Review Article

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Leishmania/HIV co-infections in the second decade

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***Leishmania*-HIV co-infection has been globally controlled in Southern Europe since 1997 because of highly active anti retroviral therapy (HAART), but it appears to be an increasing problem in other countries such as Ethopia, Sudan, Brazil or India where both infections are becoming more and more prevalent. Most of the scientific background on *Leishmania*/HIV co-infection has been dropped from the Mediterranean experience and although the situations among countries are not fully comparable, it is of high importance to take advantage of this knowledge. In this review several aspects of the *Leishmania*/HIV co-infection are emphasized *viz.*, epidemiological features, new ways of transmission, pathogenesis, clinical outcome, diagnosis, treatment and secondary prohylaxis. An extensive review of the literature on *Leishmania*/HIV co-infection has allowed the inclusion of a comprehensive and updated list of bibliographical references.**

Key words Co-infection - HIV - immunotherapy - *Leishmania* - pathogenesis

The development of the HIV/AIDS pandemic during the last 20 yr has modified the spectrum of leishmaniasis in both the clinical and epidemiological fields. From middle of the 1980s, when the first case of leishmaniasis associated with human immunodeficiency virus (HIV) infection was published[1], an increase in the cases of co-infection has been registered; to date cases in 35 countries have been detected[2] (Fig. 1).

The increase in the number of cases of co-infection arises from the overlap between the AIDS epidemic, basically from urban transmission, and the areas in which leishmaniasis is endemic, fundamentally from rural transmission. Epidemiological changes, such as the increase of the population in suburban areas where the vector and the reservoir abound, have increased this overlap.

To date, the greatest prevalence of *Leishmania*/HIV co-infection has been in the Mediterranean basin. Of more than 2,000 cases notified to the WHO, 90 per cent of them come from Spain, Italy, France and Portugal[2-5]. From the beginning of the AIDS epidemic up to the era of highly active antiretroviral therapy (HAART), between 25-70 per cent of all the cases of visceral leishmaniasis (VL) in these countries were associated with HIV infection, and it

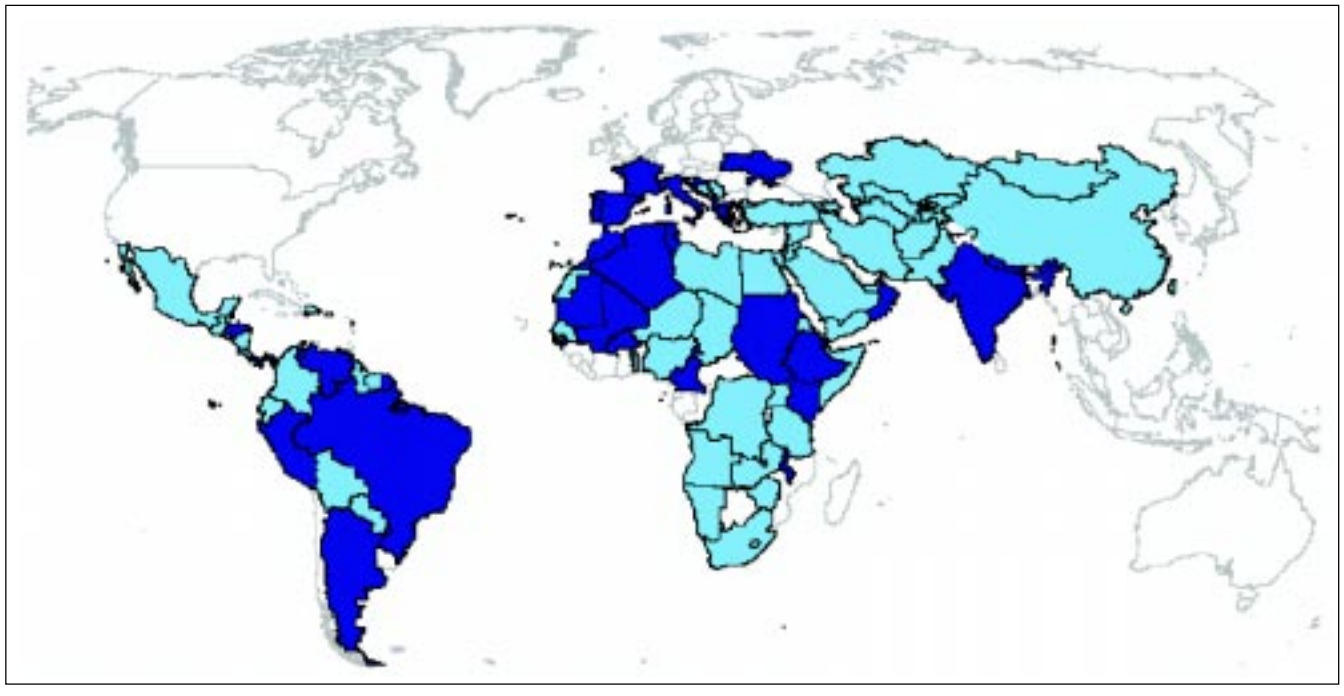

Fig. 1. Countries with endemic leishmaniasis and with *Leishmania*-HIV co-infection. (reproduced with permission[2]). ■ global distribution of leishmaniasis; ■ countries that have reported co-infection.

is anticipated that up to 1.5-9 per cent of AIDS patients suffer a bout of leishmaniasis, either as a new infection or as revival of a latent infection[4]. The epidemiological data for Southern Europe indicates that HIV infection is a risk factor associated with VL[6]. A study carried out by Kubar *et al*[7] demonstrated that in zones of endemic leishmaniasis, such as the south of France, about 10 per cent of HIV$^+$ individuals had an asymptomatic *Leishmania* infection. Similarly, in a series of HIV$^+$ patients studied by Alvar *et al*[8] this percentage was to 17 per cent. In fact, two thirds of the cases of VL registered in Madrid in the last decade were associated with HIV infection[6].

The majority of the cases of co-infection registered in the South American continent happen in Brazil, where the incidence of AIDS has increased from 0.8 cases every 100,000 inhabitants in 1986 to 12.3 in 2001[9,10]. In Africa the number of cases has started increasing, affected by social phenomena such as mass migration and wars[11]. In Addis Ababa (Ethiopia) 35 per cent of all the cases of leishmaniasis were found to be associated with HIV infection (Berhe N, personal communication), while for Sudan this percentage was 8 per cent[12]. In Asia cases of co-infection are being registered in India, Bangladesh and Nepal, countries in which the situation will be aggravated by the phenomenon of increasing resistance to antimonials[13-15]. Also leishmaniasis has been described as an imported infection among HIV$^+$ patients from non-endemic areas like Northern Europe[16].

Nevertheless, the real impact of *Leishmania*/HIV co-infection is probably underestimated on the global scale due to deficiencies in the surveillance systems. The fact that leishmaniasis is not included among the AIDS defining diseases of contributes to this scarcity of information. For this reason, from 1998 the WHO, in collaboration with the United Nations HIV/AIDS programme (UNAIDS), has created an active surveillance network to determine the real dimension of this problem[5].

Considering principally the data from the Mediterranean basin, it can be said that the prevalence of VL in AIDS patients is between 100 and 2320 times greater than in immunocompetents or other non-HIV$^+$, immunodeficient groups of

people[2,5,6,17-19]. From this information it can be highlighted that the AIDS epidemic indirectly reflects the prevalence of latent leishmaniasis in the general population[19].

Particularly in Spain, the epidemiological view of VL has changed since the advent of the AIDS epidemic. Until 1985 the majority of VL cases occurred in immunocompetent children, while since then this proportion has inverted and up to 80 per cent of the VL cases are related to immunodeficient adults, the great majority of whom are HIV$^+$[6,20, 21]. In fact, in this country VL is the third most common parasitic disease in HIV$^+$ patients, after toxoplasmosis and cryptosporidiosis[22].

Co-infection in the HAART era

It is well known that the arrival of HAART has modified the natural history of HIV infection and its opportunistic infections, including leishmaniasis[23-25]. Moreover, it has allowed a partial but substantial recovery of several immune functions in HIV$^+$ patients[26], avoiding the need to use secondary prophylaxis[27-30].

The beneficial effect of HAART has been demonstrated on other opportunistic infections, such as toxoplasmosis, cryptosporidiosis and microsporidiosis[7, 31-33]. A study carried out in France demonstrated that the incidence of VL in HIV$^+$ patients dropped from 12 in every 10,000 people per year before 1996 to 7 per 10,000 per year after 1996, the year in which HAART therapy was introduced in this country[25]. Similar data have been published for Spain and Italy[34,35].

