# Report of the

# Fifth Consultative Meeting on Leishmania/HIV Coinfection

Addis Ababa, Ethiopia, 20–22 March 2007



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The World Health Organization gratefully acknowledges the financial support provided to this meeting by the "Agencia Española de Cooperación Internacional para el Desarrollo"

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Printed by the WHO Document Production Services, Geneva, Switzerland

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# 1. Introduction

The first case of leishmaniasis associated with human immunodeficiency virus (HIV) infection was reported in 1985, and the number of reported cases in southern Europe subsequently increased rapidly. Since that time, 35 countries have reported cases of coinfection.

In 1991, in order to achieve a better evaluation of the magnitude of the problem, WHO established a global network of 28 institutions. Initially, the sites involved in the network were predominantly European, reflecting the epidemiological situation at the time. Meetings held in Montpellier (1991), Rome (1994), Minorca (1998) and Catania (2001) documented the spread of *Leishmania*/HIV coinfection and covered topics that included routes of infection, pathogenesis, microbiological aspects, details of diagnosis and treatment, and secondary prophylaxis. Some documents produced by the network were:

- Report on the Consultative Meeting on Leishmania/HIV coinfection, Rome, 6–7 September 1994. Geneva, World Health Organization, 1995 (WHO/LEISH/95.35).
- Leishmania/HIV coinfection. Weekly Epidemiological Record, 1997, 72:49–56.
- Desjeux P. Leishmania and HIV in gridlock. Geneva, World Health Organization, 1998 (WHO/CTD/LEISH/98.23).
- Desjeux P et al. *Leishmania/HIV coinfection, south-western Europe 1990–98*. Geneva, World Health Organization, 2000 (WHO/LEISH/2000.42).
- Desjeux P, Alvar J, eds. Leishmania/HIV co-infections. Annals of Tropical Medicine and Parasitology, 2003, 97(Suppl.1).

After the introduction of antiretroviral therapy (ART) for HIV, the number of coinfected cases reported in European *Leishmania*-endemic countries fell sharply. However, because of the increasing overlap of the two diseases, the problem of coinfection spread to the countries that are the world's major foci of leishmaniasis. The need to update epidemiological information regarding coinfection around the world and to prepare specific guidelines for case management in developing countries led WHO to organize the Fifth Consultative meeting on *Leishmania*/HIV Coinfection, which was held in Addis Ababa, Ethiopia, from 20 to 22 March 2007.

# 1.1 HIV/AIDS: statistics and features

Statistics for December 2006 show that some 39.5 million people are living with HIV/AIDS, of whom approximately 95% reside in developing countries. 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 several countries of this region, the impact of HIV has been to reduce adult life expectancy by 50%.

Although lack of access remains a major challenge, the number of people receiving ART in sub-Saharan Africa recently exceeded one million for the first time. Weakness of the health systems has been identified as a key obstacle to expanding treatment, care and preventive services. Ensuring compliance is another important issue: one of the lessons learned is that adherence to long-term therapy is improved by simplifying treatment and treating concurrent infections.

#### 1.2Leishmaniasis: statistics and features

As recognized in a resolution of the sixtieth World Health Assembly in 2007, leishmaniasis is among the most neglected of the tropical diseases: more than 12 million people are currently infected worldwide, there are 2 million new cases every year (a number that is growing), and 350 million people are considered to be at risk. The disease affects the poorest populations in 88 countries (mostly developing countries). Two basic clinical forms are recognized – cutaneous leishmaniasis (CL), a disfiguring and stigmatizing disease, and visceral leishmaniasis (VL) or kala-azar, which is fatal without treatment.

The visceral form is present in 70 countries. The largest focus of VL is in the south-east Asian region, with an estimated 300 000 cases in 2006. East Africa has approximately 30 000 cases per year, and the third largest focus is in the Americas with 4000 cases reported in 2006. New foci are appearing at an alarming rate, and incidence in east Africa is on the increase. A lack of surveillance systems and the frequency of misdiagnosis (especially confusion with malaria) mean that true incidence is underestimated; failure to diagnose the disease leads to increased case-fatality rates. Post-kala-azar dermal leishmaniasis (PKDL) is a complication of VL, often occurring after apparently successful treatment of kala-azar. It requires a prolonged course of treatment: patients are highly infectious to sandflies and thus form an important component of the infection reservoir, especially in areas where transmission cycles are solely or predominantly anthroponotic.

Cutaneous leishmaniasis is present in at least 82 countries, with an annual estimated incidence of 1.5 million cases worldwide. Several clinical forms are recognized: localized CL, which often heals without treatment, diffuse CL, which is very difficult to treat, and mucosal leishmaniasis, which is the most severe form, producing disfiguring lesions and mutilation of the face.

#### 1.3 Leishmania/HIV coinfection: statistics and features

The HIV/AIDS pandemic has modified the natural history of leishmaniasis disease. HIV infection increases the risk of developing VL by a factor of between 100 and 1000 in endemic areas, reduces the likelihood of therapeutic response, and greatly increases the probability of relapse. At the same time, VL promotes clinical progression of HIV disease and the development of AIDS-defining conditions. Because the two diseases target similar immune cells, together they exert a synergistic damaging effect on the cellular immune response. Atypical presentations of leishmaniasis are reported in HIV patients, including visceralization of CL and cutaneous involvement in VL.

Coinfection has been reported in 35 countries worldwide. *Leishmania*/HIV coinfection is currently reported in 2–12% of all VL cases – a proportion that is likely to increase dramatically. Among selected populations, such as in Humera, Ethiopia, the proportion with coinfection is 15–30%. It is generally accepted that the reported global incidence is underestimated, at least in part because VL is not on the CDC list of opportunistic infections and thus is rarely reported in AIDS notification systems.

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<sup>&</sup>lt;sup>1</sup> http://ftp.who.int/gb/ebwha/pdf files/WHA60/A60 10-en.pdf

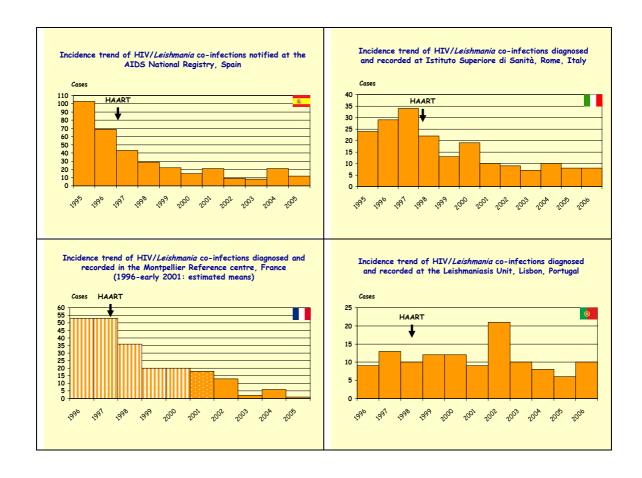
The introduction of ART has radically altered the course of HIV/AIDS infections and the outcome of associated opportunistic infections. However, access to ART in developing countries remains limited. In addition, there are many unresolved questions relating to the management of coinfected patients.

# 2. Epidemiological information on *Leishmania/HIV* coinfection

To date, most reports to WHO of coinfection cases have come from southern Europe: 90% of the more than 2000 cases notified up to the end of 2001 were in France, Italy, Portugal and Spain. However, these figures are misleading, because a large proportion of cases in many countries of Africa or Asia are missed through a lack of diagnostic facilities and poor reporting systems. Most coinfections in the Americas are reported from Brazil, where there are good surveillance systems for both diseases. Brazil is home to 620 000 people living with HIV; the cases of VL in the country increased from 700 cases in 1980 to 3500 in 2005, and for tegumentary leishmaniasis from 4000 cases in 1980 to 32 000 in 2005. The number of coinfection cases has increased dramatically in some areas of Africa as a result of social upheaval (such as mass migration and war). In some areas of Ethiopia (e.g. Humera), 30% of all VL patients are also infected with HIV. In Asia, coinfections are increasingly being reported from India – a country that is also confronting a high rate of resistance to antimony-based anti-leishmaniasis drugs.

