disease in the 1970s was most likely due to residual post-kala-azar dermal leishmaniasis (PKDL) patients harboring parasites in the skin. Currently, PKDL is reported to occur in only 1% of Indian VL patients, but reliable data are lacking (228). In addition to this phenomenon, a loss of acquired immunity may partially explain the periodicity of VL incidence peaks every 10 to 15 years. In the past, most cases were in adults, although now there are reported to be many more children and adolescents, i.e., immune-naïve individuals, affected (41, 201).

The first case of HIV infection in India was reported in 1986 in Chennai (Madras) and was quickly followed by reports from all major cities; there has been a steady rise in the number of cases since (63, 164). The new 2006 estimates by the National AIDS Control Organization (<http://www.nacoonline.org>), supported by UNAIDS and WHO, indicate that the national adult HIV prevalence in India is approximately 0.36%, which corresponds to an estimated 2 million to 3.1 million people living with HIV in the country (<http://www.nacoonline.org>). The disease is concentrated in states in the south (Tamil Nadu, Andhra Pradesh, Maharashtra, and Karnataka), where effective interventions have caused HIV prevalence to begin to decline or stabilize, and to a lesser degree in the northeast (West Bengal, Orissa, Rajasthan, and Bihar), where overlap with leishmaniasis occurs. HAART was initially introduced in 25 centers in India during 2004, but because the implementation of the national HIV plan has been rapid, HAART is now widely available all over the country.

In Bangladesh, there are 11,000 people living with HIV; a national adult prevalence of <0.1% was estimated for 2006 (http://www.unaids.org/en/HIV_data/epi2006/default.asp). In Nepal, an estimated 75,000 people are living with HIV, and the national adult prevalence was estimated to be 0.5% for 2006 (http://www.unaids.org/en/HIV_data/epi2006/default.asp).

In India, the first *Leishmania*-HIV coinfection case was reported in 1999 from the sub-Himalayan region (Kumaon) (245). Various case reports of coinfection have since been published (150, 203). The problem of coinfection seems to be exacerbated by economic migrants who acquire HIV in urban settings and then return to their rural homes in areas where VL is endemic, where they acquire or reactivate leishmaniasis infection due to declining immunity. At the Kala-Azar Medical Research Centre in Muzaffarpur (Bihar), the number of HIV-positive patients increased from 339 in 2000 to 776 in 2006, and this was accompanied by an increase in the VL-HIV coinfection rate, from 0.88% in 2000 to 2.18% in 2006 (S. Sundar, personal communication). In a case series from India, rates of HIV positivity among VL-PKDL patients ranged from 1.5 to 6.3% (150, 258). One hospital-based study in India reported a VL prevalence of 2.84% among HIV-positive patients in 2006 (247).

In a study conducted in 39 hospitals in Nepal from June 2003 to May 2004, 140 of 854 febrile individuals studied had VL, and 8 of these were HIV positive (5.7%) (112). No data on *Leishmania*-HIV coinfection are available from Bangladesh.

As characterized by the PKDL patient, coinfecting patients may represent an important reservoir of parasites with a high potential to maintain infection rates in sand flies at the community level (158). Although the extent of overlap between both infections is still limited, the potential for coinfection in this focus is very high, especially if one considers that these patients are a source of drug resistance due to their unresponsiveness to any antileishmania drug, in an area already threatened by a high rate of resistance to antimonials (251).

Brazil

In the 2006 Pan American Health Organization report for the Americas region, 62,000 cases of CL and 5,000 cases of VL were reported, of which the majority were from Brazil (<http://www.paho.org/English/AD/DPC/CD/leish-2007.htm>). However, linked databases from the National Surveillance Systems in Brazil for the period 2002–2003 yielded a corrected estimate of 10,207 VL cases for the same period, indicating that 40 to 50% of cases are underreported (<http://www.aids.gov.br>). Both CL and VL have been increasing for some years in Brazil; 32,000 (4.75 to 22.94 cases for every 100,000 inhabitants) and 3,500 (0.96 to 2.66 cases for every 100,000 inhabitants) cases of CL and VL, respectively, were reported in 2005. CL is widely distributed, whereas VL is endemic in the northeast areas, accounting for 87% of all VL cases, and is expanding to central west, north, and southeast areas of the country, and more worryingly, following a process of rural-to-urban transmission displacement, VL is spreading to important cities, such as Rio de Janeiro, Belo Horizonte, Santarém, Teresina, Natal, and Fortaleza (47a).

Brazil is the epicenter of the HIV/AIDS epidemic in South America and accounts for one-third of all persons living with the virus in South America. AIDS was first reported in Brazil in 1983, and in the 2006 UNAIDS report, it is estimated that 620,000 (range, 370,000 to 1 million) people in Brazil are living with HIV (<http://www.aids.gov.br>). HIV/AIDS diagnostic and treatment services are provided free for all patients throughout Brazil. The country's emphasis on prevention and treatment has helped to keep the national adult prevalence steady at

roughly 0.5% since 2000, although the spread of HIV transmission to regions with lower urbanization rates and to small- and medium-sized cities, where leishmaniasis can be transmitted, has been observed.

From 2001 to 2005, 16,210 cases of VL were reported in Brazil, and 315 (2%) patients were coinfecting with HIV (92). Of the coinfecting patients, 78% were male, and the median age was 38 years (86% of patients were in the 20- to 49-year age group); 56.3% of the HIV infections were attributed to heterosexual transmission, and 53% and 29% of the cases were reported from the northeastern and southeastern regions, respectively. The clinical forms found among coinfecting cases are 43% MCL, 37% VL, and 20% CL (199), a clinical pattern that differs from that found in southern Europe, where typical VL represents 88% of coinfecting cases; 8% of cases are atypical because of intestinal, lung, or other parasite colonizations, 4% are DCL, 0.4% are MCL, and 0.3% are combined cases of VL and CL (83). In the absence of HAART, the fatality rate during the first VL treatment course was 24%.

Of 150,000 CL cases reported from 2001 to 2005, 150 (0.1%) patients had HIV coinfection (92). MCL is frequently observed in HIV-coinfecting patients in Brazil (43%), a clinical pattern rarely seen in southern Europe (0.4%).

In 2002, the Brazilian *Leishmania*-HIV Coinfection Network was established with the following aims (<http://www.cpqrr.fiocruz.br/leish-hiv>): (i) mapping of the geographical distribution of leishmaniasis-HIV coinfection; (ii) evaluation of the epidemiological, clinical, immunological, and therapeutic characteristics of each identified case; (iii) increasing the availability and ensuring appropriate usage of diagnostic tests for leishmaniasis; (iv) establishment of reference laboratories for the isolation and characterization of the parasites; (v) improvement in the dissemination of relevant information; (vi) enhanced training and provision of appropriate guidelines for the health system; (vii) broadening the participation of grassroots and nongovernmental organizations; and (viii) planning and evaluation of coinfection control interventions and follow-up activities. The *Manual de Recomendações para Diagnóstico, Tratamento e Acompanhamento da Co-Infecção Leishmania-HIV* was made available in 2004 by the Brazilian Ministry of Health (47a; <http://www.saude.gov.br/bvs>).

Sub-Saharan Africa

Burkina Faso. The HIV seroprevalence in Burkina Faso is 2%. Sporadic cases of CL due to *Leishmania major* were reported from many localities from 1953 to 1984, but the number of cases has increased in recent years (61 cases in 1996, 552 cases in 1997, 1,218 cases in 1998, 1,595 cases in 1999, 712 cases in 2000, 1,063 cases in 2001, 1,152 cases in 2002, 1,194 cases in 2003, 901 cases in 2004, and 827 cases in 2005). A major increase in the number of cases since 2000 followed the development of a new district in a previously rural area near the capital Ougadougou; the disease is now known as "Ouaga 2000" (111). The peak incidence months are August and September, overlapping with the malaria peak season. Cases occur across all age groups and are equally prevalent among men and women.

Ten of 70 (14.3%) "Ouaga 2000" patients were coinfecting with HIV. Many unusual clinical manifestations of CL, including lepromatous and diffuse ($n = 15$), ulcerative ($n = 14$),

infiltrative ($n = 12$), papulonodular ($n = 9$), psoriasis-like ($n = 5$), Kaposi's sarcoma-like ($n = 1$), cheloid ($n = 1$), and histioid ($n = 1$) forms, were found in a prospective study of 32 coinfecting patients. Treatment with a standard dosage regimen of meglumine antimoniate intramuscularly for 21 days achieved a 50% cure rate, but rapid relapses were observed in HIV-coinfecting patients, for whom repeated courses of treatment were required (111, 172). Three treatment cycles increased the cure rate to 75%. Visceralization occurred in one HIV-positive patient, with disseminated papulonodular leishmaniasis and a bone marrow aspirate positive for *L. major*. Similar findings were observed in French Guiana, where nine coinfecting patients had more lesions and less adenopathy or lymphangitis than immunocompetent patients, with a poorer response to treatment and higher recurrence and/or reinfection rates (66).

Ethiopia. There is incomplete information on the epidemiology of leishmaniasis in Ethiopia, as no surveillance system is yet in place. However, reports of VL endemicity date back to the 1940s. The disease is prevalent mostly in lowland arid areas, and the parasite involved is mainly *L. donovani*, with an estimated annual incidence of 4,000 cases. The six areas of endemicity identified are northwestern Ethiopia (Metema, Humera, Wolkayit, and Libo/Fogera), northeastern Ethiopia (Awash Valley and Ethio-Djibouti border), south and southwest Ethiopia (Dawa, Genale, Gelana, Segen, Woito, Konso, and Omo River Valley), the Kenyan border, and the Gambella-Sudan border (113). However, leishmaniasis is spreading to areas where it was previously nonendemic, as exemplified by a recent outbreak in the Libo and Fogera districts (Amhara State), a highland area close to the focus in the northwest (8).

The first HIV-positive patient was reported in 1984, and the first AIDS case was reported in 1986. Based on antenatal sentinel surveillance projections, the estimated national HIV prevalence is 3.5% (10% in urban areas and 1.9% in rural areas). The urban prevalence is thought to have stabilized during the period 1999–2001, with a current estimation of 1.3 million HIV-positive people. HAART was introduced in 2003, but programs do not yet adequately address the needs of the rural poor. Approximately 65,000 HIV patients are now receiving HAART.

In Humera (northwest Ethiopia), the proportion of VL patients who were coinfecting with HIV increased from 18.5% in 1998–1999 to 40% in 2006. In a retrospective review of 791 cases in Tigray, the case fatality rate among VL-HIV-coinfecting patients was four times higher than that for VL patients not infected with HIV (143). In Libo, 15 to 18% of all VL patients are HIV positive (Médecins Sans Frontières (MSF)—Greece, personal communication). The groups at highest risk of coinfection are seasonal migrant laborers, sex workers, young adults in resettlement areas (particularly in the northwest and along the Sudan border), truck and other public transport drivers, and military personnel deployed in border areas (19).

Kenya, Uganda, and Somalia. Data quality is poor for Kenya, Uganda, and Somalia. There are well-established foci in semi-arid, poor, and remote districts where termite hills are colonized by the sandfly vector (*Phlebotomus martini*).

