Visceral leishmaniasis in immunocompromised: diagnostic and therapeutic approach and evaluation of the recently released IDSA guidelines

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SUMMARY

Visceral Leishmaniasis (VL) is a chronic infectious disease endemic in tropical and sub-tropical areas including the Mediterranean basin, caused by a group of protozoan parasites of the genus *Leishmania* and transmitted by phlebotomine sandflies. Typically, VL is classified as a zoonotic infection when *Leishmania infantum* is the causative agent and as an anthroponotic one when *L. donovani* is the causative agent. Immunocompromised patients, in particular HIV positive, are considered at risk of VL. They may present atypical signs and poor response to the treatment due to a compromission of T-helper and regulatory cells activity. Also pregnancy can be considered a condition predisposing to *Leishmania* reactivation and to the changes in immune response, due to a switch toward a Th2 response reported in this condition of the life. Laboratory diagnosis is based on microscopy for parasites detection on bone-marrow or spleen aspirates. Value of serology remains high in term of sensibility, but a positive test has to be confirmed by microscopy or molecular tests. Hypergammaglobulinemia and pancytopenia are the main alteration identified by blood examination. Treatment is based on use of liposomal amphotericin B (L-AmB) whose administration is associated to lower incidence of side effects, in respect to antimonials and other formulations of AmB. Use of Miltefosine needs further investigation when *L. infantum* is the causative agent. Relapses to treatment are observed in coinfected HIV patients. They can benefit of a second cycle, but cumulative efficacy of the treatment can be low.

Keywords: Visceral Leishmaniasis, HIV, Immunocompromised, steroids, guidelines

■ INTRODUCTION

Visceral leishmaniasis (VL) is a chronic infectious disease endemic in tropical and subtropical areas including the Mediterranean basin, caused by a group of protozoan parasites of the genus *Leishmania* transmitted to human via the bite of phlebotomine sandflies [1].

The vast majority of cases are recorded in the Indian subcontinent, East Africa and northeastern Brazil, where *Leishmania donovani* and *Leishmania infantum* (syn.: *Leishmania chagasi*) are the main aetiological agents [2].
In the Mediterranean area, where the incidence is estimated to be at least 1000 cases per year and dogs provide the reservoir for *L. infantum*, the disease affects patients of all ages, but children and immunodepressed are more frequently involved because of a relative inability to contain the infection [3-5] . Typically, VL is classified as zoonotic when *L. infantum* is the causative agent and as

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anthroponotic when *L. donovani* is the causative agent [6].

Many factors concur in determining VL, but an immunocompromised status and a poor nutritional status should be considered the main risk factors for an overt VL [7]. Immunocompromised frequently present with atypical signs and low-grade fever, reporting poor response to the treatment [8]. Patients living with Human Immunodeficiency Virus (HIV) are historically considered at risk of VL, although the total number of cases in such population is decreasing after the introduction of the High Active Antiretroviral Therapy (HAART). A correspondent increase of patients with VL and other conditions associated to immunodepression is currently reported [9].

In this review, we focus on current epidemiologic findings of VL and on the challenges in term of diagnosis and treatment in immunocompromised evaluating the findings highlighted by the recently released Infectious Diseases Society of America (IDSA) guidelines.

Epidemiology

Current guidelines underline that immunocompromised persons such as HIV positive, organ transplant recipients, and person treated with biologic immunomodulating agents are at the highest risk of leishmaniasis, justifying a high grade of suspicious for VL, when suggestive symptoms are reported.

Areas with high prevalence of HIV and *Leishmania* largely overlap. Up to 35% of VL cases may be co-infected with HIV in Ethiopia and an increasing number of co-infected is reported both in India, where co-infected increased to 2.18% in 2006, and in Brazil where co-infected accounted for 8.5% in 2012 [10, 11]. In southern Europe, HIV contributed to a significant number of cases, as demonstrated by the progressive increase of VL reported in Spain from the mid-1980s to the late 1990s, sustained mainly by *Leishmania*/HIV co-infected [12]. In Italy, HIV-VL cases were recorded starting from 1985, with a sharp increase reported in 1991 and two peaks in 1994 (33 cases) and 1997 (34 cases) [13].

VL in HIV positive can be sustained by non-human pathogenic strains of *Leishmania* which are favoured by the immunocompromission. HIV positive males aging between 29 and 49 years report the highest frequency of VL. Beside the bite of phlebotomine sandflies, HIV positive reporting intravenous drug abuse may acquire VL by an anthroponotic transmission cycle mediated by the infected blood transmitted by needle sharing. The introduction and generalised use of HAART resulted in a clear decrease in the incidence of HIV-VL co-infections. Before HAART era, those living with HIV were demonstrated to report an increased risk of developing active VL by 100 to 2320 times, after the introduction of HAART the VL-associate mortality and the zymodeme heterogeneity lowered [14-17].

