Visceral Leishmaniasis in HIV-Infected Patients: The Challenge of Relapse and Treatment Failure

Leishmaniose Visceral em Doentes com Infeção VIH: O Desafio da Recaída e Falência Terapêutica

ARTIGO ORIGINAL

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ABSTRACT

Introduction: Visceral leishmaniasis is an endemic disseminated infection, considered to be the third most frequent opportunistic parasitic infection in Europe. It is especially prevalent in patients co-infected with human immunodeficiency virus, in whom it poses a great therapeutic challenge due to increased risk of relapse. The goal of this study is to characterize a population of co-infected patients, as well as the efficiency of the adopted treatment strategies.

Material and Methods: Retrospective study with a sample composed of all patients with visceral leishmaniasis and human immunodeficiency virus admitted in an Infectious Diseases ward over a period of 10 years.

Results: Of the 23 enrolled patients, two were female (8.7%). The mean TCD_{4+} cell count was 104.4 cells/uL (± 120.3cells/uL), only two patients had undetectable viral load (< 20 copies/mL) and 16 (69.6%) were not under antiretroviral therapy at the time of diagnosis. Treatment-wise, liposomal amphotericin B was used in 18 patients, meglumine antimoniate in four and miltefosine in one. Fourteen (60.9%) were adherent to secondary prophylaxis protocol. A relapse rate of 26.1% was observed (six patients).

Discussion: Co-infection is responsible for higher treatment failure rates and more relapses. TCD_{4+} cell count is the main predictive factor of relapse, and strict adherence to chemoprophylaxis protocols unequivocally results in a reduction of relapse rate. Combined treatment strategies using liposomal amphotericin B and miltefosine yield fewer therapeutic failures than the classic approach.

Conclusion: We therefore conclude that alternative, combined therapeutic protocols seem to be a viable solution for these patients.

Keywords: HIV Infections; Leishmaniasis, Visceral; Recurrence; Treatment Failure

RESUMO

Introdução: A leishmaniose visceral é uma infeção disseminada endémica, considerada a terceira infeção parasitária oportunista mais frequente na Europa. É mais prevalente nos doentes co-infetados por vírus da imunodeficiência humana, em que acarreta um grande desafio terapêutico pelo risco acrescido de recaída. O objetivo do estudo é a caraterização de uma população co-infetada e da eficácia dos esquemas terapêuticos.

Material e Métodos: Estudo retrospetivo de uma amostra seleccionada de todos os doentes com leishmaniose visceral e infeção pelo vírus da imunodeficiência humana internados num período de 10 anos num serviço de Infeciologia.

Resultados: Foram incluídos 23 doentes co-infetados, dois do sexo feminino (8,7%). A contagem média de células TCD_{4+} à data do diagnóstico da leishmaniose visceral foi de 104,4 cels/uL (± 120,3 cels/uL), apenas dois doentes tinham carga viral indetetável (< 20 cópias/mL) e 16 (69,6%) não cumpriam terapêutica antirretroviral à data do diagnóstico. Como terapêutica optou-se por anfotericina B lipossómica em 18 doentes, antimoniato de meglumina em quatro e miltefosine num caso. Catorze (60,9%) aderiram a esquema de profilaxia secundária. Verificou-se uma taxa de recaída de 26,1% (seis doentes).

Discussão: A co-infeção leishmaniose-vírus da imunodeficiência humana está associada a maior taxa de falência terapêutica e recaída, sendo a contagem de células TCD_{4+} o principal fator preditivo de recaída e o cumprimento de esquemas de quimioprofilaxia inequívoco na redução da mesma. A terapêutica combinada com anfotericina B lipossómica e miltefosine regista taxas de falência inferiores ao esquema clássico.

Conclusão: Conclui-se que esquemas alternativos e combinados parecem ser uma alternativa nesta população.