In Kenya, there are several historical foci, in West Pokot (representing 70% of all patients in Kenya), Baringo, Mandera, Garissa, and Mwingi. There was a recent outbreak in

2006 in northeast Kenya, at Wajir and Issiolo, which mainly affected children ($n = 48$; 95% of patients were <5 years of age). In 2006, 15 cases of VL-HIV coinfection were reported, but the risk of coinfection is increasing (268a).

In Uganda, the only documented area where VL is endemic is Pokot County, in the Nakapiripirit district in the northeastern region of Karamoja. This is an extension of the focus of endemicity of the West Pokot and Baringo districts of the Rift Valley province in Kenya. There is no national leishmaniasis control program, and most VL cases are monitored by MSF—Switzerland, who treated more than 2,500 patients in Amudat Hospital from 2000 to 2006. Approximately 70% of treated VL patients came from Kenya (West Pokot and Baringo districts). The male-to-female ratio of the patients was 3:1, and >60% of the patients were under 15 years of age. Eight cases of VL-HIV coinfection were confirmed (MSF—Switzerland, personal communication); no other data on VL-HIV coinfection are available. The HIV prevalence in Karamoja, on the Ugandan-Kenyan border, reached 3% in 2006; local conflicts, with associated rape, and cattle trading in towns may exacerbate the situation and elevate the potential for VL-HIV coinfection.

In Somalia, MSF—Belgium is currently dealing with an outbreak of VL and treated 263 and 1,300 cases in 2005 and 2006, respectively (MSF—Belgium, personal communication). No coinfection has been reported yet.

Sudan. The main area of endemicity is the southeast of the country, bordering Ethiopia. There have been recurrent epidemics with high mortality rates, most recently in 1989 (236). The number of VL cases in 2006 was unexpectedly low in the states of Gedaref ($n = 1,918$) and Sennar ($n = 476$), and similar figures were observed in Upper Nile, but the mean annual incidence in the whole country is approximately 15,000 to 20,000 VL cases (S. H. El-Safi, personal communication). The parasite identified is mainly *L. donovani*, although *L. infantum* also occurs (81). Transmission is basically anthroponotic, with PKDL cases occurring in up to 50% of VL patients, forming an important human reservoir (91).

The first case of AIDS was reported in 1986, and by December 2006, the adult national prevalence was 1.6%, indicating that Sudan is now in a generalized epidemic (http://www.unaids.org/en/HIV_data/epi2006/default.asp). The estimated number of HIV/AIDS cases is around 350,000 (range, 170,000 to 580,000). The transmission is mainly heterosexual (97%), and vertical transmission from mother to child also plays a role. The most affected areas are Khartoum and the eastern and southern states, due to population movements (refugees and internally displaced populations). The 19- to 39-year age group is the most affected, and among registered patients, males predominate (male-to-female ratio, 3:1). The prevalence of HIV infection in the surveillance sentinel clinics in 2002 and 2004 was 1.1% and 1.9%, respectively, for sexually transmitted infection clinics, 1.6% and 2.3%, respectively, for tuberculosis patients, and 2% and 0.95%, respectively, for antenatal care clinics (Sudan National AIDS Program). The prevalence in other groups was as follows: street children, 2.3%; university students, 1.1%; refugees, 4%; prisoners, 2.5%; and sex workers, 4.4%. The strategic objectives of the National Policies on HIV/AIDS are to maintain the prevalence of HIV/AIDS below 2% and to provide care, treatment, and support to infected

TABLE 2. Zymodemes of *L. infantum* found in HIV-positive patients in southern Europe and North Africa

Country	No. of isolates with zymodeme						Total no. of isolates
	MON-1	MON-24	MON-29	MON-34	MON-183	Other (<i>n</i> = 23 zymodemes)	
France	158	5	1			5	169
Greece	2	1					3
Italy	58	7	1	2		13	81
Portugal	72	1	2			1	76
Spain	152	63	15	36	19	54	339
Algeria	12	9				1	22
Morocco	1						1
Tunisia	3	1					4
Total	458	87	19	38	19	74	695

patients. In 2006, there were 40 centers for treatment services (voluntary counseling and testing units) and around 1,000 patients receiving HAART. Screening of military personnel and students indicates that the number of cases of HIV is increasing.

By 1998, three cases of coinfection had been identified. Since then, the reported prevalence in hospital-based studies was 5% (3/60) in Khartoum between 1998 and 1999, 9.4% (5/53) in Khartoum in 2002, and 8.1% (3/37) and 3.6% (3/84) in 2002 and 2003, respectively, in Gedaref State (Sudan National AIDS Program).

MICROBIOLOGY

Since the first case of *Leishmania*-HIV coinfection (76), methods to identify *Leishmania* have greatly evolved. Beside multilocus enzyme electrophoresis, a large number of molecular methods have been introduced for the characterization of *Leishmania* from the genus to strain levels, but most of them lack standardization.

Three main cryobanks and identification centers, in France (Montpellier), Italy (Rome), and Spain (Madrid), which use the same reference identification technique by 15 enzymatic systems with starch gel electrophoresis, according to Rioux et al. (213), have identified a large series of isolates from *Leishmania*-HIV coinfection cases since 1986, on which the present review is focused. The data presented are based on a compilation of several papers, mainly three papers published in a 2003 special issue of *Annals of Tropical Medicine and Parasitology*, namely, those by Pratlong et al. (195) for southern France, Chicharro et al. (59) for Spain, and Gramiccia (108) for Italy, and a recent paper by Campino et al. (54) for Portugal.

A total of 1,037 *Leishmania* strains have been isolated and identified from HIV-positive patients, which represents the largest sample studied around the world. The majority of the isolates came from VL cases in southwest Europe, and a few isolates were obtained from other foci and other leishmaniasis forms (Table 2).

Leishmania infantum Identification during Coinfection

Using isoenzyme electrophoresis, 695 isolates were identified as *Leishmania infantum*, mainly from patients infected in

Spain (*n* = 339), France (*n* = 169), Italy (*n* = 81), and Portugal (*n* = 76), with a few isolates from patients in Algeria (*n* = 22), Tunisia (*n* = 4), Greece (*n* = 3), and Morocco (*n* = 1). Almost all of the leishmaniasis clinical forms were VL (99.6%), with occasional cases of CL (Table 2).

Of 28 different *L. infantum* zymodemes detected, MON-1 has the widest geographical distribution, being present in all eight countries mentioned above, and it is the most frequent zymodeme in the studied sample (65.9%), as also seen for immunocompetent VL patients and canine leishmaniasis cases from the Mediterranean countries. The MON-24 zymodeme is present in seven of eight countries and represents 12.5% of the isolates. Five zymodemes (MON-1, MON-24, MON-29, MON-34, and MON-183) account for 621 isolates (89.3%). The remaining 74 isolates (10.6%) are divided into 23 different zymodemes. The observed enzymatic polymorphism is based on the latter zymodemes, which are almost exclusively present in Spain (*n* = 21) and Italy (*n* = 15), indicating high enzymatic polymorphism, whereas enzymatic polymorphism is reduced in France (*n* = 5) and Portugal (*n* = 4).

Within the countries themselves, the polymorphism shows geographical variations. The best example is given by Gramiccia (108) in Italy, who showed that the polymorphism index (PI; the number of zymodemes detected divided by the number of patients investigated) for HIV-positive patients in the whole of Italy was higher (PI = 0.13) than that for HIV-negative patients (PI = 0.04). The PI for strains isolated from both HIV-positive and HIV-negative patients decreased from the south (Sicily) to the north (rest of Italy).

Zymodeme MON-1 is the usual agent of VL in both HIV-negative and -positive patients in the entire Mediterranean basin and can occasionally be responsible for CL in immunocompetent patients, although zymodeme MON-24 is the most frequent cause of CL in this area. However, several dermatotropic zymodemes, usually causing CL in nonimmunodepressed patients (MON-11, MON-24, MON-29, MON-33, and MON-78), are directly responsible for VL in HIV-positive patients because of the anergic status of these patients (58).

The higher enzymatic polymorphism in HIV-positive than in HIV-negative patients implies that several zymodemes (MON-136, MON-183, MON-185, MON-188, MON-190, MON-198, MON-199, and MON-228) exist only in HIV-positive patients (120). Curiously, these zymodemes associated with HIV infection are located at the ends of the branches of the phylogenetic

TABLE 3. Numbers of isolates identified according to zymodemes found during VL relapses

Region ^a	No. of isolates with zymodeme																		
	1	11	24	28	29	33	34	72	78	108	136	183	185	188	201	228	253	283	
A	13		2	2	1	1				1									
B	8		1	1	1	1	1												
C	20		10		1		5			1		2				1	2	1	
D	20	1	1		1			1	1		1		1	1	1				
Total	61	1	14	3	4	2	6	1	1	2	1	2	1	1	1	1	2	1	

^a Region A, south of France (195); region B, Barcelona, Spain (100); region C, Madrid, Spain (59); and region D, Italy (108).

tree, as if they had recently appeared. The question is whether these zymodemes can survive and cause symptomatic disease in immunocompetent patients (195).

VL occurring during HIV infection cannot be cured, even after extensive antileishmanial treatment. The likelihood of a relapse occurring is dependent on the level of CD4⁺ cells in the patient. In patients ($n = 80$) from southern Europe, two or three stocks were isolated from each patient following successive episodes of relapse, with time intervals of 1 to 4 years, and were subsequently identified by enzymatic electrophoresis. All of the isolates were *L. infantum*, and 18 zymodemes were found repeatedly during the various episodes. They were mainly MON-1 (58.1%) and MON-24 (13.3%), but a large number of other zymodemes were also identified in the global sample (Table 3). These results suggest that there is no specific zymodeme related to a particular severity of VL, as several zymodemes are expressed in further relapses. However, all but two patients had the same *L. infantum* zymodeme during the successive episodes. In two patients, two distinct zymodemes were obtained, at time intervals of 20 months (MON-1 and MON-34) and 29 months (MON-1 and MON-33) (100). By using a more powerful tool for molecular tracking (seminested PCR-restriction fragment length polymorphism analysis) that is capable of discriminating within the same zymodeme, Morales et al. (162) showed that 7.5% of the so-called relapses in 40 patients with VL-HIV coinfection had a different band pattern from that found in the primary attack. From these studies, it appears that repeated leishmaniasis episodes in HIV-positive patients could be due mainly to relapses of the first infection. For the two cases of different zymodemes, these occurred due to either a reinfection or the existence of a mixed infection by two zymodemes which were differently selected during culture of the two episodes.

More recently, *L. infantum-L. major* hybrid strains were described for HIV-coinfected patients from Portugal, by sequencing of the gene for polymerase II RNA and by isoenzymatic identification (202).

Other *Leishmania* Species during HIV Coinfection

No data from anthroponotic VL foci are available, with the exception of a single strain from Djibouti isolated and identified as *L. donovani* MON-268 (194).

Although *Leishmania*-HIV coinfection concerns mainly VL, some cases of CL have been reported from countries where it is endemic in Africa and South America. In the sub-Saharan Sahelian zone of Africa, several *L. major* isolates obtained from patients in Mali ($n = 4$), Burkina Faso ($n = 7$), Senegal

($n = 1$), and Mauritania ($n = 1$) belonged to zymodeme MON-26 and its small variants, MON-74 and MON-117, a group of zymodemes extending from west Africa to central Asia. An outbreak of CL in Ouagadougou (Burkina Faso) due to *L. major* MON-74 included HIV-positive patients (111).