It is considered that the co-infected patients who receive HAART have a better rate of survival than those who do not receive it[6,36]. Once the immune response has been restored, the patient shows better possibilities of controlling the initial *Leishmania* infection[37]. Unfortunately, HAART does not turn out to be so beneficial when it comes to preventing relapses. It has been determined that between 38 and 70 per cent of the co-infected patients who receive HAART end up relapsing in the 24 months after the anti-*Leishmania* treatment[34,38], the relapses being able to happen independently of an increase in the number of CD4$^+$ cells and even with an undetectable viral load. However, it should be noted that the appearance of relapsing happens an average of 7 months later in patients who receive HAART than in those who do not receive it.

In spite of the positive data associated with the use of these drugs, the benefits of this therapy are only available to 5 per cent or less of the HIV$^+$ patients in the world[39]. In developing countries where HAART is not available, the incidence of opportunistic infections like *Pneumocystis carinii* is increasing[40], which leads to the belief that the same thing can happen with *Leishmania*/HIV co-infection[4,41-43].

Aspects of transmission

Although the geographical distribution of *Leishmania* infection is restricted to the distribution areas of the *Phlebotomus* and *Lutzomyia* vectors, the HIV infection modifies the traditional zoonotic/ anthroponotic transmission patterns. The poor therapeutic success rates, the high level of relapsing and the high parasitaemia of VL in HIV$^+$ patients, as well as the atypical manifestations of the illness that complicate the diagnosis and the difficulty of access of these patients to the sanitary resources, mean that the co-infected patients increase the number of human reservoirs in areas where the transmission is anthroponotic. In addition, these same characteristics may help to create new foci of anthroponotic transmission in areas where the transmission of leishmaniasis has been traditionally zoonotic[44].

In the countries of the Mediterranean basin, up to 70 per cent or more of the cases of VL in adults are associated with infection by HIV. Of these patients between 50 and 90 per cent, according to the country (Fig. 2), are intravenous drug users (IVDUs)[21,45]. A common factor between these individuals is the sharing of syringes and doses, such that Alvar *et al*[8] proposed that this habit could be considered an alternative cycle of *Leishmania* transmission, characterized by being artificial, epidemic and anthroponotic. A series of data support this hypothesis, such as the presence of *L. infantum*

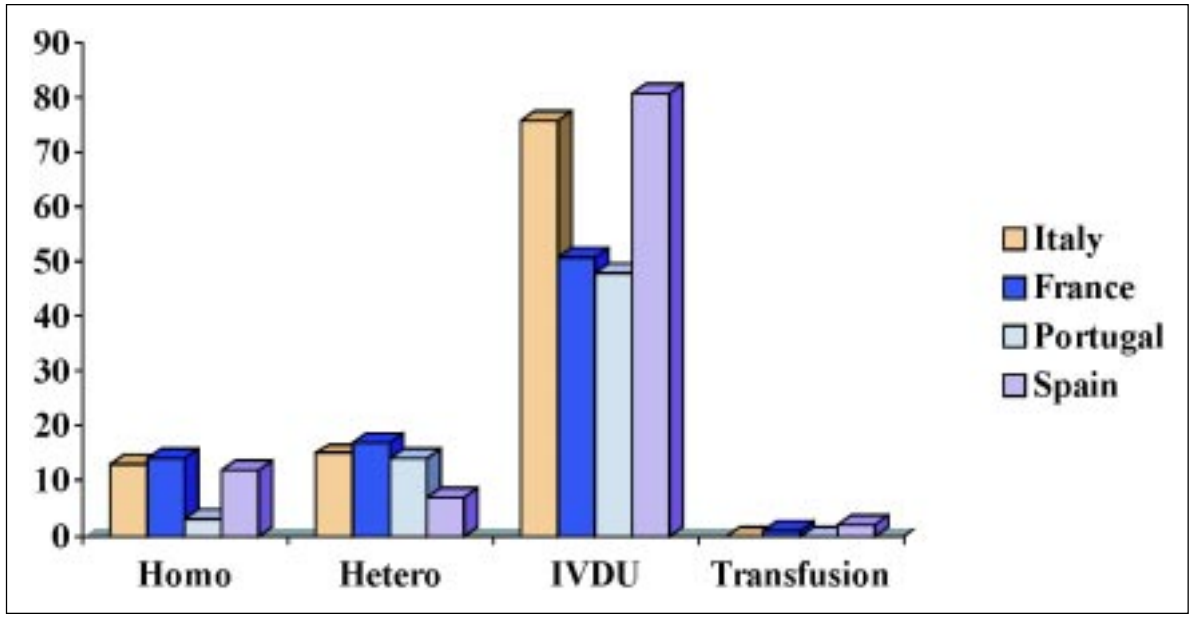

Fig. 2. Distribution of co-infected patients by risk group and country (n=1911). (reproduced with permission[2]).

zymodemes in co-infected IVDUs that have not been isolated in either immunocompetents or in dogs (the natural reservoir of *L. infantum*). Also, 52 per cent of the co-infected patients exhibit amastigotes in peripheral blood, which is rare in immunocompetent patients[46,47]. Entomological studies suggest that transmission is physically possible via shared syringes, since even the small volume usually engorged by a sand fly (0.3-0.5 µl) was sufficient to initiate the spread of the parasite, in 100 per cent of the studied cases. IVDUs commonly can exchange up to 0.3 ml of blood when sharing syringes, a quantity that can assure the transmission of the parasite and explain the high prevalence of VL between HIV^+ IVDUs[48]. This hypothesis was reinforced by the findings of Cruz *et al*[49] that demonstrated the existence of *Leishmania* DNA in the residual blood in syringes used by IVDUs.

Pineda *et al*[50,51] and Del Giudice *et al*[25] came to the same conclusions after conducting different epidemiological studies in which they measured the prevalence of antibodies and the cellular response to *Leishmania* in IVDUs, that the prevalence of the indicators of *Leishmania* infection was greater in the IVDU population, independently of whether HIV infection was present or not. The results obtained by these teams indicate the epidemiological importance that this group can have in endemic zones of leishmaniasis.

Occasionally, the transmission of *Leishmania* has been described by alternative methods that are also shared by HIV infection, including blood transfusion, congenital transmission, and in-laboratory infection[52]. Therefore, even though no case of *Leishmania*/HIV co-infection due to any of these situations has been described, it is necessary to pay attention to these possible methods of transmission.

Population at risk

Globally, the majority of leishmaniasis cases happen in the HIV-negative population, where it is considered to be principally a childhood disease[23,53-56]. In fact, in the Mediterranean basin, the majority of the paediatric patients with leishmaniasis are HIV-negative[57], with 3 yr old being the average age of those diagnosed with VL[58]. In Bangladesh the average age is higher, with the greatest incidence in 3-14 yr old, possibly due to associated malnutrition (Bern, C., personal communication). However, the association of infection by *Leishmania* and HIV has resulted in a significant change in the age of the at-risk population, leading to the largest number of cases

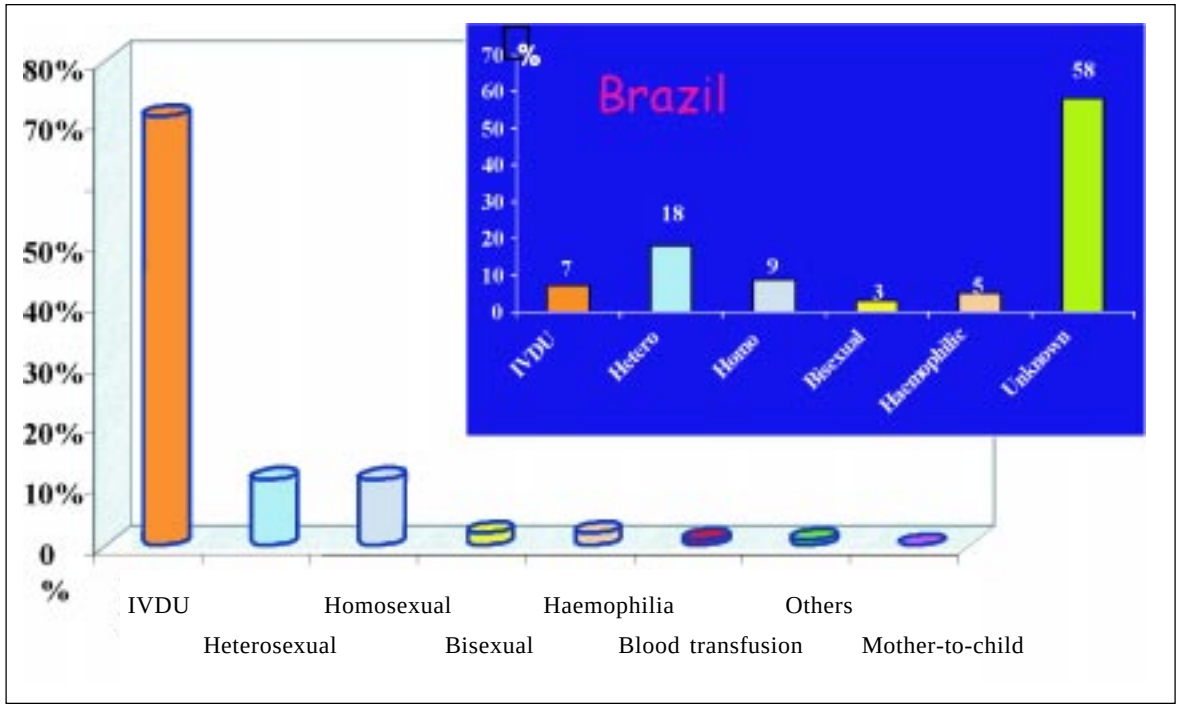

Fig. 3. Comparison by risk group of the distribution of *Leishmania*/HIV co-infection (n=1911) in southwestern Europe and in Brazil. (reproduced with permission[10]).

in people aged between 31-50 yr currently, coinciding with the predominant age of the principal at-risk group, the IVDUs[2].