# 2.1 Southern Europe

Up to the end of 2001, a total of 1911 coinfection cases had been reported to the WHO database from France, Italy, Portugal, and Spain; 717 of these cases occurred during the "1996–98 peak". Since the advent of ART in Europe in 1997, the number of coinfection cases reported from southern Europe has fallen dramatically in all countries except – for unknown reasons – Portugal. A further 241 new cases (primary infections: 95 from Spain, 64 from Portugal, 52 from Italy, and 30 from France) and a number of persistent cases were reported to WHO during the period 2001–2006. The main clusters of coinfection cases occur in large urban areas and are reported by referral centres; underreporting, however, especially of rural cases, may still occur. Other countries, such as Germany, Greece, Switzerland and the United Kingdom, report sporadic imported cases.



Coinfected patients commonly have many episodes of active VL: Molecular methods confirm that more than 90% of these cases are relapses, rather than reinfections. During the period 2001–2006, Portugal reported 95 primary infections (PI) and 34 relapses (R) (ratio R:PI 0.36), and Spain reported 64 primary infections and. 21 relapses (R:PI 0.33). There was no change in the sex or age distribution of cases during that period (males 85%, females 15%, mean age 38 years) compared with the period before 2001. The distribution of risk groups, however, did change, which may reflect the general decline in AIDS among intravenous drug users (and parallel increase among heterosexuals) in southern Europe as a consequence of health education campaigns addressing intravenous drug abuse.

Risk group	1990 –2000	2001–2006
IVDU	72%	64%
Heterosexual	13%	22%
Homosexual	10%	9%

Note: three main risk groups count for 95% of cases in both periods.

The trend in CD4+ counts between the two periods can be seen in the table below; it reflects a higher level of immunity among the patients in the more recent period at the time of presentation for health care:

CD4+/µl	1990 –2000	2001–2006
<200	789 (92.4%)	113 (88.3%)
200-500	59 (6.4%)	13 (10.1%)
>500	10 (1.2%)	2 (1.6%)

#### 2.2 South-east Asia

After the near-elimination of VL in India during malaria eradication campaigns of the 1960s, a major resurgence occurred during the early 1970s, with continued transmission since that time. South-east Asia is currently the world's principal focus of kala-azar, accounting for 67% of the total leishmaniasis disease burden: in 2005, Bangladesh reported 7000 cases, India 32 800 and Nepal 3000. There has been an upward trend in all three countries over the past several years, and 147 million people are estimated to be at risk of infection.

However, a house-to-house survey in Bihar revealed that only one in eight VL cases was included in official government surveillance data. More realistic annual incidence estimates are 250 000 cases in India, 40 000 in Bangladesh and 3000 in Nepal. Interestingly, VL is concentrated in only 94 districts of the three countries as a whole, and in just four states in India (Bihar, Jharkhand, Uttar Pradesh and West Bengal). Bihar accounts for more than 90% of cases in India and contributes to the spread of the disease into neighbouring areas of Nepal.

The re-emergence of the disease in the 1970s was most probably due to parasites harboured in the skin of residual PKDL cases. Currently, PKDL is reported to occur in only 1% of Indian VL patients but there is a lack of reliable data. In addition, loss of acquired immunity may partially explain the periodicity of VL incidence, which peaks every 10–15 years. In the past, most reported cases were in adults, but many more children and adolescents, i.e. immune-naive individuals, are now reported to be affected.

The first case of HIV infection in India was reported in 1986 in Chennai (formerly Madras) and was quickly followed by reports from all major cities; there has been a steady rise in the number of cases since that time. The new 2006 estimates by the National AIDS Control Organization, supported by UNAIDS and WHO, indicate that adult national HIV prevalence in India is approximately 0.36%, which corresponds to an estimated 2–3.1 million people living with HIV. The disease is concentrated in states in the south (Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu), where effective interventions have led to stable or declining HIV prevalence, and in the north-east (Bihar, Orissa, Rajasthan and West Bengal). Since the introduction of ART in India in 2004,

initially in 25 centres, the roll-out of the national HIV plan has been rapid and ART is now widely available throughout the country.

In Bangladesh, there are 11 000 people living with HIV; an adult national prevalence of less than 0.1% was estimated for 2006. In Nepal, an estimated 75 000 people are living with HIV, and the adult national prevalence was estimated to be 0.5% for 2006.

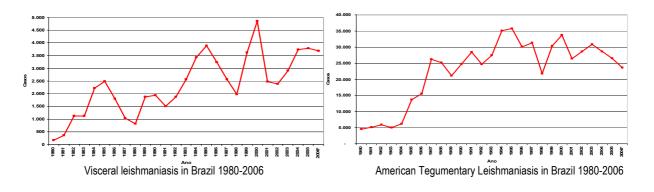
The first coinfection case in India was reported from the sub-Himalayan region (Kumaon) in 1999. A number of case reports of coinfection have been published since then; the problem seems to be exacerbated by economic migrants who acquire HIV in urban settings and then return to rural homes in VL-endemic areas where they acquire or reactivate leishmaniasis infection as a result of declining immunity.

At the Kala-azar Medical Research Centre in Muzaffarpur, Bihar, the number of HIV-positive patients rose from 3 in 2000 to 17 in 2006, and the VL/HIV coinfection rate from 0.88% in 2000 to 2.18% in 2006. In case series from the Indian subcontinent, proportions of HIV coinfections among VL patients ranged from 1.5% to 6.3%. One hospital-based study in India reported 6 coinfections among 25 VL patients (24%) and a VL prevalence of 2.84% among HIV-positive patients in 2006.

The National Elimination Programme in India has yet to acknowledge the importance of VL/HIV coinfection or develop a plan to address this issue – an important omission, since coinfected patients may represent an important reservoir of parasites, with the potential to maintain infection rates in sandflies.

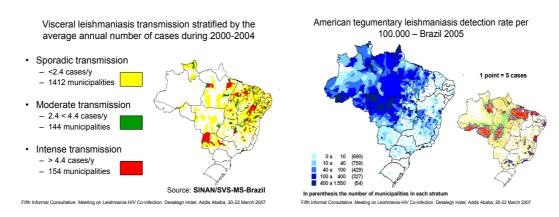
# 2.3 Brazil

The 2006 Report for the Americas Region<sup>1</sup> cites 72 000 cases of CL and 4000 of VL, mostly from Brazil. However, use of linked databases from the National Surveillance Systems in Brazil for the period 2002–2003 yields a corrected estimate of 10 207 VL cases, indicating significant underreporting. For some years, there has been an increase in both CL and VL, current annual incidence being 32 000 and 3500 cases respectively. Whereas CL cases are widely distributed, VL is endemic in the north-east and central areas of the country.



<sup>&</sup>lt;sup>1</sup> http://www.paho.org/leishmaniasis

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Source: SVS- Ministério da Saúde do Brasil

Brazil is the epicentre of the HIV/AIDS epidemic in South America and accounts for one-third of all persons living with the virus in the region. AIDS was first reported in Brazil in 1983, and the 2006 UNAIDS report estimates that there are some 620 000 people living with HIV in the country. The country's emphasis on prevention and treatment – HIV/AIDS diagnostic and treatment services are provided free throughout Brazil – has helped to keep the adult national prevalence steady at roughly 0.5% since 2000. National trends in HIV/AIDS patients are:

- spread to the regions with the lowest urbanization rates and to small- and mediumsized cities;
- increased predominance of low educational levels among the cases reported in recent years;
- an increase in AIDS among heterosexual men and women; and
- a marked decline in opportunistic infections and deaths after the introduction of HAART (highly active anti-retroviral therapy).