Among Brazilian HIV-coinfected patients, Rabello et al. (199) reported eight cases of CL by *L. braziliensis* and *L. guyanensis*, three cases of MCL by *L. braziliensis*, and one case of VL by *L. chagasi*. In French Guiana, five strains isolated from HIV-positive patients with CL were typed by multilocus enzyme electrophoresis and shown to belong to *L. guyanensis*, and four zymodemes were detected, including MON-43 ($n = 1$), MON-45 ($n = 1$), MON-131 ($n = 2$), and MON-148 ($n = 1$) (66).

Moreover, various New World *Leishmania* dermatropic species, such as *L. braziliensis*, *L. mexicana*, and *L. amazonensis*, have been identified as causes of VL in HIV-positive patients (23, 24, 200, 241), while conversely, viscerotropic variants of *L. infantum/L. chagasi* have been found in cutaneous lesions or even in the healthy skin of HIV-positive patients (44, 105, 191).

The Trypanosomatidae family includes the protozoa of plants, insects, and mammals, but only *Leishmania* and *Trypanosoma* species are usually found in humans. However, in some cases, nonhuman monoxenous trypanosomatids have been isolated from HIV-positive patients, mimicking both VL (121, 180) and CL (40, 75, 227). These infections suggest that the immunocompromised patient could be vulnerable to other currently non-human-pathogenic trypanosomatids due to their anergic status (58, 74).

PATHOGENESIS

VL-HIV Coinfection

Early after the appearance and report of the first cases of *Leishmania*-HIV coinfection, it was evident that the presence of both pathogens concomitantly in the same host cell (the macrophage) might have enhanced reciprocal effects that influence the expression and multiplication of either one or both pathogens (36, 269). It is thought that the parasite infection found concurrently with HIV induces chronic immune activation, and therefore an increased HIV load and accelerated progression of AIDS (27), whereas immunological disturbances caused by HIV are particularly favorable for the uncontrolled multiplication of the parasite.

During the last 10 years, attempts have been made to discover those mechanisms that are involved in the immunopathogenesis of *Leishmania*-HIV coinfection and to confirm

that there is a specific *Leishmania*-HIV interaction at the cellular level that might affect the course of infection for each pathogen. It has been determined that such interactions are patent in the macrophage as well in other cells, such as dendritic cells (DCs). The involvement of other cells might explain the systemic effects observed in coinfecting patients.

The main problem of coinfection is that parasitic infection may induce the activation of latent virus. Clinical studies have shown that leishmaniasis promotes an increase in serum HIV type 1 (HIV-1) load (49, 196) and a more rapid progression to AIDS that reduces life expectancy in HIV-infected patients (72). Experimental studies have demonstrated the participation of *Leishmania* in the pathogenesis of HIV-1 through the induction of viral replication and disease progression. Initial reports proved that *Leishmania* can upregulate virus expression in two monocytoid cell lines latently infected with HIV-1 (36), and evidence was provided that *Leishmania* promastigotes (LPG) and the intramembrane structural component of LPG (core-PI) present in amastigotes were both capable of promoting virus replication in T cells through complex biochemical pathways that involve the translocation of the transcriptional factor NF- κ B to the nucleus (35). Although the exact mechanism remained unclear, it was thought that LPG molecules could interact with the HIV-infected T cell during antigenic presentation between the *Leishmania*-infected macrophage and the T cell (177). Furthermore, *Leishmania* may induce HIV replication in infected cells through antigen-specific and non-antigen-specific mechanisms in which tumor necrosis factor alpha (TNF- α) plays a key role (270).

More recently, it has been shown with human tonsillar tissue cultured ex vivo (275) and primary human monocyte-derived macrophages (274) that *Leishmania* does not directly affect virus gene expression but instead modulates the life cycle of HIV-1 through an indirect phenomenon that is linked to the induction or elevation of TNF- α and interleukin-1 α (IL-1 α) and that may function in an autocrine/paracrine manner to upregulate virus gene expression mediated through induction of NF- κ B (109).

For patients coinfecting with HIV-1 and *Leishmania*, it has been proposed that the increase in cytokine production induced by the parasite will affect virus production not only in macrophages but also in HIV-1-infected CD4⁺ T cells (275).

Different clinical studies performed recently illustrate the complexity of the interplay between the pathogens. It has been observed that *Leishmania* infection may affect the life cycle of the virus through the increased expression of specific chemokine receptors. *Leishmania*-HIV-coinfecting patients have a significantly higher level of CCR5⁺ CD3⁺ T cells than do HIV-negative patients with VL or HIV-positive patients without VL (176). CCR5 is a major coreceptor for HIV entry in target cells, and its high expression in subjects is related to a high virus load and accelerated progression to HIV disease (206). Another study with coinfecting patients showed a marked increase in circulating levels of the soluble form of the human leukocyte antigen G (HLA-G), a nonclassical histocompatibility complex class I molecule with immunosuppressive properties (88).

Interactions between HIV and *Leishmania* are not only restricted to the replication of the virus. Clinical studies have demonstrated that the high incidence of disseminated leish-

maniasis in AIDS patients (141) and the high peripheral parasitemia (45) are indicative of uncontrolled parasite growth. In concordance with this condition, experimental in vitro coinfection of monocyte-derived macrophages with HIV-1 and *L. donovani* (271) or *L. infantum* (276) promastigotes showed a significant enhancement of intracellular parasite growth compared with parasite infection alone. The lack of control of intracellular multiplication is probably related to the HIV-1-mediated impairment of an effector function carried out by macrophages, such as phagocytosis, intracellular killing, chemotaxis, and cytokine production (126). Despite the fact that HIV-1-infected monocyte-derived macrophages display an impaired capacity to phagocytose numerous pathogens, *Leishmania* uptake is increased following virus infection (276).

In immunocompetent individuals, the protective *Leishmania*-specific immune response is associated with a Th1 cytokine profile, while susceptibility to *Leishmania* infection and disease progression are related to a Th2 cytokine response (127). It has been confirmed that HIV-1 inhibits the proliferative response to *L. donovani* (269); this reduced cellular response might be because the known inductive signal for gamma interferon (IFN- γ) is lacking or could be due to the direct influence of anti-inflammatory Th2 cytokines. Wolday et al. (272) confirmed that HIV-1 can modulate in vitro and ex vivo cytokine production in response to *Leishmania*; the addition of HIV-1 to cultures decreased IFN- γ , IL-12, and IL-18 production and increased IL-4 and IL-10 production in response to *Leishmania* by peripheral blood monocyte cells from healthy donors, while cells from HIV-1-infected and coinfecting patients failed to secrete IFN- γ in response to *Leishmania*. Analysis of the ex vivo production of cytokines by peripheral blood monocyte cells from coinfecting patients showed that greater quantities of IL-4 and IL-10 were released than those from HIV-positive patients not infected with *Leishmania*, and production of IFN- γ showed no changes after stimulation with phytohemagglutinin (174). In addition, patients with VL and HIV-1 infection have decreased circulating levels of IL-15, a cytokine that enhances the Th1 response and potentiates the immune response to intracellular human pathogens (85). All of these data suggest that a shift toward the Th2 type of specific T-cell immune response to *Leishmania* occurs in patients with T-lymphocyte defects induced by HIV-1, which would partly explain the high susceptibility to *Leishmania* infection, the high severity of the disease, and why reactivations occur in these patients (49).

Barreto-de-Souza et al. (25) recently studied the role of the transcriptional transactivator (Tat) protein, an essential viral gene product for HIV-1 replication, in the exacerbation of *Leishmania* proliferation in human macrophages. Tat is secreted in large amounts by cells infected by HIV-1 and is able to stimulate secretion of IL-1 β , IL-4, IL-6, IL-8, transforming growth factor beta (TGF- β), TNF- α , and TNF- β . The results showed that Tat protein was able to override the leishmanicidal effect of IFN- γ and to enhance *Leishmania* replication in human macrophages through the production of prostaglandin E₂ in the macrophage, which in turn increases the synthesis of TGF- β 1 and the augmentation of *Leishmania* growth.

A review of the literature confirms that there is a multifaceted interaction between *Leishmania* and HIV-1 in the macrophage host cell. But the macrophage is not the only cell type

capable of hosting both pathogens; DCs can also be infected by *Leishmania* parasites and HIV-1 (168, 250). There is no information on the consequence of both pathogens being present in DCs, but it has been proposed that such coinfection might modify the course of infection for each pathogen (102). Furthermore, the ability of both *Leishmania* and HIV-1 to bind to the same DC receptor, DC-SIGN (DC-specific ICAM-3-grabbing nonintegrin), to gain entry into DCs (51, 65) might affect the life cycle of the other pathogen. It has been demonstrated that HIV-1 transmission to CD4⁺ T cells is diminished when DC-SIGN-expressing cells are preincubated with *Leishmania* amastigotes before being pulsed with virions (273). Nevertheless, the consequence of both parasite and virus competing for the same DC receptor is unknown, and the importance of DCs as a virus and parasite reservoir, as well as the impact of both pathogens being present in DCs at once in coinfecting patients, remains to be determined.

CL-HIV Coinfection

CL occurs in humans as a localized (LCL), mucocutaneous (MCL), or diffuse (DCL) form, depending on the infecting species of *Leishmania* (2, 115) and the human host. The pathology of LCL in humans is similar to the pathology observed in experimental infections of resistant inbred mouse strains. The presence of amastigotes within infected macrophages is the hallmark of the disease. In early lesions, the dermal infiltrate consists mainly of macrophages filled with amastigotes (4), with lymphocytes and plasma cells appearing later, in association with edema in the superficial dermis and hyperkeratosis of the epidermis, followed by ulceration. Over the following months, a granulomatous infiltrate consisting of lymphocytes, epithelioid cells, and multinucleated giant cells emerges as the number of amastigotes and macrophages declines. Healing follows in most cases, occurring early (within 3 months) in lesions caused by species such as *L. major* or *L. mexicana* or being delayed (over 10 months) in lesions of *L. tropica* or *L. braziliensis*. Infection with a small number of parasites might lead to natural healing and immunization (2), which is the strategy applied in "leishmanization."

Resolution of disease correlates with the development of a successful Th1 cellular immune response driven by IL-12- and IFN- γ -mediated expression of TNF- α (17) and inducible nitric oxide synthase (iNOS) (240), with production of NO and parasite killing (198). It is likely that sterile immunity is not achieved and parasite persistence promotes protection against reinfection. T regulatory cells have been demonstrated in LCL lesions due to *L. braziliensis* (52).

Atypical forms of CL appear in unusual locations not normally exposed to sandfly bites, such as the genitals, palms, and feet, and CL can exhibit unusual morphology (erysipeloid, verrucoid, psoriasiform, eczematoid, or zosteriform). Failure of the immune system to control parasite multiplication might lead to infection in the superficial layer of the papillary dermis in the eczematoid, erysipeloid, and psoriasiform forms or in the reticular dermis in sporotrichoid forms of CL (2). Multiple lesions of LCL can occur because of multiple inoculations by infected sand flies but can also result from parasite dissemination (130). A chronic form of LCL develops in cases where the

protective response is insufficient and lesions persists for over 1 year (2).