In the southwest of Europe, 65 per cent of the HIV$^-$ and 83 per cent of the HIV$^+$, patients that suffer from leishmaniasis are male[6,42,43], which reflects the density of the IVDU population. This is the case in the Mediterranean basin, where within the HIV at-risk groups, the highest frequency of co-infection is shown by the IVDUs[25,59,60], who account for over 70 per cent of the cases[2]. Nevertheless, the distribution profile of co-infection for groups at risk is not homogeneous and depends on the local characteristics of HIV transmission (Fig. 3).

In certain societies, the limited access of women to medical care, due to social or cultural barriers, can lead to underestimating the real impact of the leishmaniasis among them. Though some experimental models with mice suggest that they might be less inclined to develop clinical symptoms of VL[55,61,62], it remains unclear if women are, due to their constitution, protected against leishmaniasis. In fact, in Bangladesh the mortality associated with a bout of VL is 15 per cent in women, while the same figure for men is 5 per cent, though this could be associated with poor access to medical resources[63].

Among the HIV$^+$ it is considered that between 33-78 per cent of the patients co-infected with a first bout of VL must have had previously defined criteria of AIDS[6,17,19,22,50,60,64-66]. Nevertheless, VL can be the first infection related to HIV in 13-47 per cent of patients[6,17,22].

The size of CD4$^+$ cells count is $\leq 200/\mu m^3$ in 62-90 per cent of the co-infected patients and $\leq 50/\mu l$ in 42 per cent of them[6,25,46,66]. However, VL also appears in HIV$^+$ patients with different levels of immunodepression or in ymptomatics[17,22,46,64].

There are studies that suggest that the success of the therapeutic response may be inversely related to the viral load prior to treatment[67]. Another fact that also seems clear is that the patients who do not receive HAART have more possibilities of developing a bout of leishmaniasis and they show

great risk of therapeutic failure, as well as of clinical and parasitological relapse[6,46,68].

Given that a good cellular immune response is critical for the control of the infection of *Leishmania*, two mechanisms have been proposed by which the immunodepresion might allow the appearance of a clinical VL bout[16,46]: (*i*) allowing the revival of a latent infection, as happens with a large proportion of the opportunistic infections associated with AIDS; and (*ii*) facilitating the appearance of active VL after the initial infection.

Microbiological aspects

The majority of the cases of *Leishmania*/HIV co-infection has been described in adults infected by HIV-1; however, they have also been reported in patients infected by HIV-2[46]. In these patients, the VL is caused principally by *L. infantum* or *L. donovani*[46]. Other species of *Leishmania*, such as *L. braziliensis*[69,73], *L. aethiopica*[74], *L.tropica*[75,76], and *L.major*[77], have been described as responsible for cases of co-infection, according to the geographical area concerned.

Three important considerations can be derived from the microbiological aspects of *Leishmania*/HIV co-infection: (*i*) a high variability of *L. infantum* zymodemes affecting co-infected people exists[78]; (*ii*) several new zymodemes have been described exclusively in HIV$^+$ patients, which have not been discovered in immunocompetents or dogs of the same geographical area[46,47]; and (*iii*) the anergy state of HIV$^+$ patients allows cutaneous *Leishmania* zymodemes to visceralize and *viceversa*[79]. Various dermotropic zymodemes of *L. infantum*, as well as of *L. braziliensis*, *L. mexicana* and *L. amazonensis*, have been described as causes of VL in HIV$^+$ patients[80-83]. In addition, some visceral tropic variants of *L. infantum*[84] and *L. chagasi*[85,86] have been found in cutaneous lesions, and even in healthy skin of HIV$^+$ patients[87].

Pathogenesis of the *Leishmania*/HIV co-infection

Both infectious agents, in addition to producing similar effects in the host, share target cells. *Leishmania* infects and multiplies inside the macrophages, and the HIV can also invade and replicate in these cells, in addition to T CD4$^+$ cells[88,89]. The presence of both microorganisms in the same cellular type can have important implications in their expression and spread[90]. Both infections change the predominant cellular immune response of Th1 or Th0 to Th2 through complex mechanisms mediated by cytokines, conferring susceptibility to both infections, according to the murine model[91]. Among the cytokines changes it has been recorded is an inhibition in the production of inferon gamma (IFN-r), which causes a deficiency in the leishmanicidal capacity of the macrophages[92], disabling their ability to eliminate the intracellular amastigotes by means of nitric oxide's toxic action[93]. The effects of the viral infection tend to predominate over those caused by the parasite, and the immunodepresion induced by the HIV prevails over the cellular response caused by *Leishmania*[46]. The depletion of T CD4$^+$ cells related to the HIV infection implies a loss of T cells capable of recognizing *Leishmania* antigens and of stimulating the B-lymphocytes. This would explain the high valuation of false negatives in *Leishmania* serology in co-infected patients, in contrast to those found in immunocompetent patients, who present a strong specific humoral immune response[94-96]. HIV also provokes an inhibition of the proliferative response against *Leishmania*, favouring the spread of the parasite, so that parasites can be encountered in atypical locations, and high parasitaemia is seen in these patients.

It has been observed that infection by *Leishmania* increases the replication of the HIV, as much in *in vitro* cultures as in co-infected individuals[89], principally due to chronic activation of the immune system[97,98], which is one of the principal determinants of the progression of the illness caused by the HIV. The immune activation increases the expression of viral co-receptors (CCR5 and CXR4), diminishes the secretion of B cytokines[99,100], and favours the entry, integration, formation and release of viral particles[101]. This brings with it an increase in the secretion of tumour necrosis factor-alpha (TNF-α), interleukins IL-2, IL-4, IL-6, and IL-10 and affects the cellular cycle[102,103]. All this, therefore, results in a progression of the immunodeficiency and a decline in the patient's survival rate[104-109].

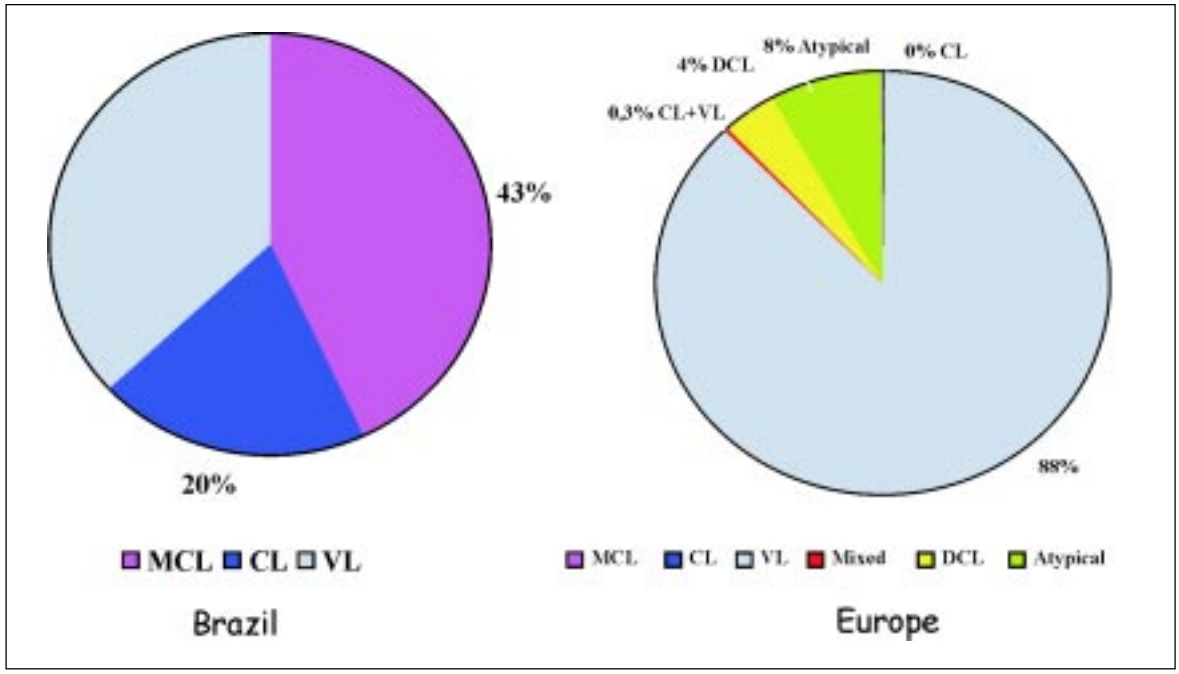

Fig. 4. Prevalence of the different forms of leishmaniasis in the co-infection, according to area (reproduced with permission[2,10]). CL, cutaneous leishmaniasis; VL, visceral leishmaniasis; MCL, mucocutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis.