Palavras-chave: Falência Terapêutica; Leishmaniose Visceral; Recidiva; Infecções por VIH

INTRODUCTION

Leishmaniasis is a protozoal infection, spread by the bite of phlebotomine sand-flies, involving over 20 species of *Leishmania* and over 90 species of sand flies.[1] Leishmaniasis can present worldwide in two different ways: the zoonotic form (*Leishmania infantum / Leishmania chagasi)* with dogs as its main reservoir host and mainly affecting patients throughout the Mediterranean Basin, in the Middle East, China and South America and the anthropomorphic form (*Leishmania donovani*) with humans as the reservoir host, more prevalent in India and along the East African coast.[2] Leishmania infection affecting immunocompetent patients remains usually unnoticed[3] even though, depending on patient's immune status, it can present as a localized or spread disease, namely leading to cutaneous or mucosal leishmaniasis or even as visceral leishmaniasis (VL) leading to a systematic disease known as *kala-azar* ('black fever' in Hindi), the most severe form of the disease, potentially lethal if left untreated.[4]

Visceral leishmaniasis is endemic in more than 60 countries and 350 million people are currently in risk.[2] More than two million patients were affected by VL in 2013, according with the World Health Organization (WHO), producing 20 to 30 thousand deaths related to the infection and an estimated 200 to 400 thousand new cases of VL

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each year worldwide. Even though widely spread, more than 90% of the patients are mostly found in six countries – India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil[5,6] and the Mediterranean region represents 5-7% of the overall number of patients, with an annual incidence of 1,200 to 2,000 new cases,[7] corresponding to the third most frequent parasitic opportunistic infection in Europe.[8] In Portugal, 173 patients with VL were described between 2000 and 2009 by the *Instituto de Higiene e Medicina Tropical*, 66 of these affecting immunocompetent patients and 107 affecting human immunodeficiency virus (HIV)-VL coinfected patients.[1]

VL incubation period usually ranges from two to six months, although it can be shorter in severely immunocompromised patients.[9] A ratio greater than 1:30 has been found between the presence *vs.* the absence of symptoms (subclinical infection) and most asymptomatic patients have lifelong viable parasites which may arise as a reactivation in the presence of any immunosupression.[6] Fever, enlargement of liver and spleen and pancytopenia are included into the classical clinical presentation, sometimes with a constitutional syndrome with asthenia, weight loss, lymphadenopathy or with mucocutaneous, digestive, respiratory or kidney involvement. There is a similar clinical presentation in immunocompromised and in immunocompetent patients, even though the former usually present with more exuberant clinical manifestations. In addition, a lower enlargement of the spleen can be found in immunocompromised patients.[10]

The identification of amastigote forms of the parasite in blood smear or culture, in bone marrow aspirate or organ biopsies is required for the definitive diagnosis. Giemsa coloration has an approximately 70% sensitivity in bone marrow aspirate, while the culture in Novy-McNeal-Nicolle medium (up to four weeks) has a 60-85% sensitivity.[4] Serological testing [indirect fluorescent antibodies (IFA), enzyme-linked immunosorbent assays (ELISAs), direct agglutination test (DAT), recombinant kinesin antigen (rK39)] and urinary antigen detection (Kala-azar latex agglutination test – Katex] are also available. Serology remains positive upon therapeutic success and therefore should not be considered in isolation for a diagnosis. Unlike serological testing, urinary antigen detection quickly turns negative upon therapeutic success, therefore with higher sensitivity. However, the Polymerase Chain Reaction (PCR) molecular technique has higher sensitivity in bone marrow aspirate than in blood smear or culture,[11] with a 92% sensitivity and a 96% specificity in HIV coinfected patients.[7]

Different drugs were approved for the treatment of VL. Antimoniate compounds (sodium stibogluconate and meglumine antimoniate), with an historical relevance and significant toxic effects, were the first to be introduced and its use led to the development of resistances mainly in India, currently leading to only a 50% cure rate. Liposomal amphotericin B is currently recommended as the gold standard, due to its favourable therapeutic use, less secondary effects, usually with an indication for the immunocompetent patient, on a 5-day, 3 mg/kg/day regimen.[12] In fact, a cumulative dose of 20mg/kg, regardless of the regimen, has been considered adequate by the WHO in order to allow for a high cure rate in the immunocompetent population worldwide.[13] Liposomal amphotericin B is used with different regimens of 20-60 mg/kg (evidence BI-III) in HIV patients, namely in the Mediterranean region.[7] Different second-line drugs have subsequently emerged, namely paromomycin, pentamidine and miltefosine, the first oral formulation ever approved for VL.[12]