CL has been compared to leprosy as a "spectral disease" (263). CL recidivans (2) and LCL would correspond to tubercloid forms of leprosy, with a well-developed cell-mediated immune response characterized by a positive skin test, low levels of antibody, small numbers of parasites in the lesions, and a Th1 cytokine pattern dominated by IFN- γ and the presence of NO in lesions. At the other extreme of the cutaneous forms would be DCL, analogous to lepromatous leprosy, with a failure to mount a delayed-type hypersensitivity response characterized by a negative skin test, dissemination of lesions in the skin with large numbers of parasites, and a Th2 regulatory type of cellular response with increased IL-4 (14, 242), IL-10, and TGF- β (86), with the associated inflammatory dermal chemokines (217). Nevertheless, an explanation for the variable spectrum of CL based on the host response may only be valid within an area where the same species of *Leishmania* are distributed. Even then, the virulence of the parasite would be expected to play a role. It has been reported that CL caused by *L. aethiopica* manifests as either LCL or DCL depending on the propensity of the parasite strain to induce IFN- γ or IL-10 (3, 4), although this could not be substantiated in subsequent studies investigating the genetic variability of *L. aethiopica* strains (231).

HIV infection promotes a Th2 immune response (61) which is detrimental to leishmaniasis. In *Leishmania*-HIV coinfection, particularly in later stages of HIV disease with marked CD4⁺ T-cell depletion and reduced production of IFN- γ , lesion healing may be compromised because of the impaired capacity of macrophages to kill amastigotes. Appropriate recognition of *Leishmania* antigens and stimulation of B lymphocytes depend on an effective CD4⁺ T-cell response which, when compromised, manifests in an inhibition of the in vitro proliferative response to leishmanial antigens and a failure to mount a delayed-type hypersensitivity response in vivo, with a predominant humoral response from an oligoclonal B-cell population that favors parasite multiplication, dissemination, and unusual localization.

Most of the literature on the clinical course of CL-HIV coinfection highlights atypical presentations, usually of severe and unusual forms. It is likely that the impact of the HIV-driven Th2 shift on the pathogenesis of CL in coinfecting patients depends on the degree of immunosuppression and the loss of CD4⁺ T cells. For example, the probability of ulceration is reduced in coinfecting patients with low CD4⁺ T-cell counts (256). However, the relationship is not necessarily linear. Among six African HIV-positive patients with cutaneous *L. major* infection seen in an outpatient clinic in France between 1997 and 2002, five had DCL and none showed parasite dissemination beyond the lesion, despite a high viral load and very low CD4 levels in the peripheral blood (<10 cells/ μ l) (99). In DCL, where the tendency for a Th2 response is already established, synergy could emerge early with severe negative consequences for the course of leishmaniasis.

Unusual clinical presentations of CL have been reported from sites of endemicity for probably all *Leishmania* species. These are consistently associated with clinical polymorphism and difficulty in differential diagnoses (80) in the different geographical areas (111, 137, 151, 219, 244). Some patients

manifest with several forms (papulonodular, ulcerative, infiltrative, lepromatous and diffuse, psoriasis-like, cheloid, histioid, or Kaposi's sarcoma-like) at the same time. "Dermotropic" variants of *L. infantum* as well as *L. braziliensis*, *L. mexicana*, and *L. amazonensis* have been reported to cause visceral disease in HIV-positive patients. Cutaneous involvement occurs in 8 to 12% of VL-HIV-coinfected patients (268). "Viscerotropic" *Leishmania* sp. variants, such as *L. infantum* or *L. chagasi*, can be found in cutaneous lesions and even in apparently healthy skin (181). In general, the dermatological manifestations of coinfection (reviewed in reference 197) tend toward dissemination and abundance of amastigotes in the dermis and in keratinocytes, with various histopathological findings depending on immune status, often with scarce mononuclear and lymphocyte infiltrates, and delayed healing.

For *L. aethiopica*, recurrence was reported from healed LCL lesions in two cases, one where the LCL reappeared as MCL after 12 years and another where a successfully treated LCL lesion reappeared 5 years after healing (30). Recurrence of lesions in LCL due to *L. aethiopica* is estimated to occur in <1% of cases (20). It is not known whether the frequency of recurrence (reactivation or reinfection) is higher in HIV-coinfected patients.

Leishmania major was reported to cause DCL in a child with AIDS (104), in four coinfecting patients with CD4 levels below 10/ μ l, with no visceralization and no relapse after successful treatment (99), and in a severely immunocompromised patient in Burkina Faso, who failed to respond to treatment courses of amphotericin B deoxycholate (AMB), sodium stibogluconate, and liposomal AMB (L-AMB) but responded to miltefosine (234). A longitudinal study in the same country reported clinical polymorphism and a variable treatment response for 32 CL-HIV patients followed over a period of 16 months, with some patients presenting with more than one clinical form (173). DCL in an HIV patient with a CD4 count of 98/ μ l responded dramatically to meglumine antimoniate treatment (171). In Sudan, *L. major* was identified in a Kaposi's sarcoma lesion in an HIV-coinfected patient (90).

In *L. guyanensis* infection, higher rates of recurrence (or reinfection) were observed for CL-HIV-coinfected patients. Coinfecting patients with moderate immunosuppression (CD4 levels above 200 cells/ μ l) had more lesions and a lower rate of recovery after one treatment cycle with pentamidine than their HIV-negative counterparts (66). Relapse was reported after repeated cycles of treatment for one coinfecting patient (CD4 count of 582/ μ l) who had mucosal and cutaneous involvement with abundant amastigotes, even in clinically healed lesions, and a negative skin test (84).

PKDL, a form of CL that generally appears after successful treatment of VL, is characterized by maculopapular or nodular lesions on the face, limbs, or trunk, with a heavy infiltrate of plasma cells and lymphocytes (118), and occurs in association with *L. donovani*. *Leishmania infantum* has also been incriminated in PKDL lesions in coinfecting patients (223, 249).

In summary, HIV coinfection has the potential to negatively affect the course of CL as immunosuppression progresses by promoting parasite dissemination, atypical localization, unusual and more severe clinical presentations, delayed healing, poor responses to treatment, more frequent recurrences, and difficulty in diagnosis, irrespective of the etiologic species of

Leishmania involved. The major pathophysiologic basis for this appears to involve an enhanced shift to a predominant Th2 response driven by the HIV-mediated immune dysregulation in the human host, which undermines the mainly Th1 response that is characteristic of CL in patients who are not immunocompromised. On the other hand, CL might be unmasked or show deterioration during the course of immune recovery following HAART (192) in HIV-coinfected patients.

DIAGNOSIS

For HIV patients, the diagnosis of leishmaniasis may be difficult because the clinical manifestations typical of the disease are not always present and/or patients may present with nonspecific clinical signs. Moreover, splenomegaly is less frequent in coinfecting patients (189), and 42 to 68% of these patients have other associated opportunistic infections with similar symptoms, which further complicates the clinical diagnosis (224). The prevalence of different forms of leishmaniasis depends on the particular geographical area. VL is the most prevalent clinical form, especially in southwestern Europe and Ethiopia, where the majority of coinfection cases have been recorded, although cases of CL and MCL, with or without an association to VL, have been reported.

Parasitological Diagnosis

The detection of leishmanial parasites by microscopy or culture in different tissue samples is considered the gold standard for the diagnosis of leishmaniasis in HIV-coinfected patients and may be a useful way of detecting treatment failures.

Microscopy. Bone marrow aspirate microscopy has been the most frequently employed diagnostic technique for VL-HIV cases, with reported sensitivities of 67 to 94% (7, 82, 160, 189). Observation of amastigotes in peripheral blood-stained smears is a noninvasive method that yields a positivity rate of 50 to 53% for coinfecting patients (146, 153). Splenic aspiration is considered the most sensitive method for the diagnosis of VL, but the risk of hemorrhages cannot be discounted (approximately 0.1%). Therefore, this procedure has to be performed in equipped settings by experienced clinicians (124). Demonstration of the parasite in spleen or lymph node aspirates is a common procedure in countries where leishmaniasis is endemic and has also been applied to the diagnosis of coinfecting patients (30, 143). Lymph node aspiration is less sensitive but still frequently used in Sudan, where lymph node enlargement is a common sign in VL patients (21, 277). Microscopic examination of hepatic biopsies has shown a sensitivity of 87% (160). Amastigotes can also be found in unusual locations, such as the lungs, larynx, tonsils, digestive tract, rectum, spinal fluid, and others (9, 175, 220).

A good microscopic diagnosis should be done on the basis of the observation of two slides of smears or touch preps from samples obtained by needle aspiration or biopsy stained with Giemsa stain or other Romanowsky-type stains. They should be examined for at least 1 hour, looking for amastigotes. The experience of the microscopist is crucial to the accurate diagnosis of VL. Nevertheless, false-negative results can be obtained due to the small number of *Leishmania*-infected cells as a consequence of pancytopenia. Parasitic load can also be

affected by previous treatments with pentamidine or AMB against concomitant infections.

Culture. The culture of splenic aspirates has shown a high sensitivity (63 to 100%) (141, 189) for *Leishmania*-HIV-coinfected patients, but due to the risk of internal bleeding, bone marrow aspiration is recommended because it is safer and has a similar sensitivity (50 to 100%) (9, 188). The culture of mononuclear peripheral blood cells reaches a sensitivity of 64 to 67% (140, 149). Cultures have to be examined every week by light microscopy, looking for promastigote forms, and subcultured into fresh medium. Cultures are considered negative if no flagellates are observed after 4 weeks.

One of the most remarkable features of coinfecting patients is their tendency to relapse, which often causes them to refuse successive bone marrow aspirations, and therefore the option of noninvasive samples, such as blood, is extremely useful in the diagnosis of leishmaniasis for these patients.

Serological Diagnosis

Serological tests are simple, noninvasive methods but have limited diagnostic value because over 40% of coinfecting individuals have no detectable specific antibody levels against *Leishmania* (9). This is probably the consequence of an oligoclonal B-cell response due to the absence of T cells that can recognize specific *Leishmania* antigens and make the presentation to the B cells. Nevertheless, seropositivity varies with the serological test employed. Therefore, it is recommended that at least two different serological tests are used for each patient in order to significantly increase the sensitivity of antibody detection (83).

The sensitivities of different serological techniques for the diagnosis of coinfecting patients reported in the literature can be summarized as follows: 11 to 67% for indirect immunofluorescent antibody test (152, 163), 76 to 89% for enzyme-linked immunosorbent assay using crude antigen (80), 22 to 62% for enzyme-linked immunosorbent assay using rK39 antigen (80, 152), and 74 to 85% for immunoblotting (80, 152, 163). A high sensitivity (95%) of the direct agglutination test (DAT) for Ethiopian VL-HIV-coinfected patients has also been reported (114). Similar DAT results for Ethiopian patients were obtained by Oskam et al. (179) and Schoone et al. (233), using a fast agglutination screening test (a modified version of DAT performed within 3 hours with only one serum dilution), with sensitivities of 89% and 90% reported in the respective studies. The DAT has been validated in several areas of endemicity (57). Unfortunately, the technical requirements of the DAT procedure (e.g., micropipettes, microtiter plates, etc.) restrict its use to referral hospitals or well-supported health centers.