From 2001 to 2005, 16 210 cases of VL were reported in Brazil. When the databases of AIDS and visceral leishmaniasis were related, it was found 176 cases of co-infection VL-aids. The estimate is that there are 315 cases of LV-HIV co-infection without aids. Among coinfected patients, 78% were male; the median age was 38 years (86% were aged 20–49 years). Heterosexual transmission was the source of 56.3% of the HIV infections. Of coinfected cases, 53% were reported from the north-eastern region and 29% from the south-eastern region. The clinical picture of coinfected patients was similar to that observed among VL patients without HIV, with fever, weight loss and splenomegaly. In the absence of ART, the case-fatality rate during the first VL treatment course was 24%.

Among the 150 000 CL cases reported from 2001 to 2005, HIV coinfection was present in 150 (0.1%). Mucocutaneous leishmaniasis is frequently observed in HIV-coinfected patients in Brazil (43%), a clinical pattern seen only rarely (0.3%) in southern Europe.

The Brazilian leishmaniasis/HIV coinfection network was established in 2002 with the following aims:

- mapping of the geographical distribution of leishmaniasis-HIV coinfection;
- evaluation of the epidemiological, clinical, immunological and therapeutic characteristics of each identified case;
- increasing the availability, and ensuring appropriate usage, of diagnostic tests for leishmaniasis;
- establishing reference laboratories for the isolation and characterization of the parasites;
- improving the dissemination of relevant information;
- enhancing training and providing appropriate guidelines for the health system;
- broadening the participation of "grassroots" and nongovernmental organizations; and
- planning and evaluation of coinfection control interventions and follow-up activities.

The Brazilian national guidelines for diagnosis and treatment of *Leishmania/HIV* coinfection were made available in 2004.

### 2.4 Sub-Saharan Africa

#### **Burkina Faso**

The national 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, with 61 cases in 1996, 552 in 1997, 1218 in 1998, 1595 in 1999, 712 in 2000, 1063 in 2001, 1152 in 2002, 1194 in 2003, 901 in 2004, and 827 in 2005. The major increase in the number of cases from 2000 onwards followed new district development in a previously rural area near the capital Ouagadougou; the disease is now known as "Ouaga 2000". The peak incidence months are August and September, overlapping with the peak malaria season. Cases occur across all age groups and in equal numbers of males and females.

Among 70 "Ouaga 2000" patients screened for HIV, 10 were CL/HIV coinfected (14.3%). Many unusual clinical manifestations were found in a prospective study of 32 coinfected patients, including lepromatous and diffuse (15), ulcerative (14), infiltrative (12), papulonodular (9), psoriasis-like (5), Kaposi-like (1), cheloid (1), and histioid (1) forms.

Treatment with standard dosage schedules of meglumine antimoniate IM for 21 days produced 50% cure rate, but rapid relapses were observed in HIV-coinfected patients, requiring repeated courses of treatment. With three treatment cycles, the cure rate increased to 75%. Visceralization occurred in one HIV-positive patient, with disseminated papulonodular leishmaniasis and bone marrow aspirate positive for *L. major*.

The main challenges in Burkina Faso are:

- evaluation of the epidemiological situation outside Ouagadougou;
- testing new strategies for the control of CL;
- improving case management in HIV-coinfected patients.

## **Ethiopia**

Information on the epidemiology of leishmaniasis in Ethiopia is incomplete as no surveillance system is yet in place, but reports of VL endemicity date back to the 1940s. The disease is prevalent mostly in arid lowland areas, and the principal parasite involved *L. donovani*, with an estimated annual incidence of 4000 cases. Migrant workers in Ethiopia who are HIV-positive but not VL-infected are being suggested by MSF to move out of VL-endemic areas to avoid becoming infected.

Leishmaniasis is spreading to previously non-endemic areas, as exemplified by the recent outbreak in Libo and Fogera districts (Amhara State), a highland area close to the northwest focus. Cutaneous leishmaniasis is endemic in the highlands; the predominant parasite is *L. aethiopica* with the rock hyrax as reservoir. *L. major* is infrequent.

The first HIV-positive patient was reported in 1984 and the first AIDS case in 1986. Antenatal sentinel surveillance projections yield an estimated national HIV prevalence of 3.5% (10% in urban areas, 1.9% in rural areas). Urban prevalence is thought to have stabilized since 1999–2001. Currently, an estimated 1.3 million people are infected. Although ART was introduced in 2003, programmes do not yet adequately address the needs of the rural poor. Some 65 000 HIV patients are currently receiving ART, with 10.5% coverage in urban areas and 1.9% in rural areas, but an estimated 277 000 patients are still in need of this treatment. There are an estimated 777 000 AIDS orphans (out of a total 4.9 million abandoned children or orphans, in a population of 77 million).

Six endemic areas have been identified:

#### north-west:

- Metema, Humera, Wolkayit
- Libo/Fogera

#### north-east:

- Awash valley
- Ethiopia-Djibouti border

#### south and south-west

- Dawa, Genale, Gelana, Segen, Woito, Konso and Omo river valley
- Kenva border
- Gambella–Sudan border

In Humera (north-west Ethiopia), the proportion of VL patients coinfected with HIV increased from 18.5% in 1998–99 to 40% in 2006. In a retrospective review of 791 cases in Tigray, the case-fatality rate among VL/HIV coinfected cases was four times higher than that for VL patients not infected with HIV. In Libo, 15–18% of all VL patients are HIV-positive. The groups at highest risk of coinfection are seasonal migrant labourers, sex workers, young adults in resettlement areas (particularly in the north-west and along the Sudan border), truck and public transport drivers, and military personnel deployed in border areas

Major challenges in Ethiopia include:

- the high national HIV prevalence;
- high turnover of staff trained in HIV (ART) and VL treatment, which is exacerbated by the remoteness of endemic areas;
- difficulties in providing diagnostic and treatment supplies to health facilities in endemic areas.

Nevertheless, several factors will facilitate the management of coinfection at the national level:

- ART is provided free and there is a plan in place to increase the number of health facilities providing ART.
- New partners have joined the effort to implement both HIV and leishmaniasis control programmes, including Médecins Sans Frontières (MSF) groups and WHO.
- There is increased government commitment, at both national and regional levels, and the political will to establish a *Leishmania*/HIV coinfection surveillance network in selected sentinel sites.

# Kenya/Somalia/Uganda

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

In Somalia, MSF-Belgium is currently dealing with an outbreak and treated 263 cases of VL in 2005 and 1300 in 2006. No coinfection has yet been reported.

There are several historical foci of VL in Kenya – West Pokot (70% of all patients in Kenya), Baringo, Mandera, Garissa, and Mwingi. A recent (2006) outbreak in Wajir and Issiolo, in the north-east of the country, mainly affected children (48 confirmed cases, 95% of them under 5 years of age). In 2006, 15 cases of VL/HIV coinfection were reported, but the risk of coinfection is increasing.

The only documented area of Uganda endemic for VL is Pokot county, Nakapiripirit district, in the Karamoja region of the north-east. This is an extension of the Kenyan endemic focus of West Pokot and Baringo districts in Rift Valley province. There is no national leishmaniasis control programme. Treatment of most VL cases is undertaken by MSF-CH, which prepared the guidelines *The diagnosis and treatment of visceral leishmaniasis in Uganda, 2007*. From 2000 to 2006, more than 2500 patients – some 70% of whom came from Kenya (West Pokot and Baringo districts) – were treated by MSF-CH in Amudat hospital. The male to female ratio of cases was 3:1 and more than 60% of the patients were under 15 years of age. Eight cases of VL/HIV coinfection were confirmed; no other data on VL/HIV coinfection are available. In 2006, HIV prevalence in Karamoja reached 3%; local conflicts (with associated rape) and population movements (e.g. for cattle trading in towns), may exacerbate the situation and increase the potential for VL/HIV coinfection.

The challenges in this region are advocacy for control of VL, HIV/AIDS and coinfection within national health systems, strengthening of surveillance systems, and creation of new partnerships.