However, the approach to coinfected HIV patients is particularly challenging as apart from a higher chance of atypical presentation, these are also in higher risk for progression to chronicity, in high risk of relapse and poor response to therapy, requiring an accurate secondary prophylaxis and follow-up (clinical and biological).[8] Our study aimed at the analysis of a group of VL-HIV coinfected patients, with an emphasis on treatment failure, the prevalence of relapse and the need for new therapeutic approaches in this population. It also aimed at the characterisation of VL-HIV coinfected patients admitted to the Department of Infectious Diseases of a tertiary hospital over a 10-year period and at the assessment of the efficacy of treatment regimens.

MATERIAL AND METHODS

This was a retrospective and observational study involving a convenience sample of non-probabilistic and intentionally selected patients, including all VL-HIV coinfected patients admitted to the Department between Jan 2006 and Dec 2015.

The patients whose clinical records were unavailable were excluded from the study, as well as one VL non-HIV patient. The main variables were as follows: patient's age, gender, comorbidities, clinical staging (assessed by CD_{4+} T-cell count), plasma HIV RNA levels, the use of antiretroviral therapy (ART), whether or not containing protease inhibitors (PI), signs and symptoms, laboratory abnormalities, VL diagnostic method, VL treatment approach, prophylaxis and first relapse.

Study's database and the statistical analysis were based on the Statistical Package for Social Science - SPSS® software for Windows® version 20.0.

RESULTS

In total, 23 VL-HIV coinfected patients were admitted over this 10-year period of time [mean age 37.2 years - (± 6.3 years); range 27-48 years, only two female patients (8.7%)]. Three patients were at the same time diagnosed with HIV and VL. Most (78.3%, n = 18) patients presented with stage C3 CDC (Centers for Disease Control)-classification disease before being diagnosed with VL, with a mean CD_{4+} T-cell count of 104.4 cells/μL (± 120.3 cells/μL) at the time of VL diagnosis.

Most patients were not on ART (69.6%, n = 16) at the time of VL diagnosis. Only two patients from those on ART showed undetectable blood plasma viral load, with HIV

Table 1 – Demographic and Immunological characterisation of VL-HIV coinfected patients at the time of VL diagnosis

Case	Gender	Age	ART	Viral load (copies/mL)	CD_{4+} T-cell count (cells/uL)	Therapy	Prophylaxis upon the 1st episode	Time from the end of therapy to relapse (in months)
1	Male	30	No	287,771	14	Liposomal amphotericin B	Pentamidine	12
2	Male	40	No	414,122	53	Liposomal amphotericin B	No*	19
3	Male	39	Yes, with PI	< 20	281	Liposomal amphotericin B	Liposomal amphotericin B	16
4	Male	35	Yes, without PI	< 20	100	Liposomal amphotericin B	Liposomal amphotericin B	14
5	Male	31	No	28,077	362	Liposomal amphotericin B	Pentamidine	18**
6	Male	28	No	1,034,958	28	Liposomal amphotericin B	No*	24

ART: Antiretroviral therapy; PI: Protease inhibitors

* Two patients did not complete the secondary prophylaxis and were lost to follow-up, even though they had a formal indication for it;

** Patient no. 5 died over the first relapse episode and cause of death was not directly assigned to VL as, even though remaining on chemoprophylaxis, was not compliant with the ART and progressed to severe immunosuppression, presenting with VL-HIV-disseminated tuberculosis coinfection, which has been lethal.

RNA levels < 20 copies/mL and only four patients were on a PI-containing ART regimen.

As regards comorbidities, 20 patients were ex-intravenous drug users, 18 presented with HIV/HCV coinfection and nine patients were coinfected by *Mycobacterium tuberculosis* (pulmonary, lymph node or disseminated) and five patients presented with pneumocystosis. Fever (73.9% of the patients), abdominal pain and postprandial fullness (34.8 and 17.4% of the patients, respectively), nocturnal sweating and diarrhoea (13% each) were the most frequently described symptoms. The presence of an enlargement of the spleen (91.3%) and the liver (87%) and weight loss (34.8%) were the most frequent clinical signs found. Pancytopenia (39.1%) or bicytopenia (26%) as well as elevated transaminases levels (30.4%) were mostly found.