The rK39 dipstick test is a simple, fast, noninvasive serological method that has shown high sensitivity and specificity in the diagnosis of immunocompetent VL patients from different countries. Unfortunately, two different rK39 dipstick formats showed poor sensitivities, of 20% (CORIXA-IDRI, Seattle, WA) and 22% (ACON Laboratories, Bethlehem, PA), respectively, in the diagnosis of European coinfecting patients (80). Studies on *Leishmania*-HIV-coinfected patients from countries where leishmaniasis is endemic are needed in order to assess the diagnostic value of the dipsticks under field conditions.

Serological cross-reactivity between *Leishmania* and other infectious agents, such as *Trypanosoma* and mycobacteria, has been reported widely for immunocompetent patients. Patients with visceral leishmaniasis can even have false-positive HIV test results. Nevertheless, a few false-positive results are less of a problem than are false-negative results, taking into account the high percentage of coinfecting patients showing a partial, weak, or absent antibody response to *Leishmania*, as for other infections (152).

Antigen Detection

A new latex agglutination test (KATex; Kalon Biological, United Kingdom), designed to detect *Leishmania* antigens in urine, was recently developed. KATex was highly sensitive during clinical episodes when the parasitic load was high: in two studies of coinfecting patients in Spain, the sensitivity was 85.7% and 100%, and the specificity in the second study was 96% (209, 264).

Riera et al. (209) found that KATex was negative for coinfecting patients who were considered cured (without amastigotes in bone marrow aspirates or blood after treatment), indicating that this test could be used as an infection marker. Furthermore, antigenuria becomes negative after successful chemotherapy, and no relapses were observed in the majority of these patients, showing that KATex can be a useful tool for monitoring the efficacy of treatment. Coinfecting patients usually suffer numerous relapses, and it is very difficult for them to achieve parasitological cure. Nevertheless, when antigen detection in urine was repeatedly negative after treatment, the probability of relapse was only 5% at 6 months. KATex also detected *Leishmania* antigens in asymptomatic periods between relapses, indicating subclinical infection. These results are in agreement with those obtained by Sundar et al. (252) for immunocompetent VL patients from India, by El-Safi et al. (93) for Sudan, and by Cruz et al. (69) for pediatric patients from Spain. In a recent study undertaken in Spain, *Leishmania* antigen was undetected in the urine of HIV-positive asymptomatic *Leishmania* carriers by PCR, probably because of low levels of circulating parasites in these individuals (101).

Molecular Diagnosis

PCR is a useful technique that has been used for the detection of different *Leishmania* species. PCR protocols can be applied to numerous clinical samples, with a better performance than the classical parasitological methods. The analytical sensitivity is around 0.01 parasite per reaction, and home-made protocols are routinely used in many laboratories worldwide.

Leishmania PCR assays using bone marrow samples and peripheral blood as clinical specimens proved to be a reliable tool for the diagnosis of VL in HIV-positive patients. PCR sensitivity for bone marrow samples ranges from 82 to 100% (68, 131, 185), while blood samples offer a noninvasive alternative, with sensitivities ranging from 72 to 100% (53, 68, 97, 190). PCR is a useful technique for monitoring long-term efficacy of treatment and for prediction of relapses due to its high performance with noninvasive samples (16, 68, 210).

PCR has been applied to the detection and identification of

Leishmania at different taxonomic levels and can even be used directly for clinical samples (232). Infections with other monoxenous trypanosomatids have been described for immunocompromised patients who displayed symptoms of CL or VL (58), which could contribute to false-positive results obtained by microscopy, culture, or even serological techniques, such as the indirect immunofluorescent antibody test. However, the excellent specificity associated with PCR overcomes this problem (58).

Real-time quantitative PCR (RTQ-PCR) was recently applied to the diagnosis and monitoring of *Leishmania* infections. Its main advantages include a reduction in the time needed for the assay and the possibility to quantify the parasitic load of the clinical sample. Based on small-subunit (SSU) rRNA gene amplification, Bossolasco et al. (45) obtained an analytical sensitivity of 0.625 parasite/ml and estimated that an increase in the parasitic load in peripheral blood above 10 parasites/ml preceded clinical relapse in *Leishmania*-HIV-coinfected patients after treatment. Using kinetoplast DNA as a target, Mary et al. (147) developed an RTQ-PCR with an analytical sensitivity of 0.001 parasite DNA equivalents/ml. RTQ-PCR analysis of blood samples from asymptomatic carriers of *Leishmania* in the Mediterranean area was able to detect disease in 58% of healthy subjects, compared with 16% detected by immunoblotting. Q-PCR with blood samples constitutes a non-invasive alternative to bone marrow aspiration in the diagnosis of leishmaniasis and represents a very sensitive tool for monitoring drug therapy and for follow-up in coinfecting patients to confirm relapses.

An algorithm (Fig. 2) was recommended for the diagnosis of leishmaniasis in HIV-infected patients (WHO, presented at the Fifth Consultative Meeting on *Leishmania*-HIV Coinfection, Addis Ababa, Ethiopia, March 2007 [WHO/CDS/NTD/IDM/2007-7]).

CLINICAL ASPECTS OF *LEISHMANIA*-HIV COINFECTION

Clinical manifestations of leishmaniasis and *Leishmania*-HIV coinfection depend on both the infecting species of *Leishmania* and the cell-mediated immune response of the host. Data and studies on VL-HIV coinfection come mainly from Europe, where *L. infantum* is predominant: *L. donovani* is the infecting species in Africa and the Indian subcontinent. Data outside Europe are scarce, despite the increasing incidence of VL-HIV in these regions. *Leishmania* infection may remain asymptomatic, restricted to the skin in LCL and DCL and to the mucous membranes in MCL, but more commonly spreads systemically in VL. All forms, most notably VL, behave as opportunistic infections in immunocompromised HIV-infected people (9, 181).

In Southern Europe, 94% of patients with *Leishmania*-HIV coinfection suffered from VL, and 4% had CL (266). In East Africa and the Indian subcontinent, VL is the commonest form, but exact data are lacking. In Brazil, 43%, 37%, and 20% of the burden of coinfection are due to MCL, VL, and CL, respectively (199).

VL-HIV Coinfection

Clinical manifestations of VL caused by *L. infantum* and *L. donovani* in HIV-infected people are not significantly different from those in non-HIV-infected people (71, 133, 141, 143, 160, 166, 181, 188, 189, 205, 220, 224, 266). Classical features are fever, weight loss, hepatosplenomegaly, and pancytopenia. Localization of parasites in VL-HIV coinfection is broadly similar to that described for immunocompetent subjects (188, 189), although whether the frequency of leishmaniasis found in atypical locations is higher for VL-HIV-coinfected patients has not yet been confirmed with large comparative studies (220, 224).

At the time of diagnosis of the first episode of VL, 79 to 100% of HIV-positive patients coinfecting with *L. infantum* had a CD4⁺ cell count of <200 cells/ μ l (133, 141, 166, 188, 189, 205, 224), and 42 to 72% of patients met the AIDS-defining criteria before or during the first episode of VL (133, 141, 160, 166, 189, 205). The CD4 counts of those with *L. donovani* infection are not published. The clinical presentations of VL in coinfecting patients who have relapsed are comparable with initial episodes of VL (141, 189) and have not changed since HAART was introduced, except for the fact that the percentage of concomitant opportunistic infections has decreased (96, 265). Patients receiving HAART showed significantly lower frequencies of thrombocytopenia and leukopenia, as well as a lower frequency of increased liver enzyme levels, than did patients who did not receive HAART during episodes of VL (96).

In contrast to the clinical manifestations, the clinical course and prognosis of VL in HIV-infected individuals differ importantly from those in non-HIV-infected individuals. VL-HIV coinfection is characterized by significantly lower cure rates (143, 189, 216), higher drug toxicity (189, 215), higher relapse rates (9, 141, 188, 189, 215), and higher mortality rates (141, 189, 216) than those for VL in non-HIV-infected individuals.

In the period before HAART was available, cure rates in Europe and Africa after the first episode of VL in HIV-infected patients ranged from 56 to 90% (133, 141, 143, 189), but the impact of HAART on cure rates of VL in HIV-infected patients has not been established clearly.

Drug toxicity in HIV-related VL has become a well-documented problem. In an evaluation of pentavalent antimonial treatment, adverse effects were observed in 56% of patients, and therapy had to be discontinued permanently due to serious adverse effects (mainly acute pancreatitis) in 28% of patients. A further 12% of patients died due to severe pancreatitis directly attributable to treatment (78). Although data from Africa and India are lacking, the high frequency of vomiting and mortality among antimonial-treated HIV-positive patients in Ethiopia suggests that similar toxicity occurs (215, 216). In addition, it has been demonstrated in vitro that sodium stibogluconate stimulates HIV-1 replication (22).

Relapses are an important feature of VL-HIV coinfection. If a patient does not receive HAART, relapse rates increase to 60% within 6 to 9 months posttreatment and to 90% within 12 months, with the mean interval length between subsequent relapses becoming shorter (138). In a comparative study by Pintado et al., a relapse rate of 56.6% occurred within 3 months after apparently successful treatment of VL (189). HAART with immune reconstitution and undetectable viral RNA loads does not prevent VL relapse (55, 96, 265). In

patients receiving HAART, 80.6% had undetectable viral loads at the time of VL relapse (96). The exact impact of HAART and other factors on reducing relapse rates and the length of the interval between relapses has still to be determined. Some studies indicate that the period between relapses is prolonged by HAART (138, 156). Suffering a relapse increases the risk of a further relapse (265). This may be related to the poor increase in CD4⁺ cells observed in relapsing patients compared with that in nonrelapsing patients, despite complete suppression of HIV replication (77, 156).

HAART has greatly reduced the incidence of symptomatic first episodes of VL in Europe (89, 96, 222, 262), but this effect has not yet been seen in affected regions in South Asia or Africa, where access to free HAART is very limited. Demonstrated risk factors independently associated with the emergence of first episodes of VL, besides HAART, are CDC clinical category C and a CD4⁺ cell count of <300 cells/ μ l (262).

The mortality rate for VL-HIV-coinfected patients is much higher than that for HIV-negative VL patients. During a 1-year follow-up in a comparative study, 53.7% of HIV-positive patients died, compared with 7.5% of HIV-negative patients ($P < 0.001$). Factors that predicted survival of HIV-positive patients were HAART, a high CD4⁺ cell count, and the use of secondary prophylaxis for VL (189). Mortality during VL treatment is significantly higher among coinfecting patients; a relative risk of 9.25 ($P = 0.00005$) was found in Ethiopia (216). Mortality during treatment is fourfold higher when antimonials are used than when miltefosine is used (215).

CL-HIV Coinfection

HIV-positive patients with CL should be examined carefully for the coexistence of VL. LCL in HIV-infected individuals tends to be associated with the initial stages of HIV infection and minimal immunosuppression and is clinically and histopathologically identical to LCL in non-HIV-infected individuals, with isolated papules or plaques observed on exposed areas. HIV-induced immunosuppression facilitates dissemination of amastigotes, leading to DCL that is usually, but not always (50, 62, 89), associated with VL (116, 170).