#### Sudan

Visceral leishmaniasis in Sudan was first reported in 1904. The main endemic area is the south-east of the country, bordering Ethiopia. There were many epidemics throughout the 20th century with high mortality, most recently in 1989. In 2006, the number of VL cases was unexpectedly low – 1918 in Gedaref state and 476 in Sennar state, with similar numbers in Upper Nile; however, the mean annual VL incidence in the whole country is between 15 000 and 20 000 cases. The principal causal parasite is *L. donovani*, although *L. infantum* also occurs. Transmission is basically anthroponotic, with PKDL cases occurring in up to 50% of kala-azar patients and forming an important human reservoir.

The first case of AIDS was reported in 1986. The adult national prevalence in December 2006 was 1.6%, indicating that there is now a generalized epidemic in Sudan. The estimated total number of cases is around 350 000 (170 000–580 000). Transmission is mainly heterosexual (97%) and vertical transmission from mother to child also plays a role; the 19–39-year age group is most affected and males predominate among registered cases (male:female ratio is 3:1). The worst affected areas are Khartoum and the eastern and southern states as a result of population movements (refugees and internally displaced persons – IDPs).

In 2002 and 2004, prevalence of HIV infection in the surveillance sentinel clinics was 1.1–1.94% among patients at clinics for sexually transmitted infections, 1.6%–2.3% among tuberculosis (TB) patients, and 0.95-2% in antenatal care clinics. Prevalence in other population groups was as follows: street children 2.3%; university students 1.1%; refugees 4%; prisoners 2.5%; 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 for infected people: in 2006, there were 40 centres for treatment and VCT (voluntary counselling and testing) services, and around 1000 patients on ART. Screening of military personnel and students indicates that HIV is emerging in these groups.

Up to 1998, only 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), respectively, in 2002 and 2003 in Gedaref state. Because of the spread of both VL and HIV, an increase in the prevalence of coinfection is expected. Most coinfected cases present with typical manifestations of VL.

The main challenges in the country are:

- the need for further studies to assess the magnitude of the problem at both hospital and community levels;
- screening for HIV in VL patients unresponsive to antileishmanial treatment, with relapses or with opportunistic disease;
- serological and parasitological screening of AIDS cases with a history of residence in, or travel to, VL-endemic areas; and

- raising awareness of VL/HIV coinfection as an emerging problem in Sudan.

#### 2.5 Conclusions

As a general conclusion, it is crucial to establish ART services and VL treatment centres and to strengthen the referral system for ART for coinfected patients.

# Epidemiological trends in VL/HIV coinfection

- There is a declining trend in coinfection cases reported in southern European countries with the exception of Portugal.
- In areas where transmission is anthroponotic, VL/HIV coinfected patients should be targeted as potential reservoirs of infection.
- Currently, there is no significant overlap of the distribution of leishmaniasis and HIV in India. However, as India has the highest burden of VL in the world and HIV is spreading in the country, VL/HIV coinfection is a serious concern and the following recommendations should be considered:
  - HIV prevention and control programme activities should cover VL-endemic areas to raise public awareness.
  - Leishmania/HIV surveillance activity should be instituted in VL-endemic areas.
  - The control programmes should clearly define VL/HIV coinfection and learn from the TB/HIV collaborative initiative in instituting the surveillance system.
  - The two programmes should start integrated, well-coordinated intervention activities, particularly in VL-endemic areas
  - Collaboration between, and integration of, the two programmes should be strengthened, particularly at local government/district level, and should focus on the specific circumstances of endemic areas.
  - VCT services should be established in all centres providing VL treatment services, to encourage HIV screening of all VL cases.
  - Criteria for screening HIV-positive individuals for VL should be developed, based on the national case definition and other prevalent signs and symptoms in the area.
  - A locally applicable strategy for migrant and seasonal workers should be developed.
- Urbanization process in leishmaniasis transmission in Brazil:
  - VL transmission in urban Brazil had been reported since 1980 but accelerated in the 1990s with the prevalence of leishmaniasis in urban dogs the main reservoir host as high as 25%. The prevalence of HIV infection in cities is also very high, contributing to the risk of coinfection.
  - Other factors that exacerbate urban spread should be investigated.

- An urban epidemic of CL due to *L. major* started in Ouagadougou, Burkina Faso, in 1996 and has continued, affecting all parts of the city. The cause of the spread is not known; support should therefore be provided to the country to investigate the reasons for the spread of the disease and to plan appropriate local interventions.
- Population movement is a major factor in the spread of disease (Ethiopia, Sudan).
  - Population movements take place for a variety of reasons, e.g. seasonal movements of migrant workers, resettlement from non-endemic to endemic areas, refugees and internal population displacements. Public health measures related to such movements should include:
    - increasing public awareness activities, political commitment, and sensitization of communities and all levels of administration;
    - building health institution capacity for active case-detection, prevention, diagnosis, treatment and follow-up;
    - strengthened collaboration with HIV prevention and control programmes and integration of leishmaniasis intervention activities in endemic areas;
    - establishment of VL/HIV coinfection sentinel surveillance sites because of HIV prevalence and the overlapping endemicity of the two diseases;
    - support for countries in the form of more resources for the establishment and strengthening of surveillance and notification systems.
- WHO (headquarters and the Regional Offices for Africa and the Eastern Mediterranean Region) should support health ministries in Kenya, Somalia, Sudan and Uganda in documenting the disease burden, establishing a surveillance system and designing appropriate prevention and control programmes in these countries.

#### Research areas

- Appropriate intervention strategies for migrant communities should be developed.
- Mortality rates among coinfected patients should be documented.
- Factors that contribute to the transmission and urban spread of VL should be investigated.
- A cost-effective strategy for preventing further spread of VL should be developed and tested in Brazil.
- The urban spread of *L. major* in Ouagadougou in relation to both HIV and the reservoir hosts should be investigated and the implications for west Africa examined.
- Vector distribution and ecological and environmental factors that facilitate the spread of VL in Ethiopia and Sudan should be investigated.
- All VL foci should be mapped.

# 3. Pathogenesis in *Leishmania/HIV* coinfected patients

Both *Leishmania* and HIV-1 can occur in the same geographical area, the same person and even in the same cell. Both can infect, interact with, invade and multiply within cells of myeloid, monocytic or lymphoid origin and both can deregulate the immune system. In individuals who are coinfected, complex mechanisms – involving cytokine secretion and cellular signalling events – play pivotal roles in the *Leishmania*/HIV-1 interaction. Recent studies indicate that *Leishmania* can induce the activation of HIV in latently infected monocytic and T-cells. Moreover, HIV can enhance intracellular growth of *Leishmania* in macrophages. HIV infection may be modulated by coinfection with *Leishmania*, and *Leishmania* can hasten the progression of HIV disease in coinfected patients – supporting the notion that dual infection plays an important role in the pathogenesis, clinical latency and disease progression of either infection.

# 3.1 Pathogenesis in VL/HIV coinfected patients

Coinfected patients generally present very late WHO stage 4 disease, very low CD4+ counts and high viral loads (1000–1 million copies). Clinical presentation of VL in HIV-coinfected patients with CD4+ counts below 200 cells/µl could be atypical and relapses very common.

Both live and heat-inactivated HIV can enhance *Leishmania* growth and inhibit macrophage killing capacity. This increases the risk of active VL and explains the huge parasite burden in HIV-infected patients.

Several studies have suggested that pretreatment HIV viral load influences the response to antileishmanial chemotherapy and that active VL is associated with increased viral replication in vivo. The HIV infection progressively weakens the Th1 immune response and switches immune response (from Th0 or Th1) to Th2. HIV can abrogate the effective cellular immune responses during natural infection; it may be associated with reactivation of VL in previously asymptomatic persons through reduced antileishmanial activity of macrophages (as evidenced by enhanced intracellular growth of *L. donovani* in monocyte-derived macrophages). Coinfection with HIV reduces in-vitro cellular and Th1 cytokine responses to *Leishmania* and increases Th2 cytokine responses.