Compared to HIV$^+$ patients without leishmaniasis, co-infected patients show a cytokine profile with high levels of IL-4, IL-10 and IL-2 receptor (IL-2R), and a decrease in the production (post-stimulation) of IFN-γ[97,98,110].

It has been proved that the lipophosphoglicon (LPG) of *L. infantum* can induce the expression of HIV in mononuclear cells of peripheral blood infected in a latent manner[111], probably due to the secretion of TNF-α[112,113]. The initiation of the expression of HIV has been suggested from observations that mark a progressive increase of the load of viral ribonucleic acid (RNA) in co-infected patients, associated with an increase in the levels of IL-4, IL-6 and IL-10[97]. In fact, the success in the treatment of cutaneous, mucocutaneous and visceral leishmaniasis has been correlated with the decrease of the levels of TNF-α[114]. The response to the leishmaniasis treatment in co-infected patients depends on the initial viral load. Patients with good response to the antileishmanial treatment showed a marked reduction[67] in post treatment viral load, in contrast, post-treatment HIV viral concentrations were markedly increased among patients with poor response to anti-leishmanial therapy.

Therefore, it is seen that VL promotes the development of the defining conditions of AIDS[43] and its clinical progression. Equally it reduces the life expectancy of HIV$^+$ subjects. On the other hand, infection by HIV raises the possibility of developing VL by between 100 and 1,000 times (and sometimes even more) in endemic areas[41,43], it reduces the possibility of post-treatment recovery, and it increases the probability of relapsing[6,21,41,43,46,68].

In this way, *Leishmania*/HIV co-infection emerges as a very serious process, in which both pathogens act in synergy.

Clinical presentation

Though the majority of infections by *Leishmania* in HIV$^+$ individuals show the classic signs of visceral, cutaneous and mucocutaneous leishmaniasis in immunocompetents[115,116], the leishmaniasis associated with HIV infection possesses several principal characteristics: (*i*) parasitic dissemination via the reticulo endothelial system without visceral involvement, creating diffuse cutaneous leishmaniasis; (*ii*) it has been suggested that almost any organ that contains phagocytic cells could become infected[117];

(*iii*) atypical locations are affected as a consequence of the parasite's spread and deficiency in the cellular immunity[46]; (*iv*) chronic progress and relapse[115], with each patient experiencing between two and three relapses independently of the treatment received; (*v*) poor response to classic therapy[118]; (*vii*) low presence of anti-*Leishmania* antibodies, a factor that can be observed in many endemic areas[119]; and (*viii*) the prevalence of the different forms of leishmaniasis is particular to each zone, in relation to the causative species of *Leishmania* (Fig. 4).

The clinical aspects are comparable to those of the classic disease[19,65,115,119-121]. The period of incubation is variable and can be related to age[46,122]. During a bout of VL, other concomitant opportunistic diseases can be diagnosed in 42-68 per cent of HIV^+ patients[17,64]. The visceral form is widespread and generally not only affects the organs of the reticulo endothelial system but also unusually in many other organs[66].

A very important aspect in *Leishmania*/HIV co-infection is the appearance of post-treatment relapses, as happens to 27 per cent of patients in the first six months after treatment and up to 60 per cent in first twelve months[19,123]. These bouts are caused by the reactivation of latent parasites because the immune system is incapable of eradicating the infection, although initially the patient may seem to have recovered clinically[22].

The prognosis of VL in HIV^+ patients is very different from that for immunocompetents. The levels of relapsing and of mortality are greater in the cases of co-infection. Before the introduction of the new antiretroviral therapies, the mortality of co-infected patients reached 60 per cent in the first year, of which 27 per cent died during the first month after the diagnosis of VL[124]. Though the majority of the patients die due to other diseases related to AIDS, it is considered that leishmaniasis contributes to this, causing immunosuppresion independently of the HIV, and stimulating the virus replication.

Systemic signs and symptoms: The majority of co-infected patients display fever, hepatomegaly and/or splenomegaly, hypergammaglobulinaemia and pancytopenia[22,122].

Typically the splenomegaly is less frequent in the HIV^+[125]. The majority of co-infected patients present some type of haematological cytopenia, the frequency and the grade of anaemia, leucopoenia, lymphopenia and thrombocytopenia have been observed to be greater in this type of patient than in the immunocompetent[6,121]. The hypergammaglobulinaemia has limited diagnostic value because not only is it frequently found in VL, but also in HIV infection *per se* and in other chronic infections. The constitutional syndromes (weakness, anorexia and loss of weight) are frequent in 50-70 per cent of patients. The lymphadenopathy affects 15-60 per cent of patients. The disease tends to spread to the skin and other organs, so that the presentation outside of the reticulo endothelial system can lead to clinical errors[8,126,127]. Between 7-17 per cent of the fevers of unknown origin in HIV^+ patients are due to *Leishmania* infection, and 45 per cent of the HIV^+ patients diagnosed with VL present fever of unknown origin[6].

Gastrointestinal implications: Gastrointestinal implications are among the most frequent complications in HIV^+ patients[128,129]. *Leishmania* has been identified in the gastrointestinal tract of 50 per cent of HIV^- patients with VL[130], and also in those who are HIV^+. The parasite can appear in any section of the gastrointestinal tract[130-139]. In HIV^- patients the principal digestive signs are diarrhoea, malabsorption, hypoalbuminaemia and loss of weight. However, these symptoms are not specific in the AIDS patient, and therefore could have been caused by another source[140]. Besides, *Leishmania* can co-exist with other pathogens in the same stretch of the digestive tract[141]. Therefore, the endoscopy and routine biopsy are the best diagnostic tools in HIV^+ patients who display gastrointestinal symptoms of unknown origin[137-139,141]. Endoscopic examination gives very varied views, such as normal mucous, oesophagus mucous with diffuse erythemas, oesophagus mucous with extensive ulceration, erosive gastroduodenitis, gastric ulcers and multiple petechial lesions in the colon[137,141]. The co-existence of *Leishmania* in Kaposi's sarcoma lesions, ulcers caused by cytomegalovirus, or in the oesophagus caused by *Candida* or *Herpes simplex* have been described[137,141].

Cutaneous and mucocutaneous implications: The cutaneous involvement in VL is rare[142]; nevertheless it is more frequent in VL associated with AIDS, being observed in 4.8 per cent of co-infected patients[2]. These lesions can occur simultaneously with a bout of VL and can be papular, macopapular or nodular. On occasions, the discovery of *Leishmania* is associated with changes attributable to other dermatological processes, such as dermatofibromas, psoriasis, Reiter's syndrome, bacillary angiomatosis, cryptococcosis and oral aphtha, though its presence does not necessarily imply a causal role[84]. Cases of cutaneous, mucocutaneous, and diffuse cutaneous leishmaniasis and post kala-azar dermal leishmaniasis (PKDL) have also been described[73,143-145]. On the other hand, primary cutaneous lesions may spread to the viscera in patients with severe immunodepression[80,81,83].

The mucocutaneous forms appear in 0.3 per cent of European co-infection cases[2]. Practically all the species of *Leishmania* could be responsible for mucocutaneous lesions in HIV^+ patients, and they are also not unknown in HIV^- subjects due to other species, including *L. infantum*[146], distinct from the subgenus *Viannia*. Although nasal septum and the soft palate could appear to be involved due to metastasis, they might also be affected as consequence of a primary lesion[147]. In the latter case, taking a nasal biopsy is usually necessary to establish the definitive diagnosis of mucocutaneous leishmaniasis, a greater number of amastigotes being observed than in the case when the lesion is produced by species of the subgenus *Viannia* in immunocompetent patients.

If the opposite is not found, for any cutaneous specimen from HIV^+ patients in whom there are *Leishmania* amastigotes, the first option to be considered should be a disseminated form of VL rather than a primary cutaneous lesion.