LCL and DCL are characterized by a higher rate of recurrence or reinfection and are more difficult to treat than CL in HIV-negative patients (29, 66). The incubation period does not seem to be affected by HIV status (99). The impact of HAART has not been studied.

MCL-HIV Coinfection

MCL is prevalent mainly in South America, but reports of coinfection with HIV are scarce. Sporadic cases have been described in Brazil (13, 42, 84) and other South American countries, as well as India (244), Spain (157), and Ethiopia (29). MCL may be severe in HIV-infected patients, with dissemination and a relapsing course.

PKDL and HIV Coinfection

PKDL is common in HIV-negative patients, appearing shortly after or even during treatment in 50% of VL cases in Sudan and, after a longer period, in 5 to 10% of VL cases in

India (279). PKDL manifests as maculopapular or nodular lesions on the face, trunk, and limbs. Only a few cases of PKDL in HIV-positive patients have been reported in the literature (26, 211, 249). In Ethiopia, severe PKDL was more common in HIV-positive (27.3%) than in HIV-negative (13.3%) VL patients at a 6-month follow-up (216). Case reports of VL-HIV-coinfected patients have described cutaneous lesions that precede (159), accompany (18, 44, 64, 105), and follow (50, 56) VL after treatment. It is unclear whether these are forms of PKDL or parasitized skin lesions formed during the course of VL. In addition, an association between PKDL and immune reconstitution disease (IRD) soon after the start of HAART has been described (26, 103, 208). Interestingly, PKDL-IRD also occurs after VL caused by *L. chagasi* (38) and *L. infantum* (47, 208, 249), which are usually not associated with PKDL.

IRD in *Leishmania*-HIV Coinfection

One recent study suggests that patients with untreated subclinical VL do not develop symptomatic disease when started on HAART (77). There are, however, small numbers of reported possible cases of IRD. In France, two asymptomatic patients developed VL (*L. infantum*) within 2 weeks of HAART (37), while another report described a patient who had not been in a area of leishmaniasis endemicity for 8 years but who developed a first episode of VL following initiation of HAART (5). Furthermore, first episodes of VL were described for three severely immunocompromised patients, all with undetectable viral loads, who had just started HAART (122). A few possible cases of IRD with DCL (192), CL (128), and PKDL (26, 103, 208) have been described.

TREATMENT OF LEISHMANIASIS IN HIV-POSITIVE PATIENTS

The treatment of leishmaniasis in HIV-positive patients is characterized by a reduced number of therapeutic options. Drugs are inaccessible because of their price, lack of registration, toxicity, or ineffectiveness or because they have not yet been tested in these patients (Table 4) (10). There are few clinical trials analyzing the efficacy of the different treatments for *Leishmania*-HIV-coinfected patients (Table 5). In general, these patients have lower cure rates, higher drug toxicity rates, and higher fatality rates for leishmaniasis than do immunocompetent patients. Low CD4⁺ cell counts may prevent a full recovery, and multiple relapses often become unresponsive to all the previously used drugs. The treatment of coinfecting patients is confounded by the scarcity of formalized clinical data. Another potential pitfall occurs when extrapolating treatment for *L. donovani* in Africa or Asia to the experience in Europe, where *L. infantum* probably has different drug susceptibilities and less virulence and seems to occur at lower CD4⁺ cell counts. Optimal treatment regimens have yet to be established for any region. A parasitological response to treatment is not indicative of an absence of future relapses (134, 135, 189), while a clinical response does not necessarily indicate parasitological clearance (141). Because recovery without relapses in immunodepressed patients has not yet been achieved, secondary prophylaxis must be considered, but there is little evidence of its efficacy.

TABLE 4. Drugs used for treatment of VL

Product	Brand name	Manufacturer	Areas of licensure for VL ^a	Price per unit	Standard treatment protocol ^d
Sodium stibogluconate (pentavalent antimonial)	Pentostam	GlaxoSmithKline	Af, Am, Ind, Eur	40 GBP (public sector price from GSK) per vial at 100 mg/ml (100 ml)	20 MKD for 30 days
		Albert David (India)	Af, Am, Ind, Eur	3.25 Euros per vial at 100 mg/ml (30 ml) (via International Dispensary Association Amsterdam)	20 MKD for 30 days
Meglumine antimoniate (pentavalent antimonial)	Glucantime	Sanofi-Aventis	Af, Am, Ind, Eur	US\$1.20 per ampoule at 85 mg/5 ml (no profit-no loss price, agreed with WHO, for treatment of leishmaniasis)	20 MKD for 30 days
AMB	AmBisome	Combinopharm (Spain)	Af, Am, Ind, Eur	US\$1.91 per vial of 50 mg (236a)	1 MKD for 15 days (alternate days)
L-AMB		Gilead	US, Eur	US\$20 per vial of 50 mg (reduced price, agreed with WHO, valid for public sector in developing countries for treatment of leishmaniasis)	20-mg/kg total dose
ABLC	Abelcet	Zeneus	No	82.13 GBP per vial of 100 mg/20 ml ^b	No standard treatment protocol but has been used in doses similar to those for L-AMB
L-AMB in colloidal suspension with cholesteryl sulfate	Amphocil	Beacon	No	104.10 GBP per vial of 50 mg ^c	No standard treatment protocol but has been used in doses similar to those for L-AMB
L-AMB in emulsion	Amphomul	Bharat Serums and Vaccines (India)	Ind	US\$10 per vial of 50 mg	15-mg/kg total dose
Paromomycin	Impavido	Gland Pharma (India)	Ind	US\$10 for full course	15 MKD for 21 days
Miltefosine		Aeterna Zentaris (Germany)	Af, Am, Ind, Eur	108.50 Euros for 56 capsules of 50 mg for the public sector (www.medeor.org)	100 mg/day for 28 days
Pentamidine mesylate	Pentamidine	Sanofi-Aventis	No	Pentamidine is free for the treatment of leishmaniasis (agreement with WHO)	Used for secondary prophylaxis (no known treatment protocol)

^a Af, Africa; Am, America; In, Indian subcontinent; Eur, Europe; US, United States.

^b British National Formulary, March 2007.

^c P. Desjeux, Initiative for One World Health (www.iowh.org), personal communication.

^d MKD, milligrams per kilogram per day.

Antileishmania Drugs

Pentavalent antimonials. Injectable pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) (Table 4) have remained the mainstream treatment for VL since their introduction in the 1940s and are still highly effective, except in regions in India (Bihar) and Nepal, where resistance has rendered them almost useless (212, 255).

A first course of antimonials, at the WHO-recommended regimen of 20 mg/kg of body weight/day for 28 to 30 days, led to clinical and/or parasitological response in 33 to 82% of European coinfecting patients, with relapses being common. The success rates in published studies vary greatly due to high levels of drop-out and early death (78, 134, 135, 141, 189). Drug-induced pancreatitis was the most common cause of treatment discontinuation and death (78, 134, 135, 189). Serious electrocardiac changes have also been reported (135, 141). In Ethiopia, one-third of coinfecting patients died during antimonial treatment (216). Mortality is clearly associated with antimonial therapy, which has a sixfold higher risk ($P = 0.0003$) of mortality than does miltefosine (215). Antimonials were also associated with a higher incidence of severe vomiting (215).

The cause of increased clinical pancreatitis with antimonials in coinfecting patients is not clear (78). It is possible that antimonial toxicity selectively affects the sickest patients, such as

those with VL-HIV coinfection, because the pentavalent antimonial is reduced to the toxic trivalent antimonial to a greater extent in these patients. Toxicity and high mortality during treatment render antimonials unsuitable as a first-line treatment for coinfecting patients.

An additional disadvantage of antimonials is that they were shown to stimulate HIV-1 replication in vitro, a finding that merits further investigation (22).

AMB. AMB has been used widely for the treatment of VL, mainly in India, where it is still highly effective after 10 years of extensive use (178). The risk of nephrotoxicity and hypokalemia necessitates hospitalization and, preferably, biochemical monitoring during treatment, which is difficult to achieve in resource-poor settings. AMB has already been abandoned in Western countries for the treatment of VL, in favor of safer and more effective lipid amphotericin preparations.

In vitro, AMB is a more potent antileishmanial than the antimonials, as its activity does not depend on the patient's immune response (9, 94, 169). However, in coinfecting patients, its superiority over antimonials could not be demonstrated. Initial cure rates ranged from 58 to 82%, with regular relapses. The toxicity rates of both drugs were shown to be similar, with AMB causing renal toxicity in 18 to 36% of patients (134, 141, 189). As with the antimonials, the side effects of AMB in

TABLE 5. Treatment studies for *Leishmania*-HIV coinfection

Region, parasite	Reference	Drug ^f	Regimen	No. of patients with initial cure (clinical and/or parasitological)/total no. of patients (%)	Severity of side effects	No. or % of dropouts	No. of recorded relapses/total no. of patients (%)	Study type
Europe, <i>L. infantum</i>	134	SbV	20 mg/kg, 28 days	29/44 (66)	Most serious (cardiotoxicity in six cases [one patient died] and pancreatitis in three cases)	10	11/24 (46)	Randomized comparative trial
		AMB		28/45 (62)	Most serious (severe chills in 1 case, venous thrombosis in 1 case, and nephrotoxicity in 16 cases)	15	8/24 (46)	
	78	SbV	20 mg/kg, 28 days	5/25 (33) ^a	Most serious (pancreatitis in five cases, acute renal failure in one case, and leucopenia in one case)	10	3/15 (20)	Retrospective study
	135	SbV	20 mg/kg, 28 days	7/19 (37)	Most serious (pancreatitis in four cases [all four patients died], renal failure in two cases [both patients died])	9	4/7 (57)	Randomized comparative trial
		ABL/C	3 mg/kg, 5 days	6/18 (33)	Overall mild (seven cases of infusion-related reactions, one case of renal failure)	1	2/4 (50)	
		ABL/C	3 mg/kg, 10 days	8/20 (42)	Overall mild (eight cases of infusion-related reactions)	2	3/8 (38)	
	189	SbV	20 mg/kg, 3–4 weeks	61 ^b	Hyperparanasal sinusitis in 15.9% of patients, anemia in 14.7% of patients	19%	No data per individual drug	Retrospective study
		AMB	0.5–1 mg/kg, 3–4 weeks	58 ^b	Anemia in 29.4% of patients, renal failure in 17.6% of patients	7%	No data per individual drug	
Ethiopia, <i>L. donovani</i>	31	SbV	20 mg/kg, 28 days	17/23 (74)	No data	0	Not recorded	Prospective study
	141	SbV	20 mg/kg, 28 days	42/51 (82) ^c	Hyperparanasal sinusitis in eight cases and cardiotoxicity in nine cases (one death)	Data not given	No data per individual drug	Retrospective study
Europe, <i>L. infantum</i>		AMB	At least 15-mg/kg total dose	14/17 (82) ^d	Anemia in six cases and renal failure in five cases	Data not given	No data per individual drug	
		PENT	4 mg/kg, 28 days	2/3 (67)	One case of insulin-dependent diabetes	Data not given	No data per individual drug	
	73	L-AMB	100 mg, 21 days	11/11 (100)	No serious side effects; well tolerated	0	8/11 (73)	Open-label dose finding
	226	L-AMB	4 mg/kg on days 1 to 5, 10, 17, 24, 31, and 38	9/10 (100)	No serious side effects; well tolerated	0	7/7 (100)	Open-label dose finding
Ethiopia, <i>L. donovani</i>	215	MILT	100 mg, 28 days	56/63 (89)	Vomiting in 41 cases, 3 of which were serious; diarrhea in 34 cases	3	16 of 52 (31)	Randomized comparative trial
		SbV	20 mg/kg, 40–60 days	40/44 (90)	Vomiting in 20 cases, 7 of which were serious; diarrhea in 32 cases	0	5/35 (14)	
India, <i>L. donovani</i>	243	MILT	100 mg, for a mean of 35 days ^e	25/39 (64)	Overall mild (vomiting in 10 cases, diarrhea in 4 cases)	Data not given	22/25 (88)	Case series

^a One of 25 cases was a relapse.^b Fifty-one patients were started on pentavalent antimonials, and 17 were started on AMB, among whom 8 received L-AMB.^c Allopurinol (three cases) and IFN- γ (one case) were added to therapy.^d In four cases, L-AMB was used.^e For two patients, the daily dose was 150 mg.^f SbV, pentavalent antimonial; AMB, amphotericin B deoxycholate; L-AMB, liposomal AMB; ABL/C, AMB lipid complex; PENT, pentamidine; MILT, miltefosine.

coinfected patients are more severe than those in noncoinfected patients.