The *Leishmania* promastigote and its lipophosphoglycan (an important ligand for attachment) induce HIV-1 replication, which correlates with CD4+ T-cell loss by apoptosis. If the clinical response to antimonials is good, HIV replication decreases – if it is poor, HIV replication increases.

# 3.2 Pathogenesis in CL/HIV coinfected patients

The inhibition of proliferative responses to *Leishmania* spp. in HIV-infected patients causes dissemination of leishmaniasis, atypical localization of *Leishmania* parasites, and negative leishmanin test results in coinfected patients. The depletion of CD4+ T-cells reduces the probability of ulcer development.

The clinical interactions of CL and HIV are expressed in three clinical manifestations:

- clinical polymorphism (papulonodular, ulcerative, infiltrative, lepromatous and diffuse, psoriasis-like, cheloid, histioid or Kaposi-like);
- association in the same patient of more than one clinical form; and
- parasite dissemination with visceralization of "dermotropic" variants of L. infantum,
   L. braziliensis, L. mexicana and L. amazonensis, and cutaneous involvement of "viscerotropic" Leishmania spp.; L. infantum or L. chagasi can be found in cutaneous lesions and even in apparently healthy skin in 2–12% of VL/HIV coinfected patients.

Cases of *L. aethiopica*/HIV coinfection have been reported as showing:

 recurrence from healed lesions, variable progression of lesions at presentation, and poor response to treatment.

The few reported cases of *L. major*/HIV coinfection have shown:

 diffuse CL, no response to treatment, and cutaneous immune reconstitution inflammatory syndrome (IRIS) with appearance of *Leishmania*-free subcutaneous nodules after treatment of ulcer.

Reports of coinfection with *L. major* in Burkina Faso describe clinical polymorphism, some patients with more than one clinical form, and treatment success in 75% of patients after the third cycle (but a 35% relapse rate after the first cycle).

A study of coinfection with *L. guyanensis* reported a higher rate of reinfection/recurrence, lower rate of recovery after one treatment cycle with pentamidine, more lesions, less lymphangitis and moderate immunosuppression (CD4+ levels above 200 cells/µl).

It is important to interpret reported cases with caution: the literature tends to be biased by reporting only unusual and severe cases of CL/HIV coinfection.

# 4. Diagnosis and microbiological aspects of the Leishmania/HIV coinfection

# 4.1 Diagnosis in VL/HIV coinfected patients

Disease manifestations in HIV-infected individuals without severe immunosuppression are similar to those in immunocompetent persons. Among those with advanced immunosuppression and low CD4+ T-lymphocyte counts ( $<200~cells/\mu l$ ), manifestations of leishmaniasis may be atypical and more severe, with a chronic and relapsing course after treatment. In 42–68% of coinfected individuals, other concomitant opportunistic infections can complicate the clinical diagnosis. The prevalence of clinical forms depends on the geographical area. Cutaneous or mucocutaneous forms can be associated with VL, and visceralization of cutaneous forms can occur in coinfected patients.

#### Microscopy and culture

The "gold standard" for the diagnosis of leishmaniasis in HIV-infected patients remains the isolation or identification of the parasite. Direct examination of amastigotes in splenic and bone marrow aspirates can yield false-negatives because of the low number of *Leishmania*-infected cells that is the result of the characteristic pancytopenia in coinfected patients or of previous pentamidine or amphotericin B treatment for concomitant infections. Amastigotes can be found in the peripheral blood of approximately 50% of HIV-infected individuals and in unusual locations such as lungs, larynx, gastrointestinal tract, rectum and spinal fluid. Cultured mononuclear peripheral blood cells and spleen or bone marrow aspirates from *Leishmania*/HIV coinfected patients have a sensitivity of 64–67% and 63–100% respectively. In Sudan, parasitological diagnosis is mainly by lymph node aspirate – lymph node enlargement is a very common sign there.

# Immune-based diagnosis

The deficit in host humoral and cellular response induced by both HIV and *Leishmania* infections means that serological and delayed-type IV hypersensitivity-based tests are of limited value in coinfected patients. Only 40–50% of VL/HIV coinfected patients have detectable levels of specific antibodies against *Leishmania* (positive *Leishmania* serology). The sensitivity of different tests in coinfected patients is as follows:

- 11–67% for indirect immunofluorescence test;
- when using rk39 antigen, 20–22% for immunochromatographic rapid test and 22–62% for ELISA form;
- 90% for direct agglutination test (DAT); and
- 74–85% for immunoblotting.

# **Antigen detection**

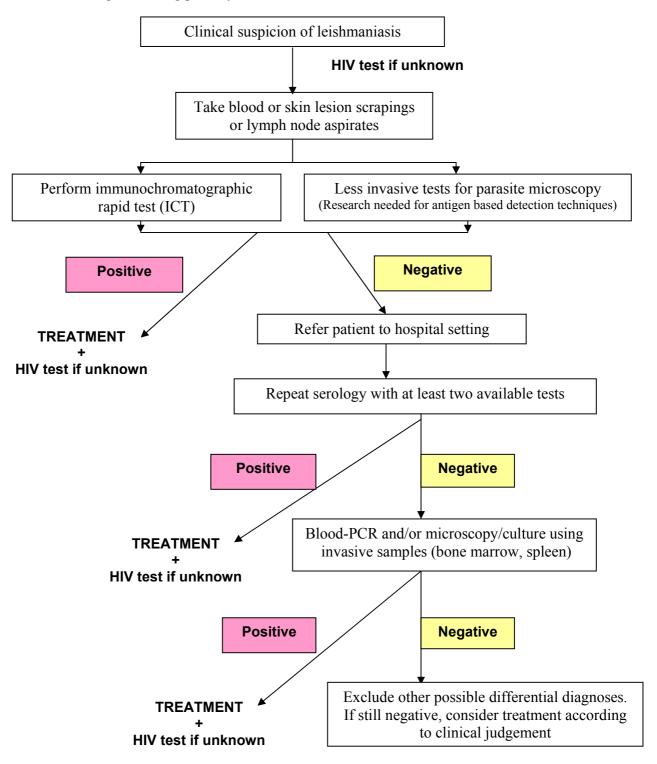
The high parasitic burden of coinfected individuals permits the detection of *Leishmania* antigens in urine samples by latex agglutination test with a sensitivity of 85.7–100%. This seems to be an active phase marker, decreasing after treatment and becoming positive with relapses; however, more studies are needed for confirmation.

# Molecular diagnosis

Polymerase chain reaction (PCR) has proved extremely useful in coinfected patients; it has the advantage that it can be applied to non-invasive samples. This technique has shown 72–100% and 82–100% sensitivity in peripheral blood and in bone marrow samples respectively. However, the sophisticated technology required for PCR precludes use of this technique in resource-poor settings. PCR has been applied to the detection and identification of *Leishmania* at different taxonomic levels (genus, species and strain). The use of real-time quantitative PCR (RTQ-PCR) allows the quantification of parasitic load in a reduced assay time and the follow-up of coinfected patients for diagnosis of relapses.

# Diagnostic algorithm

Field settings including primary health care facilities:



#### Test of cure

A test of cure (TOC) should be performed at the end of treatment when there is no improvement in terms of fever disappearance, spleen size reduction and weight gain, and when it is decided to stop the treatment for prophylaxis or because of relapse. However, more research is needed to link the clinical condition and the lack of cure. Laboratory diagnosis to determine the presence/absence of parasites may also be predictive of cure.

Field settings, including primary health care facilities	Hospital setting	Referral setting
Enlarged lymph node	Enlarged lymph node microscopy,	PCR on buffy coats
microscopy and antigen	bone marrow and antigen detection	
detection in urine <sup>a</sup>	in urine <sup>a</sup>	

<sup>&</sup>lt;sup>a</sup> Research needed for other antigen detection tests.