Beside HIV infection, other immunosuppressive conditions can increase the risk of VL. Patients living with organ transplantation, receiving immunosuppressive treatments due to rheumatologic diseases, and those with haematologic or oncologic malignancy are considered at the highest risk of VL [5]. In a previous study, we demonstrated that in a population of 64 adult patients HIV-negative with VL, any condition associated to immunocompromission was reported in 19%. Infection in these cases was sustained by *L. infantum* zymodeme Montpellier 1 and 72 [18].

On the basis of an extensive review of the cases of VL among those living with organ transplantation, it was estimated that they report a four-fold increase of the risk of VL and that kidney transplantation accounted for the majority of cases followed by liver transplantation. VL usually occurs as a late complication after transplantation, with a median delay depending on the transplanted organ (6 months for liver transplantation as opposed to 19 months for kidney transplantation) [19].

Treatment commonly adopted for rheumatologic diseases including steroids, methotrexate, azathioprine and cyclosporine are associate to an increase of the risk of VL [20]. Similar increase is reported among those receiving modern immunosuppressive drugs such as tumor necrosis factor-α (TNF-α) antagonist [21]. Whereas initial data focused on the risk of tuberculosis, current evidences demonstrate that beside tuberculosis, TNF antagonists increase the risk for severe invasive infections sustained by *Listeria*, VL, and bacteria [22-25].

On the basis of data previously reported, we demonstrated that the risk of VL is high in those living with chronic liver disease and that the annual incidence of VL among patients with cirrho-

sis was 0.5-1/10 000, 8-17-fold higher than the incidence [0.06/10 000] among the adult population in the same area. In our small series of patients with cirrhosis and VL, we demonstrated that symptoms and laboratory presentation largely overlap with those reported during liver decompensation, making diagnosis of VL troublesome [26].

In patients living with malignancies, VL has been associated to the administration of a number of chemotherapeutic agents and monoclonal antibodies [9].

Immunity

Predisposition to VL in immunocompromised is related to many factors, but the exact mechanism causing VL susceptibility is not known exactly. *Leishmania* infection is subclinical in most cases without progression to an overt disease. Passage from infection to the overt disease was shown to be related to individual factors (age, nutritional status), parasitic virulence, immune status, and genetic predisposition [27]. Main factors supporting immune response against *Leishmania* are the activated macrophage and a specific T-helper (Th) cell type I activation. Indeed, an active response against *Leishmania* include proinflammatory cytokines enhancing Th1 response and an active macrophage response mediated by TNF-α and Interferon-γ. Overt disease is related to a mixed Th1/Th2 response and expression of regulatory cells is thought to play a key role in VL-induced immunodepression [28].

HIV and *Leishmania* reinforce their pathogenic effect on macrophage and dendritic cells and the drugs commonly active against HIV do not reduce the replication index of *Leishmania*. In HIV/ *Leishmania* co-infected patients the benefit of HAART seems to be based on the cytokine-mediated activity of Th1 cells whose number improves after treatment [29]. However, a number of studies demonstrate that *Leishmania* may increase immune activation resulting in any case in progression of HIV disease and poor CD4 cells improvement. In HIV European patients whose infection is mediated by *L. infantum*, median Th cell count at the time of VL diagnosis frequently falls below 200 cell/μL. In Ethiopia *L. donovani* infection occurs at a higher number of Th cells. Nutritional status, pathogenic effect of the parasites and different availability rate of antiretroviral therapy

could influence the relationship between HIV and *Leishmania* [10, 30].

In non-HIV immunocompromised, a relevant group of conditions concur to immunodepression and different mechanisms are responsible of the susceptibility to VL in those receiving immunosoppressive drugs or in those suffering haematologic malignancies. Many cases of *Leishmania* in these patients are related to parasite reactivation rather than to a recent infection [8].

Glucocorticoids affect the effector, suppressor, and cytotoxic T cells functions through the blockade of cytokine expression, with the result of an increased susceptibility to infections, particularly to intracellular microbes such as occurs with *Leishmania* [31]. In a murine model the prolonged use of steroids has been associated to a decreased production of IL-2, IFN-γ, IL-4 and TNF-α and to 3-fold increase in amastigote burden in the spleen [32]. Susceptibility to *Leishmania* of patients on steroids treatment can be sustained only on theoretical basis, because clinical data are not conclusive.