Lipid formulations of AMB. All lipid formulations of AMB show a better safety profile than that of AMB, with L-AMB (AmBisome; Gilead USA) considered superior to the other formulations. L-AMB has the highest therapeutic index of all antileishmanial drugs, including the other lipid AMB formulations (33). It is therefore indicated for the treatment of coinfecting patients. The limited data on the use of L-AMB in coinfecting patients were generated mostly in Europe. In other regions, L-AMB is prohibitively expensive, even though it is now available for a nonprofit cost of \$20 per vial for VL in the developing world (Table 4).

For immunocompetent patients, no treatment failures have been observed with the currently recommended total dose of 20 mg/kg (33), except in Sudan. Sudanese patients without HIV were less responsive to L-AMB than were Europeans or Indians: the cure rates for total doses of ≤ 15 mg/kg, 16 to 20 mg/kg, and > 20 mg/kg were 50%, 64%, and 88%, respectively (235). In another study, 10 of 64 Sudanese patients did not respond to treatment with L-AMB (20 mg/kg). This was thought to be related to high initial parasite loads and immunosuppressing underlying diseases (HIV infection and tuberculosis), and it was suggested that higher doses should have been used (165). In Europe, total doses of up to 30 to 40 mg/kg have been evaluated in small numbers of coinfecting patients and were well tolerated, but they did not prevent relapses (73, 226). A dosing recommendation for coinfecting patients cannot be made based on the limited data available. Since the half-life of L-AMB that has accumulated in the spleen, liver, and bone marrow is estimated to be at least 2 to 3 weeks (106), a prolonged high-dose regimen may be necessary to avoid the selection of resistant parasites.

Of the other lipid forms of AMB, AMB lipid complex (ABLC) has been used for secondary prophylaxis, administered every 21 days for 12 months. ABLC was well tolerated, and after 1 year, 22% of patients had relapsed, compared with 50% of patients without secondary prophylaxis (142). Data on secondary prophylaxis with L-AMB are anecdotal (161). An inexpensive lipid emulsion is manufactured in India (Amphomul; Bharat Sera and Vaccines) (Table 4), which is encouraging news for the treatment of VL, but it has not yet been evaluated in coinfecting patients.

Miltefosine. Treatment with miltefosine has consistently been shown to be safe and effective (255a). Relatively mild gastrointestinal side effects are common. A study of 299 Indian patients showed that vomiting occurred in 38% and diarrhea occurred in 20% of patients and generally lasted 1 to 2 days (253); none of these patients were coinfecting. A major pitfall of miltefosine is its potential teratogenic effect, which impedes its use in pregnant or lactating women and women of child-bearing potential not taking contraceptives.

In Ethiopia, side effects with miltefosine are worse in HIV-positive patients; vomiting occurred in 65% of coinfecting patients, compared to 45% of noncoinfecting patients. Miltefosine was used in 39 coinfecting patients from Europe who had relapsed on previous treatments. Cure was achieved in 64% of patients, but almost all patients relapsed again and were re-treated, with moderate success. It was found that prolonged treatment was safe, with one patient receiving treatment for 2

years (243). In Ethiopia, miltefosine was found to be less effective than antimonials in coinfecting patients, with 18% versus 2% of patients failing initial treatment and 25% versus 11% relapsing within 6 months in the miltefosine and antimonial groups, respectively. Overall, mortality in the miltefosine group (6%) was lower than that in the antimonial group (12%) (215).

Miltefosine has a half-life of ~ 1 week, so especially in relapsing coinfecting patients, there is a risk of resistant parasites developing (32), as generated in vitro (239). As with other drugs, the use of miltefosine in coinfecting patients should be prolonged until parasite clearance is achieved. In India, where large-scale miltefosine distribution is planned within the context of an elimination program, controlled use and knowledge of the HIV status of patients are of crucial importance (255a). In Europe and other zones where VL transmission is zoonotic, the use of miltefosine (and other first-line drugs) should not be allowed in dogs to prevent the development of primary resistant strains (255a).

Paromomycin. Paromomycin sulfate was recently licensed for the treatment of VL on the basis of a large phase III trial, where it was shown to be as effective as AMB and was very well tolerated; the main side effects were a reversible elevation of liver enzymes, in approximately 1% of patients, and pain at the injection site (254). Paromomycin is the cheapest of all antileishmanial drugs (Table 4). Paromomycin has a good safety profile and is a broad-spectrum antibiotic active against some gram-positive and most gram-negative bacteria, many protozoa, and *Mycobacterium tuberculosis* (125). Its activity in coinfecting patients has yet to be established. Acquired resistance may easily develop (67, 98, 144) and has been generated in vitro. There has been extensive, favorable experience with combinations of paromomycin plus sodium stibogluconate, with 224 patients receiving a combination in three randomized clinical trials (60, 237, 257), as have thousands of patients treated under field conditions (154). However, data for coinfecting patients are lacking.

Pentamidine. Pentamidine was used to treat VL mainly in India but has now been abandoned due to the severity of its side effects and to poor efficacy. Irreversible diabetes mellitus is a serious complication, occurring in 4 to 12% of cases (178). After a decade of use, the efficacy has declined to $< 70\%$ (67). The scope of safe drugs to treat coinfecting patients is limited, and thus these drugs are not to be used for secondary prophylaxis but reserved for treatment of acute cases. Pentamidine, although toxic in treatment doses, has been considered for use in secondary prophylaxis (183), as intermittent low-dose maintenance therapy is unlikely to show the toxicity seen with a therapeutic dosing regimen.

Following a recent agreement between WHO and the manufacturer (Sanofi-Aventis), pentamidine is now available for free for the treatment of all neglected diseases, including leishmaniasis (Table 4).

Combinations. There is consensus that combination therapy for VL should urgently be introduced to minimize the development of resistant strains (48, 110). This is especially true for coinfecting patients, as repeated exposure to single antileishmanial drugs will inevitably generate resistance, and incurable VL patients (158) or PKDL patients (1) may transmit drug-resistant parasites. Although no clinical trials to date have investigated combination therapies in coinfecting patients, phy-

sicians may use combination therapy in patients with multiple relapses on a compassionate basis. Combinations of miltefosine plus paromomycin, pentavalent antimonials, and AMB were studied in vivo and in vitro; miltefosine plus paromomycin and miltefosine plus AMB were synergistic and should progress to clinical testing (238). In coinfecting patients, even if these combinations do not prevent relapse, they may preserve drug sensitivity in individual patients and therefore prolong survival.

Antiretrovirals

Since 1996, the introduction of HAART in Europe has led to far fewer new VL infections in HIV-positive patients. HAART has reduced the risk of relapse in coinfecting patients, although it is not clear to what extent (55, 77, 96, 138, 156, 265). These results cannot be extrapolated to the developing world, where the condition of patients is much worse and the *L. donovani* species is highly virulent. Nonetheless, it is clear that HAART reduces the morbidity and mortality of coinfecting patients in all regions. In some areas with a high incidence of coinfection (Bihar, India; Ethiopia; and Eastern Sudan), access to HAART remains minimal. HAART is frequently restricted to nongovernmental organizations (NGOs), programs that cover small numbers of patients, while government programs are absent, malfunctioning, or at the first stage of implementation (e.g., in Ethiopia). Only relatively few coinfecting patients in developing countries will be included in well-functioning HAART programs. Where CD4⁺ cell counts are difficult to obtain, VL should therefore be regarded as a valid entry point for HAART. For the African region, VL has been defined as an AIDS-defining disease, which is a pragmatic approach that should be adopted globally (<http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf>).

Indinavir and saquinavir show in vitro activity against *L. infantum* and *L. major* (33, 193, 229), but there is currently no clinical evidence of any HAART being more effective than others in preventing VL relapses in coinfecting patients. Thus, the choice of HAART for coinfecting patients does not differ from that in national programs for HIV.

Other Approaches

It has been suggested that in the absence of an adequate immune response, the addition of IFN- γ or recombinant IL-2 to VL therapy could be useful. However, both have not yet shown any additional benefit (46, 132). Selective splenectomy has been evaluated for relapsing coinfecting patients, but despite being effective in restoring hematological parameters and reducing the need for blood transfusions, it did not prevent further relapses (261); in addition, this procedure should be avoided because of the risk of overwhelming sepsis and severe malaria.

MCL and CL

Evidence to support different treatments is very limited for HIV-negative CL patients (129, 145) and is absent for immunocompromised HIV-coinfecting patients.

In general, to reduce the probability of dissemination and relapse in CL-HIV-coinfecting patients, systemic treatment is

recommended over local treatment, with close monitoring and follow-up of the patient. As long as evidence is lacking, the same treatment used for immunocompetent individuals will be offered to localized CL-HIV-coinfecting patients. Reports of the treatment of HIV-associated MCL are scarce, although the cure of MCL following treatment with L-AMB has been described for a coinfecting patient failing antimonial treatment (13). Miltefosine has been used effectively for treatment of PKDL in HIV-infected patients (26).

FOLLOW-UP OF PATIENTS AND MAINTENANCE THERAPY

Leishmania infection exacerbates the evolution of HIV through an increase of HIV replication and a decrease of the CD4⁺ lymphocyte count. In fact, about half of coinfecting patients develop full AIDS criteria within 2 months after the diagnosis of VL. Furthermore, the course of VL in HIV-positive patients is characterized by the appearance of numerous relapses despite effective antileishmanial treatment. The number of relapses and the average time between them depend mainly on the immunological status of the patient (mainly defined by the CD4⁺ cell count and definitely influenced by HAART) and on the use of antileishmanial maintenance therapy. However, the different evolution of VL among patients suggests that other factors, such as parasitological variability, pathogenic strength of the infecting strain, or the existence of any resistance to antileishmanial drugs, can influence treatment success (87, 95, 218).