# Differences in serology between Europe and Africa

There is a need for comparative analysis studies of well characterized serum samples (taking into account CD4+ counts) from various geographical areas by different serological techniques.

# 4.2 Microbiological aspects in Leishmania/HIV coinfected patients

Most cases of leishmaniasis in HIV-coinfected patients have been described in individuals with HIV-1, although there are some reports involving patients with HIV-2. Most of the leishmaniasis in coinfected cases is due to *L. infantum* and *L. donovani*; other involved species, depending on the geographical distribution, are *L. braziliensis*, *L. aethiopica*, *L. tropica* and *L. major*.

# Leishmania infantum in southern Europe

Use of isoenzymatic analysis by starch gel electrophoresis to study 993 strains isolated from 579 patients during the period 1986–2005 in France, Italy, Portugal and Spain revealed the following:

- Polymorphism is higher in isolates from HIV-infected patients than in isolates from immunocompetent patients.
- Enzymatic polymorphism varies from country to country; it is high in Italy and Spain and low in France and Portugal.
- The most common zymodemes are MON-1 (80%) and MON-24 (12%).
- Some zymodemes occurred only in HIV-positive patients.
- The identical zymodemes identified in most relapsing HIV-infected patients with repeated clinical episodes of LV infection indicate failure of parasite eradication rather than reinfection.

# Leishmania donovani identification during coinfection

Study of 13 isolates of *L. donovani sensu strictu* revealed:

MON-18 – one strain from Portugal

MON-37 – three strains from Morocco

MON-18 (the most frequent) – from Sudan

MON-82

MON-268 from Djibouti.

# Leishmania major identification in Africa

Leishmania major was identified in 65 strains from Burkina Faso, Mali, Mauritania and Senegal. Three zymodemes – MON-26, MON-117 and MON-74 (the most frequent) – were found.

# Leishmania identification in America

High polymorphism has been found among *L. guyanensis*, *L. braziliensis* and *L. chagasi* isolates in Brazil and French Guiana.

# 4.3 Conclusions on diagnosis

The diagnostic tests to be carried out depend on local conditions, hospital quality and availability of referral centres. Splenic aspirates are restricted to hospitals or health facilities that have adequate equipment and staff trained to manage complications appropriately.

	Field settings including primary health care facilities	Hospital setting	Referral setting
Rational	Early detection	Confirmation of field diagnosis	Quality control; Storage Standards; Cryobank
Samples	Lymph node aspiration, bone marrow, blood, skin scraping	Spleen, lymph node and bone marrow aspiration, blood, skin scraping	Spleen, lymph node and bone marrow aspiration, blood, skin scraping
Parasitology	Microscopy on skin, lymph nodes, bone marrow and blood samples Culture: sample collection	Microscopy on spleen, bone marrow, skin, lymph nodes, PBMC samples Culture: sample collection and temporary storage Biopsy samples can be used for PCR if available	Microscopy on spleen, bone marrow, skin, lymph nodes, PBMC samples Culture: growing and maintaining parasites, medium production PCR
Immuno- diagnosis	Antibody Immunochromatographic rapid test (ICT), DAT if cold chain available Antigen detection Latex agglutination for antigen in urine if cold chain available (if not, collection of urine) In vivo Leishmanin skin test	Antibody Immunochromatographic rapid test (ICT), ELISA DAT Antigen detection Latex agglutination for antigen in urine In vivo Leishmanin skin test	Antibody ELISA, WB, DAT Immunofluorescence Antigen detection Latex agglutination for antigen in urine Reagent production Leishmanin skin test Antigens for assays Culture media
Training	Training	Training	Training

<sup>&</sup>lt;sup>a</sup> Peripheral blood monocytic cells.

*Note:* Tests should be validated in different geographical settings.

# Treatment and secondary prophylaxis of visceral leishmaniasis in HIV-coinfected patients

Several significant issues in the management of *Leishmania*/HIV coinfected patients remain to be reviewed – such as the drug of choice, the optimal treatment duration and dosage, when to perform a TOC and which test, management of treatment side-effects and of frequent relapses, and secondary prophylaxis. There is growing concern that unsupervised treatment with antileishmanial drugs may lead to higher relapse rates.

# 5.1 Treatment of the coinfected patient in southern Europe

#### **Before HAART**

Before the introduction of HAART, the prognosis for VL/HIV coinfected patients was very poor: 18–25% of coinfected patients died during the first VL episode (3–25 months), and the median survival time was 1 year, even after effective treatment with amphotericin B or antimonials. Indicators of poor prognosis included AIDS, other severe concomitant opportunistic infection, low CD4+ count, thrombocytopenia, relapsing course and absence of secondary prophylaxis.

Adverse events related to VL treatment are more likely in VL/HIV coinfected patients than in VL patients who are not HIV-infected. Principal adverse events are those related to cardiotoxicity (15%) and pancreatitis (30%) for antimonials and to nephrotoxicity for amphotericin B (5–36%).

Treatment of VL in coinfected patients was characterized by a low rate of clinical response (38–87%) and long-term failure in parasitological cure (38–81%). In a randomized trial comparing antimonials with amphotericin B, relapse rates of 25–60% within 12 months and 79–97% over the patient's lifetime were observed, regardless of the treatment; mortality and survival rates were similar in the two treatment groups.

# **HAART** impact on coinfected patients

The introduction of protease inhibitors resulted in:

- reduced incidence of new VL cases, with an overall reduction of 50–60%;
- prevention of future symptomatic VL in bone marrow-positive patients (*Leishmania* could be found accidentally on bone marrow biopsy/culture of asymptomatic patients);
- improved survival of coinfected patients from 1 year to more than 6 years; and
- modification of VL relapsing pattern, since HAART delays VL relapses of coinfected patients (i.e. there is a longer interval between VL episodes).

However, relapses occur even in HIV patients receiving successful HAART with increasing CD4+ cell counts. The most frequent relapses occur in patients with CD4+ cell counts below  $200/\mu l$ . Among relapses in coinfected patients receiving HAART, 40%

(25–70%) occurred in those not receiving secondary VL prophylaxis. At the same time, VL negatively affects the immunological recovery of the patients on HAART even though clinical manifestations are not modified and the immune restoration phenomenon is not commonly observed.

Some reports pointed out the direct inhibitory effect of the HIV protease inhibitors indinavir and saquinavir on the protease of *Leishmania* in vitro: however, this effect has not been observed clinically, and further research is needed for confirmation.

# 5.2 Treatment of the coinfected patient in India

To date, experience of treating VL/HIV coinfected patients is very limited, although treatment is essentially similar to that of immunocompetent VL patients; relapse generally occurs within 1–8 months. In India the efficacy of sodium stibogluconate (SSG) declined from 80–90% in 1990 to 40% in 2002; alternative treatment is amphotericin B deoxycholate, miltefosine or paromomycin.

# 5.3 Treatment of the coinfected patient in Africa

Visceral leishmaniasis caused by *L. donovani* is endemic in the lowlands around Humera and Metema in northern Ethiopia, with an incidence of 1000–2000 cases annually; 20–40% of the individuals affected are HIV-coinfected.

In a large, randomized, controlled trial comparing miltefosine and SSG in the treatment of patients (n = 580) from these foci, HIV coinfection was a major determinant during treatment and outcomes. Among non-HIV-infected patients, there was no significant difference in initial cure rate, initial treatment failure or mortality between the miltefosine and SSG groups. Initial miltefosine treatment failure was experienced mainly by HIV-coinfected patients (17.5% v. 4.6% in non-HIV-infected patients). Similarly, HIV seroprevalence was significantly higher in patients who experienced initial failure than in patients who were cured (63.2% v. 26.0%).

There were 34 deaths during treatment. Death occurred within 2–30 (median 13) days during the treatment, with no significant difference in time to death between treatment groups. HIV-infection, unknown HIV status, and vomiting were the independent risk factors for death. Other risk factors for death, such as age, body mass index, haemoglobin level, diarrhoea and inability to walk unaided, were interdependent. The poor final outcomes among patients enrolled in the study who had experienced relapse after previous treatment may be attributed to the high HIV coinfection rate (90%).