Many cases of VL have been reported in patients receiving anti-TNF therapy. A number of case-reports highlight the susceptibility to VL after anti-TNF therapy, but we cannot exclude that the increased risk of developing VL can be reported to other factors related to the rheumatologic disease itself [33].

Pregnancy can be considered a condition predisposing to *Leishmania* reactivation, due to the changes in immune response reported in this condition of the life. A switch toward a Th2 response, which causes an increased susceptibility to other intracellular agents and malaria, is the main reason of an overt VL during pregnancy, which can be associated to "in utero death" or transmission of *Leishmania* to the newborn that can develop VL months after delivery [34].

Diagnosis and clinical characteristics

VL manifestation can be non-specific in immunocompromised because symptoms frequently overlap those of the underlying disease or those of other opportunistic infections. Mycobacterial infections, lymphoma or other haematologic malignancy, and histoplasmosis should be considered in the differential diagnosis. The current data do not allow a comparison of symptoms in the different population of immunocompromised. The vast

majority of diagnostic and clinical data about VL and immunocompromised regard [35, 36].

As stated by current IDSA guidelines, in HIV positive, the number of asymptomatic carriers of *Leishmania* seems to be higher than in the immunocompetent host. In HIV positive, VL can be associated to an atypical clinical presentation involving skin, lymph nodes and the gastrointestinal tract in about 15% of cases, as assessed in a study investigating secondary prophylaxis with pentamidine of VL relapses [37]. Typical symptoms of VL in HIV such as an initially intermittent fever followed by a continuous pattern, hepatosplenomegaly due to involvement of the reticuloendothelial system, pancytopenia, and concurrent infections are reported also in patients without HIV infection and are similar to those associated with other opportunistic manifestations [34]. In HIV patients frequently *Leishmania* parasites can be isolated from unusual sites including skin and *mucosae* where *Leishmania* related lesions are observed [38]. Cases receiving HAART may experience an immune reconstitution syndrome that can result in new VL symptoms including post-kala-azar dermal manifestations. When we investigated patients living with cirrhosis, we found that the main findings of clinical presentation, *i.e.* hepatosplenomegaly, ascites, jaundice, low-grade fever, overlap those of cirrhosis, making signs of VL difficult to distinguish by those of the liver disease decompensation [34].

Patients with VL report non-specific laboratory abnormalities, but the main characteristics are due to B-cell overactivation causing polyclonal hypergammaglobulinemia. Positivity of indirect Coombs test and detectable levels of anti-dsDNA or anti-nuclear antibodies are reported in patients with VL and are responsible of errors in the diagnosis. Laboratory investigations show an increase of acute-phase proteins (C-reactive protein and ferritin) and of erythrocyte sedimentation rate. Blood examination reveals reduced white blood cells and erythrocyte coupled with low platelets concentration. All the findings of laboratory presentation do not allow a presumptive diagnosis and are frequently reported in patients suffering with HIV, rheumatologic disease or other immunosuppressive diseases [14].

Laboratory diagnosis is based mainly on microscopy for parasites detection on bone-marrow or spleen aspirate, as confirmed by IDSA guidelines.

Sensibility of microscopy approaches to 80% in HIV positive and can be higher in other populations such as transplanted patients. A negative test cannot completely rule out VL diagnosis, but have to suggest alternative diagnosis both in immunocompromised and immunocompetent patients [40]. Culture of *Leishmania* obtained by bone-marrow or spleen aspirate can increase the sensitivity of the procedure, but can take several weeks, making the test of low specific impact in the real practice. In an observational study, we reported that Leishmania grew from cultures in only 13 (39%) of 33 patients with VL *Leishmania infantum* [18].

Serology tests for VL can be performed by the immunofluorescence antibody test (IFAT) which has a high practical impact in diagnosing VL in immunocompetent with a very high sensitivity. Studies investigating immunocompromised patients (both HIV positive and negative cases) reported a good sensitivity of IFAT in all HIV negative cases including those on anti-TNF-a treatment, but highlighted that a negative IFAT test could not completely rule out VL diagnosis. Search of antibodies against Leishmania by the rk39 rapid agglutination test for detection of leishmanial antigen can be useful in resource limitate settings [1, 40].

Many advantages could derive by molecular diagnosis of VL based on peripheral blood or bone marrow aspirate examination, IDSA guidelines underline that molecular diagnosis should be performed if other diagnostic testing is unrevealing. [40]. Table 1 reports accuracy of microscopy and molecular tests in VL diagnosis.