Respiratory tract forms: In anatomopathological studies *Leishmania* amastigotes have been found in the alveolar and pulmonary septums of 75 per cent of the co-infected patients[148]. Nevertheless, the clinical meaning of this finding is hard to discern due to the fact that the frequent pulmonary form is not normally accompanied by clinical symptoms or complications and, when this happens, it is difficult to differentiate the role of *Leishmania* from that of other, more frequent, pulmonary infections.

Renal form and acute renal failure: Glomerulopathy is associated with VL and not with cutaneous or mucocutaneous leishmaniasis. In 60 per cent of HIV^- patients with VL proteinuria, haematuria and leucocituria have been described[149]. The frequency of these symptoms is not known in case of HIV^+ patients. The pathological findings include glomerulonephritis, which changes from mesangioproliferative, sometimes associated with the focal and segmental collapse of the capillary handles, to tubulointerstitial damage[150]. Renal condition is not common in human leishmaniasis. Nevertheless, a case of renal failure has been described at the start of VL in an HIV^+ patient[151].

Other locations: A frequent aspect of bouts of VL in immunodeficient patients is the appearance of atypical signs. The condition of severe immunodepresion that characterizes these patients facilitates the haematogenous dispersion of *Leishmania* to tissues that are not normally affected in immunocompetent conditions[152,153]. These types of signs are more frequent when the level of $CD4^+$ cells is lower than 50 cells/µl of blood[2]. The list of infrequent signs includes, in addition to those described previously (cutaneous, mucocutaneous, gastrointestinal, renal and pulmonary), others such as pleural, laryngeal, pancreatic, adrenal, peritoneal, synovial and cerebral, as well as of the pericardium, myocardium and tongue involvement[46,59,84,117,139,148,154-160]. A retrospective study in France found amastigotes in atypical locations in 34 per cent of the HIV^+ patients with VL, while finding diagnostic evidence in only 15 per cent of the cases[161]. It is important to bear in mind that the atypical locations can be the first clinical manifestation of VL in immunodeficient patients. However, the majority of these manifestations have been described previously, though with a low frequency, in immunocompetent patients[130,148,162,163].

Diagnosis

In HIV^+ patients the clinical diagnosis of VL has an even more limited value since in the AIDS patient

leishmaniasis can first appear in an unusual way and with not very specific clinical signs[159,164,165]. To this should be added that the splenomegaly is less frequent in these patients[6]. An aspect to bear in mind is that in 68 per cent of the cases of co-infection other opportunistic infections associated with HIV also appear which display clinical and biological signs similar to those of VL[21,64,66,165].

Microscopy: In this case the experience of the microscope user is fundamental, since they might need to recognise *Histoplasma* or other organisms that could infect these patients and lead to false positive results.

For the microscopic observation of bone marrow a sensitivity has been described of between 67 and 94 per cent[6,22,166,167]. On occasion, false negatives could be present for several reasons, as for example, the presence of a low number of leishmania infected cells (as a consequence of pancytopenia) or the sample is haemodiluted due to incorrect collection. Another parameter that can affect the parasitic load is if the patients have been treated with pentamidine or amphotericin B against mycosis or pneumocystosis[168].

Amastigotes can be found in the peripheral blood of approximately 50 per cent of HIV^+ patients[119,169], although Delgado *et al*[170] established that the sensitivity of this method varies according to whether the patient presents symptomatic or asymptomatic examination leishmaniasis. The study of other samples different than biopsies from blood or bone marrow is not very common in HIV^+ patients, though Montalbán *et al*[22] found a sensitivity of 87.7 per cent for the microscopic examination of hepatic biopsies. A typical feature in this type of patient is that, occasionally, parasitation can also be observed in unusual locations such as the lungs, larynx, gastrointestinal tract, rectum, spinal fluid and others[46,153].

Culture: In patients co-infected by *Leishmania*/HIV, the culture of mononuclear peripheral blood cells has a sensitivity of 67 per cent according to López-Vélez *et al*[152]. Mathis and Deplazes[171] observed a similar percentage (64%). This is interesting since, unlike in immunocompetents, the choice of non-invasive sampling turns out to be useful in the diagnosis of leishmaniasis. The culture of splenic aspirate can reach a greater sensitivity (63-100%)[6,19], though due to the danger involved in the collection of this type of sample, myelocultivation is recommended for these patients as it has a similar sensitivity according to some other authors[46,172]. However, when using culture for diagnosis, one should remember that some special precautions are required in the routine handling of leucocytic samples from AIDS patients.

Serology: In *Leishmania*/HIV co-infection, the humoral specific response to *Leishmania* turns out to be partial, weak or absent, due to the fact that the cellular immunity is affected after infection by HIV[64], causing a decrease of total lymphocytes in the production of antibodies[95]. *Leishmania* infection affects the appearance of antigens to the B cells as much as does HIV. Thus, the process of antibodies production remains blocked[173,174]. This fact has been confirmed by Mary *et al*[175], who estimated that the level of specific anti-*Leishmania* antibodies in patients with AIDS was 50 times lower than in patients with an intact immune system. Other authors shown that the serology is positive for *Leishmania* only in 40-50 per cent of co-infected patients[22,176], this percentage being inversely related to the level of depletion of T $CD4^+$ cells. Therefore, it is recommended that at least two different serological techniques are used in the diagnosis of every patient[2].

Lymphocyte proliferation test: *Leishmania* is capable of modulating the lymphocyte function[177] causing a severe immunosupression in patients with VL[178,179]. During the course of the diseases there is a pronounced depression of the cellular response to *Leishmania* antigens[179,180] and a polyclonal activation of B cells with a high level of both specific and unspecific antibodies[181]. The immune response of T cells is crucial for the cure and protection from later infections by *Leishmania*[182]. Therefore, after a successful treatment, the proliferation of T cells and delayed hypersensitivity against the *Leishmania* antigens are developed[179,183].

Though this test is currently not practical for diagnosing a bout of VL, it is in epidemiological studies in endemic zones to determine to determine

the exposure of the population to the *Leishmania* infection[184].

In co-infected individuals this technique has allowed the verification of the finding that the absence of a cellular response to the *Leishmania* antigens, on having finished the treatment, is responsible for the high number of relapses suffered by these patients[185,186].

Indirect immunofluorescence test: Due to the immunodepresion, this test applied on the sera of *Leishmania*/HIV co-infected patients gives a low sensitivity, between 11 and 58 per cent[2,96] .

Enzyme immunoassay: Most of the assays performed on HIV$^+$ patients samples where by using the rk39 antigen, which is supposed to detect only active VL cases[187]. Medrano *et al*[96] established a sensitivity of 22 per cent for the rk39 ELISA in patients co-infected by *Leishmania*/HIV. Other authors reported a sensitivity of 20 per cent for the immunochromatographic test and 62 per cent for the ELISA form[2,188].

Agglutination tests: Hailu and Berhe[189] obtained a sensitivity of 90 per cent for the direct agglutination test (DAT) in a work carried out in Ethiopia, similar to the result the same authors found for immunocompetent patients. Vilaplana *et al*[190], in a study undertaken in Spain, found a sensitivity of 100 per cent and a specificity of 96 per cent for the detection of the *Leishmania* antigen in urine by means of KAtex.

Immunoblot: Studying the serum of 37 patients with VL and 11 with HIV/VL using immunoblot, Mary *et al*[175] showed that the antigens of 14 and 16 kDa had the highest specificity for VL. Those of 14 kDa were detected in the serum of 92 per cent of the patients with VL and 95 per cent for those of 16 kDa. The same standard was found in HIV/VL co-infected patients, although the 14 kDa band was not present in any of the cases, whereas 16 kDa appeared in a constant way for these patients. In another study undertaken with co-infected patients, Medrano *et al*[96] reported a sensitivity of 78 per cent for the immunoblot technique, confirming that this type of test provides an increase in sensitivity when detecting anti-*Leishmania* antibodies. Similar results have been presented by Moreno *et al*[186], attributing a sensitivity of 85 per cent to the immunoblot in the diagnosis of these patients.

Molecular diagnosis - PCR: PCR has been shown to be of great utility in the diagnosis of co-infected patients. Analyzing samples of bone marrow, Piarroux *et al*[168] obtained a sensitivity of 82 per cent for the PCR, whereas other studies showed 100 per cent[165]. In 165 blood sensitivity samples ranged between 72 and 100 per cent[191,192].

Due to the relapsing character of leishmaniasis in HIV$^+$ patients, the PCR can be a useful technique in monitoring patients post-treatment, as it can be applied to non-invasive biopsies so avoiding unnecessary pain to the patient when evaluating the parasitic evolution during the follow up.