Miltefosine (100 mg/day x 28 days) was less effective (higher initial parasitological failure and relapse rates), but safer (lower mortality) than SSG for treatment of VL in a population with high HIV prevalence. Miltefosine was safe and effective in HIV-negative VL patients. The safer profile of miltefosine makes it the preferred drug in HIV-coinfected patients.

The good tolerability of miltefosine observed in some Indian studies was also noted in this study; only gastrointestinal symptoms were common, which were mild to moderate and not prolonged. It has been repeatedly shown that antimonials are poorly tolerated among patients coinfected with HIV and VL in Europe. The higher mortality associated with SSG

treatment, compared with miltefosine treatment, among patients who were either HIV-positive or of unknown HIV status strongly indicates that much of the mortality among HIV-infected patients was due to the SSG treatment.

# 5.4 Drugs for VL/HIV coinfection

# Pentavalent antimony compounds

Antimonials remain the most commonly used drugs for treating VL; they can be administered IV or IM at a dosage of 20 mg antimony/kg per day for 30 days. Across all endemic areas worldwide, a response rate of 85% has been achieved. However, resistance is an increasing problem, particularly in northern India (where it may be more than 60%), and several reports show that, in HIV-coinfected patients, *L. infantum* developed resistance to antimonials after repeated treatment.

Adverse effects of these drugs – mainly pancreatitis and cardiotoxicity (prolonged Q-Tc, AV-block) – are more frequent in HIV patients. Biochemical analysis and ECG monitoring are therefore critical during the treatment. A report of stimulation of HIV-1 replication by SSG, and to a lesser degree by meglumine antimoniate, in vitro has been published, but further research on this is needed.

# Amphotericin B deoxycholate

Amphotericin B deoxycholate is a polyene antibiotic; the dosage is 0.75–1.0 mg daily or on alternate days for 15–20 infusions, with high hydration. Prolonged hospitalization is required and use of the drug is therefore restricted to hospital settings. Treatment is expensive (direct cost about US\$ 60) but cure rates approach 100%. Fever, chills, phlebitis at the injection site, renal damage and anaemia are common side-effects; hypokalaemia and myocarditis are serious but uncommon side-effects, and there is 1% acute fatal toxicity, making clinical and laboratory monitoring essential.

In India, the drug is too expensive for most patients and its supply is irregular. Newer formulations, such as liposomal amphotericin B (LAmB) and lipid complex (AmBLC) amphotericin B, are of similar efficacy to amphotericin B deoxycholate but are much less toxic and much better tolerated. Only the liposomal formulation retains the amphotericin B structure at body temperature, which may explain its good tolerability. There is little clinical information on whether one formulation is superior to another in terms of efficacy, although AmBLC appears to be associated with more infusion-related side-effects, e.g. chills.

These formulations are administered IV and the recommended dosage, though not yet well established, is 3–5 mg/kg daily or intermittently for 10 doses (40 mg/kg total dose). The price of these drugs remains an important issue. Significant cost reduction has recently been announced for public sector agencies of developing countries, but even at the proposed cost of US\$ 20 per vial, the cost of treating an adult averages US\$ 200–300. Other lipid formulations of amphotericin B are under investigation.

#### Miltefosine

Miltefosine is the only antileishmanial drug that is administered orally, at a dosage of 2.5 (2–3) mg/kg per day (100 mg/day for patients weighing more than 25 kg) for 28 days. Increasing the daily dosage to 150 mg has been suggested by some experts for HIV-positive adult patients. In patients coinfected with HIV and *L. donovani*, miltefosine is less effective than antimonials but the mortality rate is lower as a result of better tolerance and fewer adverse effects. In refractory patients previously treated with miltefosine, repeated courses increase the cure rate.

The principal side-effects are gastrointestinal toxicity and teratogenicity; thus miltefosine is contraindicated during pregnancy and in lactating women, and woman of childbearing age must use effective contraception during and for 3 months after treatment. There is serious concern that unsupervised use of miltefosine might lead rapidly to high relapse rates. A course of miltefosine treatment costs US\$ 160 in the private sector (which treats 80% of all VL cases in India); however, the price of a course of tablets purchased through WHO can be reduced to US\$ 60 (depending on the number of tablets bought).

## **Pentamidine**

Because of its severe adverse effects (including occasionally acute toxic deaths) pentamidine should be used only when no other options are available. Diabetes mellitus has been observed following use of pentamidine to treat CL and there has been a 12% incidence among VL patients treated with pentamidine in India. Thus, pentamidine is not a suitable drug for field conditions. It is being used to treat human African trypanosomiasis but over a shorter period, i.e. 4 mg/kg for 7–10 days rather than the 15–30 days necessary for VL. Use of single doses for intermittent maintenance therapy of VL could be considered.

# Paromomycin sulfate (aminosidine)

Paromomycin sulfate, or aminosidine, is an aminoglycoside antibiotic active against L. donovani. It is a very cheap drug that has recently registered in India, is produced by a local manufacturer and has been included in the Essential Drugs List. In fact, this drug has been marketed globally for more than 40 years for treating bacterial and protozoan infections and has an extensive safety profile.

In a large (n = 666) phase 3 comparative study in India, paromomycin was shown to be of similar efficacy to amphotericin B: a 94.6% final cure rate was achieved with paromomycin given by IM injection for 21 days, compared with 98.8% with amphotericin B given IV for 21 days. The efficacy and safety of the drug are unknown in patients with VL/HIV coinfection; efficacy and tolerability of paromomycin in combination with antimonials are currently being studied in VL patients. The incidence of ototoxicity is transient and reversible.

The direct cost of treatment (15 mg/kg per day for 21–28 days) is US\$ 10-15. The advantage of paromomycin IM injection over other drugs is the duration of treatment – 21 days compared with 30 days for amphotericin B and 28 days for miltefosine.

# Other drugs

Sporadic VL/HIV cases have been treated with allopurinol, ketoconazole, fluconazole, itraconazole and interferon gamma, in combination with antimonials, but the lack of experience does not allow recommendation of this treatment. Other immunotherapy options (interleukin-2, interleukin-12, tumour necrosis factor, granulocyte monocyte colony stimulating factor) are under investigation.

# **Splenectomy**

Surgical removal of the spleen or splenic artery embolization has been used for VL patients who have suffered multiple relapses despite treatment with a variety of drugs. In three reported cases further relapse has been delayed but not avoided. This approach is not appropriate for patients in tropical areas.

# Drug combinations for the treatment of VL associated with HIV/AIDS

In the future, antileishmanial drug combination therapy may be the way to delay the emergence of resistance, increase antimicrobial activity, and possibly reduce drug doses and the duration of treatment (with a concomitant reduction in toxicity and cost). However, it is probable that even drug combinations will not prevent relapses altogether, leaving ART as the key to reducing and postponing relapses.

# 5.5 Follow-up in VL/HIV coinfection

# Test of cure (TOC)

Follow-up is crucial for better information on drug efficacy and to prevent drug resistance. There is no clinical sign that correlates with a positive TOC aspirate or that predicts an increased risk of relapse; indeed, an individual with TOC 4+ parasitaemia at the end of treatment can look quite healthy. However, co-existing TB or HIV will increase the risk of treatment failure. At the end of treatment, TOC should be done for any patient whose clinical response was poor and/or whose haematological parameters are not sufficiently good to permit his or her discharge; TOC is especially important to ensure that discharge is appropriate for patients who, for any reason, may have difficulty returning for care/follow-up. When done systematically, TOC also offers a way of monitoring the development of resistance.