Treatment
Antimonials have been the first-line drugs for human leishmaniasis in many countries for more than 70 years. Their use is associated to relevant

Table 1 - Accuracy of microscopic examination and molecular methods for Visceral Leishmaniasis.

	Specificity (%)	Sensitivity (%)
Microscopic determination		
• Lymph node aspiration	High	53-65
• Bone marrow	High	53-86
• Splenic aspiration	>90	>90
Molecular methods		
• PCR	87-100	73-100
• Real-time PCR	ND	100

toxicity and high failure rate. Liposomal amphotericin B (L-AmB) is considered the most effective treatment in developed country with the highest success rate and relatively low toxicity [41, 42]. In high-income areas, the high cost of the drug is balanced by reduction of the hospitalization period [1, 18]. Other drugs including deoxycholate amphotericin B, paromomycin and pentamidine are considered active against *Leishmania* but their use is limited by the relevant toxicity. Miltefosine is considered a promising drug due to its anti-leishmanicidal activity and the proven efficacy in immunocompromised patients [43]. IDSA guidelines suggest that L-AmB is the recommended treatment of VL in immunocompromised persons in North America and that a combination therapy containing miltefosine might be considered, but the latter evidence is reported to be low. Miltefosine may fail when VL is sustained by *L. infantum* (Table 2).

In HIV positive, frequent failure after treatment and high relapse rate are responsible of the high VL related mortality either in Europe or in eastern-Africa hyperendemic areas. HIV-related immunosuppression seems to play a pivotal role in these patients where the antiviral treatment results in an improvement of immune function also due to an antitumoral effect on theoretical basis [44]. A systematic review providing comparison through the treatment regimens of HIV/VL co-infected demonstrated that L-AmB reported the highest cure rate, an acceptable toxicity profile and a low early mortality without significant impact on the relapse rate [45]. Both the efficacy and optimal duration of miltefosine therapy in HIV positive have to be established [1]. Secondary prophylaxis could be recommended in those with HIV/AIDS associated immunodepression (*i.e.* CD4 T-cells below $200/mm^3$) but current data are not conclusive [1]. HAART should be initiated either during or after the initial course of therapy for VL because incidence of immune reconstitution inflammatory syndrome related to VL is low as suggested by IDSA guidelines.

Only case-reports or small series support the therapeutic choices of VL in immunocompromised HIV negative [46, 47]. Response and relapse rates are better when observed in HIV positive, but not as good as those reported in immunocompetent subjects. In these cases, many authors recommend the use of L-AmB because of its safety profile but comparative data are not currently available. Instead, antimonials report relatively high rates of toxicity and lower efficacy, making their use attractive only considering the low cost that makes VL treatment affordable in low-income countries [8].

◼ CONCLUSIONS

VL may be a difficult task in immunocompromised subjects due to a series of factors causing a drawback of cellular immunity that can favour parasites growth and disease manifestation.
The diagnosis may be difficult due to the symptoms commonly reported such as fever and enlarged spleen and liver are not specific and can be constitutively present in some populations of immunocompromised such as cirrhotics or those living with HIV.

Table 2 - Main treatments of Visceral Leishmaniasis.

Drug	Treatment	Efficacy	Dose
L-AmB* Immunocompetent	Choice	High	3 mg/kg/day on days 1-5, 14 and 21 [total dose 21 mg/kg]
Immunosuppressed	Choice	High	3 mg/kg/day on days 1-5, 10, 17, 24, 31 and 38 [total dose 40 mg/kg]
Miltefosine	Alternative	High when caused by L. donovani; Failure reported when caused by L. infantum	50 mg bid for 28 days if 30-45 kg; 75 mg tid for 28 days if >45 kg
Antimonials	Alternative	Efficacy lower than L-AmB. Toxicity is reported	20 mg Sb^v/kg/day for 28 days

*For VL sustained by L. infantum dosage of 3 mg/kg/day on days 1-5 and 10 is currently adopted. Bioequivalence between Amphotericin B Lipid Complex and L-AmB has not been established.

On the basis of current IDSA guidelines, L-AmB is the treatment with the highest cure rate and the lowest toxicity, but its cost makes the drug unaffordable in some low-income areas. In particular populations of patients such as HIV positive, treatment may be difficult due to a reciprocal detrimental role of both HIV and *Leishmania* on the immune system. Further studies have to establish the efficacy of other drugs such as miltefosine in immunocompromised.

Conflict of interest: None

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