Real time quantitative PCR (RTQ-PCR): Although the classic PCR technique is very useful as a diagnostic tool it has some disadvantages, such as the need to analyze the amplified products using electrophoresis, and it has limited utility when the parasitic load needs to be monitored precisely as it is not able to quantify the obtained DNA.

The real time quantitative PCR allows continuous monitoring of the PCR products generated during the amplification process. Thus it is possible to identify the cycle in which the PCR product is generated in a logarithmic way and, by inference, the quantity of the target DNA present at the beginning of the reaction. Provided that the amplification product is monitored in real time as is formed cycle by cycle, no post-amplification manipulation is needed.

The absolute quantification is realized in accordance with an internal standard coamplified with the sample DNA, or in relation to a standard external curve obtained using the parallel amplification of a series of known concentrations of a sequence of reference DNA. An estimation of the parasitic load in a given sample can be obtained from the quantification of the target DNA. The advantages, compared to the classic PCR, are a

reduction in the time needed for the assay and the possibility of determining the parasitic load of the studied sample.

Different approaches exist to monitor the amplification of the products, generally based on the use of fluorescent markers. An example is the technology based on SYBR Green I, a detection system based on a fluorescent agent that can be inserted in the double stranded DNA. Another example is the TaqMan technology, which uses a specific fluorescent probe for a region of the product amplified during the PCR. The intensity of the fluorescent signal accumulates at the end of every amplification cycle and is related to the quantity of PCR-generated product.

In the recent years, different methods of quantitative PCR based on either the SYBR Green I method or on the TaqMan probes have been applied in the study of leishmaniasis. Bretagne *et al*[193] used the TaqMan system to determine the parasitic load in the liver of mice infected with *L. infantum*; whereas Nicolas *et al*[194] applied the SYBR Green I system in a study of *L. major* infection, also in mice. In human leishmaniasis Schulz *et al*[195] managed to detect and to quantify parasites in different biopsies (peripheral blood, bone marrow aspirate, skin and liver) with a model similar to the TaqMan probes, although they thought that its applicability to monitoring treatment could be affected by high variability in samples with low parasitic loads. On the other hand, Bossolasco *et al*[196] applied the real time PCR and also a fluorescent probes model to monitor the parasitic load in the peripheral blood of patients co-infected by HIV and *Leishmania* who had been under treatment. Although the sensitivity obtained with this method in the initial attack did not turn out to be very high (40%), it allowed a relationship between the parasitic load in the blood and the clinical course of the VL to be established. Mary *et al*[197] obtained similar results and improved the sensitivity, also using TaqMan probes, even though the variation in the number of copies of the target that they used could lead to a quantification calculation error between patients infected by different strains.

Though the application of real time quantitative PCR in the diagnosis and monitoring of *Leishmania* infection is in an early phase of development, the standardization of a method capable of determining the parasitic load in a sensitive and reliable way will be of great utility. It will find applications both in the study of the effectiveness of treatments and vaccines and as an aid to diagnosis.

Plasmatic markers with prognostic value: IFN-γ and TNF-α: As *Leishmania* is an intracellular parasite of the macrophages, specific immunity against VL has to come from a Th1 type cellular immune response that can produce cytokine stimulators for macrophages (including IFN-γ). On the other hand, susceptibility to this illness is associated with an impairment to generate a Th1 response, generating Th2 type cytokines, as well as high levels of TNF-α[198-207]. It has been observed that oligosymptomatic individuals or those with subclinical infection produce cytokines of types Th1 and Th2, with low or absent levels of detected IFN-γ. These individuals are in an intermediate position between illness and protective immunity[206,208].

IFN-γ : During a bout of VL the natural killer (NK) cells and the T cells produce IFN-r, influencing the development of a protective Th1 response. The production of IFN-γ, with the consequent activation of the macrophages, is crucial for the initial containment of the parasite's multiplication[205,209-211].

In the asymptomatic or subclinical cases, the production of IFN-γ influences the self-limiting evolution of the clinical symptoms and signs, reaching in the spontaneous resolution of the bout of VL[208,212-215]. Gama *et al*[206] found that 48 per cent of the children with asymptomatic infection showed detectable levels of IFN-γ, whereas this cytokine was not detected in children who suffered the acute form. Supporting this data, Carvalho *et al*[201] also observed high levels of IFN-γ in oligosymptomatic individuals, confirming the fact that resistance to the illness is associated with an effective cellular immune response.

As for the treatment of leishmaniasis, it has been proved that IFN-γ augments the therapeutic effectiveness of antimonials, both in VL and in cutaneous leishmaniasis[216].

TNF-α: TNF-α is considered to be a prognosis marker in VL, as its level is found to be significantly higher in acute and persistent cases of the illness, which then drops drastically after the parasite has been treated[217]. Though it is possible to detect TNF-α in 100 per cent of the patients who showed subclinical infection[206], during active and acute bouts of VL these levels increase notably with regard to subclinical and asymptomatic cases[80,206,218].

Moderate levels of TNF-α facilitate the elimination of intracellular parasites, whereas its increase might predispose the patient towards clinical manifestations of VL, such as fever, cachexia, anorexia, pancytopenia and polyclonal activation of B cells[199,219]. The blood pathogens induce the macrophages in liver, spleen and other locations to produce TNF-α, and the systemic release of TNF-α causes expansion of blood vessels with the consequent extravasation of fluids towards the tissues. In this way septic shock can take place, coagulating the extravased blood in the capillaries, leading to failure of vital organs such as the kidneys, liver, heart and lungs, which are compromised by the loss of blood supply[220]. For this reason, it is considered that high levels of TNF-α in serum are correlated with the severity of human VL[221], giving it a principal role in systemic alterations and, consequently, in the symptoms observed[222-224].

TNF-α plays an important role in HIV infection since it has been proved that it has a positive effect on the replication of the virus[111,225] associating high levels of TNF-α in serum with the progression of the infection from HIV to AIDS[226].

Co-infected patients display higher levels of TNF-α in serum than HIV^+ patients not infected by *Leishmania*, and these levels also remain high after recovery from a bout of VL[89]. This fact contrasts with that seen by others in immunocompetent VL patients where the levels of TNF-α normalize after successful treatment[199,227].

Treatment

Given that HIV^+ patients generally develop VL when they are in a state of severe immunodepression and are affected by other concomitant diseases, it is particularly difficult to evaluate the effectiveness and toxicity of drugs in these patients[118]. Although between 11 and 27 per cent of co-infected patients die within a month after diagnosis of VL, these deaths are rarely attributed to the leishmaniasis or to the toxicity of the drugs administered to combat the disease[6,19,124]. The clinical criteria used to verify the response to the anti-*Leishmania* treatment, useful in the immunocompetent patients, are of less value in the immunodepressed. Only the initial remedy should be considered as, if the immunodepression does not change, the probability of a full recovery is practically nil and the patient tends to relapse. The parasitological response to the anti-*Leishmania* treatment in HIV^+ patients has partially been studied[6,19,22,64,124,166]. Finally, it is necessary to consider that other medicines administered to HIV^+ patients can increase the toxicity of some leishmanicide drugs.

Pentavalent antimonials: The results of several retrospective studies indicate a high level of clinical response to antimonials in the patients co-infected with HIV[19,22,59,64,166]. However, the drug doses and the criteria used to identify the clinical recovery were not uniform, observation of the clinical cure turned out to be difficult due to the co-existence of other diseases associated with AIDS, and clinical progress does not necessarily mean that parasitological recovery has occurred[228]. In all these groups of patients a high percentage of them relapsed during the year after the treatment finished.

Moreover, pentavalent antimonials are known to be more toxic for HIV^+ patients than for HIV^- ones[6]. Effects described are; pancreatitis (becoming fatal in some patients[229]), myocarditis and renal failure[118]. In fact, these developments interrupt the therapy in 11-28 per cent of patients[6,123,124,230].

Amphotericin B (AmB): More than half of the patients treated with AmB showed a series of typical side effects related to the infusion, such as tremors, fever, nausea, vomiting, anorexia, headache, myalgia and arthralgia[231-233]. The duration of the infusion dosage must range between 2 and 6 h, to reduce these adverse effects[231,232].

The most important side effect associated with AmB is undoubtedly the nephrotoxicity, which occurs in many of the patients treated[233]. The mechanism by which these changes take place is not understood, though it is thought that it stems from an alteration in the permeability of the glomerular membrane[234]. The worsening, generally reversible, of the renal function happens during the first two weeks of treatment and occurs in 80 per cent of the patients treated with AmB. They usually recover after the treatment is terminated though it can take several months for the renal function to recuperate[234]. Daily monitoring of the levels of creatinine and other renal parameters is recommended when using AmB treatment. When the level of creatinine rises above 3 mg/dl, the therapy must be interrupted until the level returns to normal[231]. It is also recommended that the treatment be administered on alternate days to reduce the renal toxicity[232]. The use of lipidic formulations diminishes the AmB toxicity, supporting its therapeutic activity[235].