The appropriate test of cure is under discussion. It could be based on parasitological test or antigen detection techniques at 1 and 6 months after treatment. Bone marrow, spleen or lymph node aspiration smears and culture are required for parasitological confirmation, but the first two of these techniques are either painful or risky. Blood PCR is performed only in sophisticated laboratories, and urine antigen detection needs improved sensitivity and a less objectionable procedure (it currently requires the boiling or urine).

# Secondary prophylaxis – maintenance therapy

Itraconazole + allopurinol

Primary prophylaxis is not indicated. In Mediterranean countries, secondary prophylaxis after a clinical episode is recommended for patients coinfected with zoonotic VL and HIV and receiving ART, in order to prolong disease-free intervals. It has been suggested that prophylaxis be suspended when the CD4+ count has been restored and is maintained above 200 cells/µl for more than 6 months.

In anthroponotic VL, the risk of resistance development means that HIV-coinfected patients may become an important reservoir of drug-resistant *L. donovani*. Further research is needed before maintenance therapy can be recommended for these patients.

In summary, the drugs that could be used in zoonotic VL foci for maintenance therapy are:

Pentavalent antimonials
 Liposomal amphotericin B
 Amphotericin B lipid complex
 Miltefosine
 20 mg/kg per day every 3–4 weeks
 3–5 mg/kg per day every 3–4 weeks
 3–5 mg/kg per day every 3–4 weeks
 repeated 28-day courses

■ Pentamidine 4 mg/kg per day (300 mg) every 3–4 weeks

dailv

# 5.6 Conclusions on the treatment of VL in HIV-coinfected patients

- VL is an AIDS-defining condition and a valid entry point to start ART, irrespective of CD4+ count. Baseline CD4+ count is lower in VL/HIV coinfected patients since VL itself causes a reduction in CD4+ cells. It is crucial to try to restore cell-mediated immunity with ART as soon as possible to prevent further opportunistic infections and VL relapses although ART alone will not prevent VL relapses. Multiple VL relapses appears to inhibit CD4+ restoration during ART. Relapses due to *L. donovani* occur at higher CD4+ counts than is the case with *L. infantum* in Europe.
- All antileishmanial drugs are less effective in HIV-positive patients and most of the patients will relapse within 3–6 months; successive relapses become less typical and less acute, but more frequent. Moreover, with each relapse patients become less responsive to treatment and eventually unresponsive to all drugs.
- There can be added toxicity between the drugs co-administered for VL and HIV (and for TB, particularly in the case of rifampicin); diseases should therefore be treated sequentially VL, then TB then HIV.
- Amphotericin B and SSG are more toxic in HIV patients, necessitating careful monitoring for pancreatitis, cardiotoxicity (ECG every 3 days to observe Q-Tc and ventricular rhythm) and renal failure. If better drugs are available, SSG should be avoided. Miltefosine seems to be a safer drug in coinfected patients, with lower risk of mortality than SSG in HIV-positive individuals.
- A pregnancy register is needed for evaluation of the feto-toxicity of drugs in use.

- Relapses should be treated appropriately, balancing the benefits and risks of drug toxicity. Combination therapy could be a valuable option. When using an SSG/LAmB combination, it may be better to give the drugs sequentially (starting with LAmB) than concurrently.
- Secondary prophylaxis to prevent relapses should be considered in areas with zoonotic transmission, although more evidence of benefits is needed from clinical trials. More studies are also needed on secondary prophylaxis in areas of anthroponotic transmission, taking into account the risk of drug resistance. In coinfected patients from anthroponotic foci, any drug could probably give rise to Leishmania resistance: drugs used to treat relapse should therefore be avoided for secondary prophylaxis.
- After treatment of VL/HIV coinfected patients, TOC should be considered only in certain circumstances, according to the clinical response. If a patient returns with renewed clinical signs/symptoms, parasitological persistence should be reassessed to confirm VL relapse and exclude other opportunistic infections.
- The risk factors for relapse that should be considered are: previous AIDS diagnosis and no ART; low CD4+ cell count; previous VL episode; failure to achieve clinical or parasitological cure during the first episode.
- The risk factors for death that should be considered include: malnutrition, concomitant opportunistic infection (TB disease), pneumonia, diarrhoea, vomiting, anaemia, bleeding and signs of toxicity during treatment (heart failure, arrhythmia, pancreatitis, jaundice, kidney failure, anaemia, severe vomiting or diarrhoea).
- It is of utmost importance to prevent transmission of *L. donovani* by providing bed nets to all VL patients, most particularly those who are coinfected.
- The ethical issues involved in balancing compassionate treatment with experimental therapy must be considered. Appropriate background information must be available to inform decisions on new treatments.
- The protection of human drugs must be considered. The use of promising new drugs such as miltefosine in canine leishmaniasis can increase and accelerate the development of resistance and should therefore be avoided.
- The research priorities identified in the light of the Helsinki declaration (*Ethical principles for medical research involving human subjects*) are:
  - 1. Establish efficacy and tolerability of paromomycin in coinfected patients.
  - 2. Establish criteria for starting and stopping maintenance therapy, and identify the best single-dose drug for maintenance therapy
  - 3. In properly conducted clinical trials define combination therapy with currently available drugs with the aims of: shortening duration of treatment; improving compliance; increasing efficacy; reducing indirect treatment costs; protecting the drugs from resistance; reducing toxicity

5.7 Recommendations for VL/HIV treatment in coinfected patients

# **Criteria for initiation of ART (CD4+ count)**

Visceral leishmaniasis is an opportunistic infection and classification should be WHO stage IV (CDC stage C). If VL is present in an HIV-positive patient, ART should be started after VL treatment, regardless of the CD4+ cell count.

Where there is a history of VL but the patient is currently asymptomatic, a CD4+ count should be done and national HIV treatment guidelines followed.

# First- and second-line treatment

First line, first episode:

Use pentavalent antimonials in areas of no significant resistance, when amphotericin B is unavailable or not affordable, monitoring antimonials toxicity is needed. Use amphotericin B in either lipid formulation or traditional formulation; if the latter is used, toxicity monitoring is required.

Second line, first episode:

Use oral miltefosine if it is available and affordable. Conditions of use are:

- close supervision
- negative pregnancy test in a female patient
- non-lactating woman
- contraception for fertile female during and for 3 months after VL treatment.

# **Failure/relapses treatment**

After treatment of the first episode, assess fever, haematological values and splenomegaly to determine the clinical improvement.

If clinical parameters indicate improvement, do nothing further. Discharge the patient. To ensure that discharge is appropriate for patients who may have difficulty returning for care/follow-up, TOC is recommended.

If clinical parameters indicate treatment failure or relapse, reassess parasitological persistence. If positive, continue treatment or substitute second-line treatment. Exclude other opportunistic infection.

#### Maintenance

Experience of zoonotic leishmaniasis in Mediterranean areas indicates that maintenance therapy or secondary prophylaxis could be started ideally after treatment of the first episode. Drugs that have been used for maintenance, although not proven in proper clinical trials, are lipid formulation amphotericin B, pentamidine, and pentavalent antimonials. Maintenance therapy may be stopped when clinical status is stable with no deterioration and CD4+ count has exceeded 200 cells/µl for more than 6 months.

In anthroponotic transmission areas, treatment options are limited and account must be taken of the fact that frequent relapses significant increase the risk of resistance

developing. Currently, there is insufficient evidence to allow appropriate maintenance therapy to be recommended. Relapsing patients should be retreated according to the first-and second-line recommendations described above, as appropriate for individual patients.

# Management of relapses/second episode

Parasitological diagnosis or antigen detection is required in case of relapse or a second episode. The efficacy of ART should be considered.

The drug chosen to treat relapse should be different from that used to treat the first attack. To avoid the development of drug resistance as a consequence of repeated relapse, combinations of drugs should be used; however, there is still a lack of evidence for the best combination for coinfected patients.

There is insufficient evidence to allow a consistent approach to patient discharge at the end of treatment (e.g. the need for TOC) to be recommended. The physician's decision should therefore be made on an individual basis.

# Annex

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