The mortality rate for European patients during a first episode of VL is 10 to 19%, which can increase to 24% during the month following the end of treatment, and is related to the toxicity of the treatment, complications arising during the VL episode, and concomitant opportunistic infections. The survival time varies from 3 to 24 months (average survival rate of 60% after 12 months) but can triple if the patient is treated with highly active HAART. After a correctly treated initial episode of VL, 25 to 61% of patients will relapse before 6 to 9 months, and 79 to 97% will relapse during the patient's lifetime (independent of the drug used to treat the first VL episode), with the average period between relapses becoming increasingly shorter (141, 188, 189).

There are three main points to consider in following up an HIV-positive patient who was recently diagnosed and treated for an episode of VL, as follows: (i) the need for test of cure after treatment and prediction/diagnosis of relapse, (ii) prompt initiation of effective HAART, and (iii) the convenience of secondary prophylaxis with an antileishmanial drug as maintenance therapy.

Test of Cure and/or Relapse

In treating immunocompetent patients, it is recognized that tests of cure are performed 1 month and 6 months after the finish of antileishmanial treatment in order to assess parasitological cure. Bone marrow or spleen aspiration is the technique usually performed, and diagnosis of VL is made when *Leishmania* sp. amastigotes are seen in Giemsa-stained samples or promastigotes are cultured in specific medium. For HIV-positive patients, though, parasitological cure is very difficult to

achieve and relapses are very common, so the need for repeated invasive tests of cure could be very uncomfortable or dangerous for these patients. Repeated invasive diagnostic tests should be performed if there is no clinical improvement after treatment or whenever there is a suspicion of VL relapse.

PCR-based assays are useful tools for the diagnosis of VL in coinfecting patients (16, 45, 68, 131, 185, 190, 210). The analysis of peripheral blood samples using PCR can be a useful non-invasive technique for monitoring patients posttreatment, which avoids unnecessary pain to the patient in evaluating the parasitic evolution during follow-up (16, 45, 68, 131, 185, 190, 210). PCR is a sensitive technique for detecting relapses several weeks before the clinical onset of disease. *Leishmania* DNA levels correlate with the clinical course of VL, and their measurement at diagnosis and during and after treatment seems to be useful in the clinical management of VL-HIV-coinfecting patients. Positive nested PCR results during asymptomatic periods indicate the presence of the parasite, but not necessarily relapse (16, 45, 68, 131, 185, 190, 210). However, the presence of viable parasites during posttreatment follow-up increases the probability of relapse and could be a good marker of relapse (16, 45, 68, 131, 185, 190, 210). During relapse of VL, the parasite is detected more effectively in blood samples by nested PCR than by culture.

Antigen detection in urine with a latex agglutination test could represent an alternative noninvasive and easy-to-perform test for the diagnosis of leishmaniasis (sensitivity of 86%) and posttreatment follow-up in coinfecting patients. In the absence of clinical symptoms, the detection of parasite antigens in urine samples is not associated with clinical disease in more than one-half of cases, and when antigen detection in urine is repeatedly negative, the probability of relapse is very low (5% at 6 months) (209).

Antiretroviral Therapy

HAART has dramatically modified the natural history of HIV infection and associated opportunistic infections. Despite the positive data associated with the use of these drugs, the benefits of HAART apply to only a minority of coinfecting patients in the developing world. The effects of HAART on coinfecting patients can be summarized as follows (77, 138, 139, 148, 156, 225, 262, 265). (i) The incidence of new VL cases in HIV-positive patients dropped about 50 to 65% in the Mediterranean area after HAART was introduced. HAART prevents future clinical VL in asymptomatic *Leishmania* sp.-infected patients. (ii) Coinfecting patients who receive HAART have a higher survival rate than those who do not receive it (mean survival of 66 months). (iii) HAART modifies the pattern of VL relapse but is not sufficient to prevent the relapse of the disease. Relapsing VL is possible in HIV-infected patients receiving successful HAART, mainly when CD4⁺ cell levels are <200 cells/ μ l, but even if a very low viral load is achieved. About 40% (25 to 70%) of coinfecting patients receiving HAART without VL maintenance therapy experience relapse, but HAART delays VL relapse, with a longer time between VL episodes (average of 7 months), in patients who receive HAART compared with those not receiving HAART. (iv) The whole possible clinical spectrum of leishmaniasis has been observed in HIV-positive patients, and atypical clinical forms

have been observed no more frequently in patients receiving HAART. There is no correlation between the introduction of HAART and the occurrence of relapses, and the phenomenon of immune restoration syndrome is rarely observed (39, 122).

Maintenance Therapy (Secondary Prophylaxis)

Primary prophylaxis is not indicated. On the other hand, it seems advisable to use maintenance therapy systematically and soon after the first episode of VL to prevent early relapse, which would perpetuate a Th2-type response in cellular immunity and prevent the control of parasitization by *Leishmania* spp. despite effective HAART. The data about maintenance therapy come from the Mediterranean area, where VL is a zoonotic disease caused by *L. infantum* and where there are medical facilities for the follow-up of patients (136). Several questions arise when maintenance therapy is prescribed in other settings where medical care is insufficient for monitoring toxicity (mainly with antimonials) or where VL is an anthroponotic disease (Sudan, Ethiopia, and India) and there is the risk of inducing drug resistance (67). If maintenance therapy is provided in such situations, it should be conducted under a directly observed treatment program (particularly if miltefosine is the chosen drug).

There are enough accumulated data about the benefits of maintenance therapy in coinfecting patients (156, 182, 188, 189, 207), but they are based on open-label, noncontrolled studies. In studies which failed to demonstrate the benefit of prophylaxis, patients tended to have more serious infection or had relapsed more often (133). In the only prospective and randomized study to investigate the effectiveness of a maintenance therapy, ABLC (3 mg/kg every 21 days) was compared with nonmaintenance therapy. At 12 months of follow-up, the benefit of the maintenance therapy was evident in the intention-to-treat analysis; the probability of being free of illness at 12 months was 50% and 22% for the groups with and without maintenance therapy, respectively (142).

Different regimens with several drugs have been used, mainly with pentavalent antimonials (20 mg/kg/day, given every 3 to 4 weeks), AMB (either L-AMB or ABLC) (3 to 5 mg/kg/day, given every 3 to 4 weeks), or pentamidine (4 mg/kg/day [300 mg for an adult], given every 3 to 4 weeks) (43).

Miltefosine is a very effective oral drug against VL (119, 234, 253, 259). There are no data about maintenance therapy with this drug for humans, but once- or twice-weekly oral administration to T-cell-deficient nude mice infected with *L. donovani* was useful as maintenance therapy (167). However, miltefosine requires a long treatment course (28 days) and has a long half-life (around 150 h), which might accelerate the emergence of drug resistance (184). Other oral drugs, such as itraconazole and allopurinol, have some therapeutic efficacy against *Leishmania* spp. and could hypothetically be used in maintenance therapy, together with miltefosine, to increase efficacy or prevent the appearance of resistance (15).

Once a patient's immune function has recovered with HAART and VL is quiescent, prophylaxis therapy could be suspended provided that the CD4⁺ cell count is maintained (>200 cells/ μ l) for more than 6 months (28, 248).

PERSPECTIVES IN *LEISHMANIA*-HIV COINFECTION

The spread and overlap of both leishmaniasis and HIV infections in the major foci of leishmaniasis (India, Brazil, and Eastern Africa) make VL-HIV coinfection a serious worldwide concern. HIV and VL programs should develop integrated and well-coordinated intervention activities, particularly in areas where VL is endemic. Collaboration of the two programs should particularly be strengthened by local government at the district level to focus on the specific circumstances of each area of endemicity. The mobility of people, such as seasonal migrant laborers, urban migration from rural areas, resettlement activities from areas where disease is nonendemic to those where it is endemic, and the presence of refugees and internal population displacements due to war and conflicts constitute major factors for the spread of both diseases. There is a need to develop locally applicable strategies for appropriate intervention in migrant communities.

In areas where transmission is anthroponotic, VL-HIV-coinfected patients should be targeted as potential reservoirs of infection. Preventing the transmission of *L. donovani* is mandatory by providing bed nets. Furthermore, it may be prudent to encourage HIV-positive patients to move from areas of VL endemicity to prevent them from becoming infected with VL. In anthroponotic transmission areas, more studies are required to provide sufficient evidence to recommend maintenance/secondary prophylaxis treatment, and the risk of resistance must also be considered in this situation.

Both HIV and *Leishmania* infections induce a deficit in host humoral and cellular responses that limits the diagnostic value of serological tests for coinfecting patients. Only 40 to 50% of VL-HIV-coinfected patients have detectable specific antibody levels against *Leishmania*. There is a need for comparative analysis studies from different geographical areas, using different serological techniques, including CD4⁺ counts, to characterize serum samples. The high parasitic burden of leishmaniasis in HIV-infected individuals permits the detection of *Leishmania* antigens, but there is only one technique currently available for antigen detection (KAtex, used for urine samples). Research into other antigen detection tests is needed to evaluate their use as active phase markers that allow the detection of parasitological cure and relapse. In addition, more research is needed to link the clinical and parasitological conditions and to establish better criteria for cure, considering the poor prognosis of coinfection and the high risk of relapse.

VL is an opportunistic infection in HIV-infected individuals and should be considered in the definition of stage IV in the WHO HIV/AIDS classification (stage C of the CDC classification). It is crucial to try to restore cell-mediated immunity with HAART as soon as possible to prevent further relapses of VL, even though HAART alone is not enough to prevent VL relapse.

All antileishmanial therapies are less effective in HIV-positive patients. There is a high mortality rate due to concurrent illness, complications, and drug toxicity. Pentavalent antimonials and AMB are more toxic to HIV patients, who require close monitoring for pancreatitis, cardiotoxicity, and nephrotoxicity, and these drugs should be avoided if better drugs, such as liposomal formulations of AMB, are available. In considering combination therapy, drugs should be administered se-

quentially (with the safest given first) rather than concurrently. Miltefosine is a safer drug for coinfecting patients, although there are still problems of teratogenicity and adherence to consider. In general, patients who fail to clear the parasite will relapse, independent of the drug used. Relapses should be treated appropriately, and combination therapy may represent the best approach to avoid drug resistance while balancing benefit with drug toxicity. Drugs used for relapse treatment should be avoided for secondary prophylaxis, although the drug panoply is very limited. Secondary prophylaxis to prevent relapses in areas with zoonotic transmission of *L. infantum* has been reported in several publications, but more evidence from clinical trials is needed to establish a beneficial effect. Treatment options for secondary prophylaxis in VL-HIV-coinfected patients in anthroponotic transmission areas (*L. donovani*) are limited, and the risk of resistance must be taken into account. The use of promising new drugs, such as miltefosine, for canine leishmaniasis can also accelerate the development of resistance, and thus their use in dogs should be avoided to protect their efficacy in humans.

The priorities of treatment-related research should include the evaluation of efficacy and tolerability of alternative drugs, such as paromomycin, pentamidine (maintenance therapy only), and combinations with currently available drugs, in coinfecting patients. Possible benefits to achieve include a shortened duration of treatment, improved efficacy, cost reduction, protection of drug resistance, and improved compliance. The toxicity of combination therapy may be enhanced in coinfecting patients and so should be evaluated thoroughly in well-conducted clinical trials. The only combination that has demonstrated a positive result so far is sodium stibogluconate plus paromomycin. Confirmatory clinical trials are currently ongoing in East Africa to establish clear criteria on the use of maintenance therapy.

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