Treatment can be started by administering the AmB-disoxicolate in very small doses, increasing gradually (0.1, 0.25 up to 0.5 - 0.8 mg/kg, *etc*.), diluted in 5 per cent dextrose[236]. It should be administered as an intravenous (iv) infusion over 2 - 6 h, with the treatment lasting about 4 wk. The maximum daily dose must never exceed 1.5 mg/kg, but it is possible to administer a dose of 1.5 mg/kg on the alternate days because the elimination half-life of AmB is very high[237].

On comparing the parasitological effectiveness of administering maximum doses of 1 mg/kg up to a total dose of 20 mg/kg of AmB, every day or on alternate days, it was proved that the two regimens were equally effective, since all the patients recovered and 6 months later no parasites were observed in splenic aspirates, though the daily schedule was more economic as it reduced the period of hospitalization[238]. If the dose is diminished to 0.5 mg/kg in 14 administrations on alternate days, 100 per cent aseptic curing was achieved at 6 wk and 98 per cent at 6 months[239]. If the same regimen is compared with the administration of sodium stibogluconate (SGS) (20 mg/kg/day for 40 days), the level of curing is 62 per cent, as opposed to 100 per cent for the AmB, which indicates around a 40 per cent resistance to the Sb^V in patients of India[240]. AmB was also used in paediatric patients with a dosage of 1 mg/kg every 2 days for 20 days, which was tolerated perfectly, achieving 100 per cent healing. Pregnant women tolerated this dosage, without apparent damage to the foetus, and with good results against the parasite[241].

In a study undertaken by Laguna *et al*[123] on HIV^+ patients, the parasitological recovery was similar to that shown by the patients who were treated with pentavalent antimonials. However, the level of relapsing in the first year post-treatment was similar in both groups.

Delivery systems: One of the most significant advances in recent decades in chemotherapy against *Leishmania* has been the use of lipidic vehicles to transport the amphotericin B. The drug delivered in this way is rapidly captured by the cells of the endothelial-reticulum system and directed specifically to the organs rich in this type of cells, avoiding the organs in which toxicity takes place, prolonging the liberation and therefore the presence of the drug in the organism. The pharmacokinetic and pharmacodynamic differences between the transported forms and the free drugs are due to the changes which are produced in the distribution, at the different levels of plasmatic concentration, as well as to the changes that take place when the plasmatic proteins bound, in their metabolism and elimination of the transported drugs[242].

It has been proved that the effectiveness of liposomal Amphotericin B (AmBisome®) in both adults and children is independent from the form of administration (21 days with a dose of 1 mg/kg or 10 days at 3 mg/kg/day) and none of the immunocompetent patients relapsed between 12 and 24 months after treatment[243]. When the treatment was shortened by increasing the doses (5 consecutive days and then a dose on the 10^{th} day, up to a total dose of 18 mg/kg). With this regimen parasitological cures were achieved, but renal alterations occurred[244].

The liposomal AmB used in Sudanese patients with complicated kala-azar was more effective if

6 doses of 3-5 mg/kg were used (curing 88% of the patients), than if the same dose was administered only 3 times (50% cure rate), whereas if the patients were given AmBisome® (3 mg/kg) over 10 successive days, a full cure was achieved[245]. When the effectiveness and tolerance of 3 different administration protocols of AmBisome® (total doses of 14, 10 and 5 mg/kg) were compared, it was found that Indian patients (n = 30) responded to all 3 protocols. The blood, biochemical and clinical parameters returned to normal and after 12 months no parasites appeared in bone marrow or splenic aspirates and the symptoms of hepatic toxicity, especially in the group that received the larger dose, disappeared after 2-6 months[246].

The use of the amphotericin B on lipidic discs (amphotoricin B cholesterol dispersion, ABCD or Amphocil®) in leishmaniasis therapy has hardly been tested. It was used in Brazil, at a dose of 2 mg/kg over 10 or 7 days, with good parasitological results (no parasites appeared in 19 of 20 patients treated) and the clinical and biochemical parameters normalized. However, symptoms of toxicity attributed to the infusion (fever, tremors and respiratory disorders) did appear, mainly in children[247]. Amphotericin B in lipidic complexes (ABCL or Abelcet®) represent an important alternative in the treatment of VL. A dose of 3 mg/kg for 5 consecutive or alternate days achieved 100 per cent cure rate. Nevertheless, symptoms of toxicity appeared during the first infusion day in 95 per cent of the patients, and in 50 per cent in the last dose[248]. To verify the effectiveness when reducing the dose, 3 different protocols were evaluated: doses of 1, 2 or 3 mg/kg for 5 days. At day 14 parasites were not observed in the splenic aspirates, while after 6 months the cure rates were 84, 90 and 100 per cent, respectively[249].

In India, 11 patients with kala-azar were treated with AmB associated with lipidic emulsion (progressive doses of between 0.05 and 1 mg/kg every day for 25 days with a total dose of 20 mg/kg and 2 h of infusion) and 11 with free AmB (with the same regimen, but with an infusion duration of 4 h). The patients of both groups were cured clinically (biochemical parameters, weight and size of the liver and spleen) and parasitologically (splenic aspirate) during a period of at least 6 months[246]. Increasing the dose of this combination to 2 mg/kg and reducing the treatment to 8 days, also gave good parasitological results. Seven days after finishing the treatment no parasites were observed in the bone marrow and no relapse had occurred 14 months later[250].

HIV^+ patients and recipients of transplanted organs usually need greater doses and a longer treatment[251-253]. Two prospective, non-comparative studies evaluated the effectiveness and toxicity of liposomal amphotericin B in co-infected patients. In the first one, seven patients were treated with 100 mg of liposomal AmB per day for 21 days (to a total dose of 29-38.9 mg/kg). Of these, 5 patients who initially showed parasitological recovery later relapsed[243]. In the second study, in an attempt to reduce the relapsing rate, Russo *et al*[251] used high and intermittent doses (4 mg/kg/day on the 1^{st} to 5^{th} days, and then on the 10^{th}, 17^{th}, 24^{th}, 31^{st} and 38^{th} days) to treat 10 co-infected patients. This regimen gave a similar effectiveness to the low dose used by Davidson *et al*[243] and also was not able to prevent the relapses.

The experience of using ABCL to treat co-infected patients is very limited. In a study carried out by Laguna[118], in which co-infected patients were treated by ABLC (3 mg/kg/day for 5 or 10 days) or with meglumine antimoniate (20 mg Sb^V/kg/day for 28 days), found a similar level of effectiveness for the three groups, although the treatment with antimonials gave the greatest toxicity.

Pentamidine: To tackle the problem of the resistance of *Leishmania* to conventional treatments, one of the drugs that has been evaluated in the treatment of human leishmaniasis has been pentamidine. Berman *et al*[254] verified the sensitivity of *Leishmania* to this medicine. Although at the beginning of the 1980s, with 5 wk of treatment, high cure rates were obtained in endemic regions (India), in the 1990s, in the same zones resistances had already developed[255].

Its mechanism of action is not known exactly, but is believed to be related to the kinetoplast DNA[256].

In 1993, Murray *et al*[257] demonstrated that in athymic mice (deficient in T cells) pentamidine was not effective against *Leishmania* whereas it was effective in euthymic mice.

The recommended dose depends on the salt used. If it is in the isotianate form the dose is 4 mg/kg (up to a maximum of 300 mg daily), if the metilsulphonate is used, the dose is 2.4 mg/kg (up to a maximum of 180 mg daily). Short treatment regimes with low doses of pentamidine, which are used in cutaneous leishmaniasis treatments often result in myalgia, pain in the injection site, nausea, headache and, rarely, a metallic taste. The incidence and severity of these side effects are much greater in visceral leishmaniasis treatments, with their high doses over long periods, sometimes also inducing hypotension, tachycardia and hyperglycaemias, and even diabetes[255,258].

In the treatment of visceral leishmaniasis, using 4 mg/kg, three times per week up to the parasitological cure of the patient, 77 per cent of the patients were cured after 15 injections (5 wk of treatment) and 94 per cent after 27 injections (9 wk of treatment), however, 21 per cent relapsed within a year. In Colombia, a regimen of 2 mg/kg every two days for seven days was used, with 96 per cent effectiveness[259]. The treatment of VL needs high doses of pentamidine for several weeks, and is even more toxic than the treatments with antimonials. Thus, this drug should be used exclusively when there are reasons to think that it is going to be more effective than any other, in geographical zones where antimonial treatments habitually fail, or in areas where this product has not been in use for some time and therefore resistance has not been created and, of course, in individual cases where other therapies have not succeeded.

Paramomycin: This is an aminoglycosid antibiotic with a short half-life, much used against intestinal parasites. The mechanism of action against *Leishmania* is not known, though it is possible that it acts on the ribosome blocking the synthesis of proteins[260]. The resistance to paramomicine that certain strains of *Leishmania* develop is due to changes in the membrane that reduce absorption. The promastigotes maintain this resistance in the absence of the antibiotic in the culture system and it is transmitted to the amastigotes, which can infect macrophages[261].

Paramomycin can be used alone or in combination with other drugs to increase their effectiveness against the parasite, with antimonials being the preferred combination. According to Thakur *et al*[262] the use of half doses of paramomycin partnered with antimonials turns out to be the most effective combination. In the treatment of VL in Kenya, Chunge *et al*[263] used the sulphate of paramomycin (15 mg/kg/day, over an average of 19 days), healing 79 per cent of the patients. In India, with the combination of paramomycin (17 mg/kg) with Pentostan® (20 mgSb^V/kg) for 20 days, 82 per cent of the patients were cured, a percentage similar to that obtained with paramomycin alone for 40 days[264]. Similar results were obtained in Sudan[265].

Though monotherapy with paramomycin is effective, it never reaches the levels of healing obtained with antimonials or with amphotericin B[266]. However, as already indicated, the combination of antimonials and paramomycin is more effective and allows shorter treatment periods. A recent Phase III trial of paromomycin (PM) efficacy resulted in a cure rate of 94.6 per cent at 6 months after EOT[267].

Miltefosine: Miltefosine is a synthetic phospholipid (alquil phospholipid), normally used as an antineoplastic, with a very long half-life, which means that resistance to it develops rapidly. It cannot be given to women during pregnancy or lactation. A particular advantage is that it is administered orally, however a disadvantage is that this can lead to gastrointestinal side effects and, teratogenicity in the experimental model. Apparently it blocks the multiplication of *Leishmania* and alters its phospholipid and sterol composition[268]. Similarly, it seems that its anti-*Leishmania* activity is not only related to a direct cytotoxic effect on the parasite, but also to an activation of the cellular immunity[269-271].

Kuhlencord *et al*[272] verified that this drug is effective against different *Leishmania* species as much *in vitro* as *in vivo*. This effectiveness has been demonstrated in both acute and chronic murine

infections, showing a very notable reduction in the number of amastigotes after the treatment[273]. It has also been demonstrated that using topical miltefosine on experimental cutaneous leishmaniasis in mice produced good results, giving a considerable reduction of the parasitic load in the lesions, and it was found that they did not usually reappear in the lesions after the treatment[274]. The recommended dose in the various recent clinical studies undertaken on humans in India was 100 mg/day as higher doses can provoke toxicity, basically gastrointestinal disorders, and it is recommended that it be taken with food[275,276]. It is believed that total doses of between 2,100 and 3,850 mg were used in a clinical assay with 120 Indian patients with VL, which achieved parasitological cure (absence of parasites in splenic aspirates two weeks after the treatment ended) and clinic healing in 97 per cent of the patients, with no relapsing in the following six months[277]. In another clinical assay the conclusion was reached that the effective treatments must last between 21 and 28 days with a recommended dose of 100mg/day[278]. It has only been in use in a limited number of co-infected patients in a compassionate way, though the most suitable protocol has still not yet been established[279,280].

Allopurinol: In general, the reports published with regard to allopurinol are quite contradictory. Kager *et al*[281] treated 10 Kenyan patients only with allopurinol and cured three. However, Jha in 1983[282] treated 16 patients in India and cured 13. The combination of allopurinol with ketoconzol was shown to have been successful in a kidney transplanted patient with VL[283] and in cases of cutaneous leishmaniasis in the New World[284].

The combination of allopurinol and antimonials has been widely used, though the results obtained with this combination are contradictory, in the treatment of relapsing patients[285] and against Mediterranean VL[286]. In a study of 124 patients, the combination of the two medicines did not demonstrate any improvement in effectiveness when compared with the use of only pentavalent antimonials[287].

The role of allopurinol in leishmaniasis is difficult to evaluate. It seems improbable that it has leishmanicide effects by itself, though in combination with other typical drugs its effects against *Leishmania* seem to improve. Ribera *et al*[288] in a study on *Leishmania* HIV co-infected patients, in which all the subjects were previously treated with antimonials, later administered a secondary prophylaxis. The relapse rate was 65 per cent in the control group patients, who did not receive any secondary treatment, 56 per cent in the group that received allopurinol, and 18 per cent in those who were receiving antimony as a prophylaxis.

Immunotherapy

The progression of VL ends in immunosuppression, and follows the Th2 route with decrease in the levels of IL-2 and IFN-r typical of the Th1 route. Therefore, in the treatment of resistant cases, where it is more probable that they will suffer immunological depletion, it has been suggested to use a combination of chemotherapy with microbial or synthetic immunostimulants. Badaró *et al*[289] were the first to use IFN-γ in combination with pentavalent antimonials in the treatment of VL, finding that seven of nine patients with antimonial resistant kala-azar, were cured with the combination of 100 µg/m^2/day of IFN-γ and 20 mg/kg/day of pentavalent antimony over 28 days. In 1993, Squires *et al*[290], demonstrated that the same treatment produced a more rapid elimination of the parasites in Kenyan patients that had not received a previous treatment. With the same treatment, in India, Sundar *et al*[291], also observed a faster reduction in the parasitic load in the patients treated with the combination of two products, than in those treated only with antimonials. A later assay demonstrated that IFN-γ has only a partial effectiveness by itself. Four out of 9 patients in India, who previously had not received any treatment, showed no response and the others only a partial elimination of the parasites[292].

IFN-γ has pronounced side effects such as fever, tremors and granulocytopenia, in addition to a high price. Very recently, the immunomodulator tucaresol has been tested experimentally in infected mice[293]. The effectiveness of recombinant granulocyte-macrophage colony - stimulating factor (GM-SCF) has also been evaluated at a dose of 5 µg/kg/day in combination with pentavalent antimony in 24 patients. This led to parasitological

healing after three months, although three of the patients had relapses[294]. Information on the therapeutic use of IL-12 and IL-2 is still scarce and these have only been tested in experimental murine leishmaniasis in which an increase in the production of INF-γ and an important cellular response were observed[295,296].

Factors that influence the choice of therapy

Several factors have to bear in mind when choosing the most appropriate reagent for the therapeutic management of VL. Among them are not only medical factors, but also socio-economic and cultural considerations. It is necessary to have information about the endemicity of the strains of *Leishmania*, the immune and nutritional state of the population, the level and pattern of the parasite's resistance to drugs, and obviously the cost. In HIV^+ patients it is necessary to bear in mind the important problem posed by the frequent relapsing and the need of secondary chemoprophylaxis, which will depend on the level of $CD4^+$ cells[297,298].

Recommendations for the treatment of VL in immunodepressed patients

The treatment of VL in HIV^+ patients is controversial. The use of pentavalent antimonials or amphotericin B desoxycholate, though useful, results in high toxicity and the therapy takes a long time[123]. The new lipidic formulations of amphotericin B offer greater tolerability and can be used in short treatments.

The *Leishmania* infection reappears in HIV^+ patients, including those that receive HAART, after a successful treatment. Apparently this is related to low levels of $CD4^+$ cells[297,298]. Agreement does not exist with regard to the use of secondary prophylaxis, but it seems that the use of these can be suspended once the count of $CD4^+$ cells is greater than 300/ml[298,299].

Secondary prophylaxis

In HIV^+ patients, including those that receive HAART, relapsing of VL has been observed in spite of having received an effective therapy[300]. However, this is not very frequent, and is more probable due to lower level of the patient's $CD4^+$ cells[297,298]. In fact, if a stable level of $CD4^+$ is obtained after HAART, there are those who recommend interrupting the secondary prophylaxis, although this is controversial[298].

In the absence of a controlled study, no consensus exists on what type of secondary prophylaxis to administer to co-infected patients. Nevertheless, the period free of relapsing in patients treated with amphotericin B in lipidic complex (Abelcet® 3 mg/kg every 3 wk) compared to patients who do not receive any prophylaxis has been evaluated by López-Vélez *et al*[301], and after a year of monitoring it was observed that 50 per cent of the patients that received the prophylaxis did not show any new episode, whereas this percentage was 22 per cent in the group that did not receive it. In a similar study with five patients, Montana *et al*[302] also indicated the benefit of liposomal amphotericin B as the secondary prophylaxis.

The improvement of treatment of leishmaniasis in HIV-positive patients should be based on both the development of new regimens based on already tested drugs and the experimentation with oral drugs such as miltefosine[303].

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