VL definitive diagnosis was most frequently confirmed by using more than one diagnostic method: three patients presented with positive bone marrow aspirate for the presence of amastigote forms of *Leishmania spp*, 10 patients with positive culture (bone marrow aspirate and/or peripheral blood) and nine patients with positive PCR in bone marrow aspirate and / or peripheral blood. Forms of *Leishmania spp* were found in biopsies of other tissues, namely gastric/duodenal (n = 3), liver (n = 2) or even pleural or of the nasal mucosa (n = 1, respectively).

Liposomal amphotericin B was the first treatment option in 18 patients, with different schedules [five-day, 5 mg/kg/day continuous regimen and an intermittent schedule (2.5-3 mg/kg/day over five consecutive days, followed by five doses on the 7th, 14th, 21st, 28th and 35th days, as an outpatient treatment)], always aimed at reaching a minimum 20 mg/kg total cumulative dose, usually of 40 mg/kg. Two patients presented with an iatrogenically-induced acute kidney disorder. Four patients were treated with meglumine antimoniate (20 mg/kg for 21 days), one patient presented with an iatrogenically-induced acute pancreatitis and one was treated with miltefosine, 100 mg/day, for 28 days.

Fourteen patients (60.9%) showed a confirmed initial cure and were started on a prophylaxis or maintenance regimen and the remaining were lost to follow-up upon discharge from the hospital. Four patients went on a chemoprophylaxis regimen with pentamidine (6 mg/kg every three weeks) and 10 with liposomal amphotericin B (4 mg/kg every 21 days) with the aim at reaching a CD_{4+} T-cell count consistently above 200 cells/μL over a minimum six-month period, although some patients were lost to follow-up and any information on the completed time of prophylaxis was not available.

No patient on follow-up showed any initial treatment failure, even though six relapse episodes (26.1%) were described in 23 patients, i.e. showing a new identification of forms of *Leishmania spp* in direct or culture examination upon confirmed initial cure (Table 1). These results may have been underestimated due to the fact that treatment efficacy could not be ensured as no post-therapy search for the parasite was carried out in patients that were lost to follow-up. Mean time for the first relapse was 17.2 months (± 4.2; range 12-24 months) and, at the time of diagnosis, four out of the six patients that did not suffer from any relapse episode were not on ART, had no supressed viral load and showed a CD_{4+} T-cell count below 100 cells/μL. Only one patient was on a PI-containing ART regimen. Only two patients from those that did not suffer from any relapse episode were not on any chemoprophylaxis as these were lost to follow-up during treatment. One death has been described at the first relapse episode, due to immunosuppression, non-related to VL.

DISCUSSION

According with Griensven *et al.*, VL-HIV coinfection is associated with higher mortality, poorer outcome and lower relapse rate, regardless of the treatment regimen.[2] Lachaud *et al.* (2009) showed that the second episode of VL in the same patient corresponded to a relapse episode in 70% of the patients and the same strain profile was found.[14]

An even lower relapse rate (7.5%) was described in other case series.[15] The biological confirmation is needed for a clinical VL relapse diagnosis (by bone marrow or peripheral blood smear or culture) together with at least three clinical criteria among the following: intermittent fever, weakness, weight loss, sweating, enlargement of liver or spleen, respiratory or gastro-intestinal symptoms. Even considering the presence of biological relapses (transitory recirculation of parasites identified by PCR testing, which is highly sensitive and positive for levels over five parasites/mL), true relapse episodes do not occur with parasitemia under 10 parasites /mL. Therefore, a high predictive risk of relapse is only defined by at least two successive positive PCR, even with no clinical signs.[8]

A 59% relapse rate has been found in a study by Bourgeois *et al.* involving a group of 27 VL-HIV coinfected patients,[8] above what has been found in our study (26.1%). It should be mentioned that the number of relapse episodes can be underestimated, as nine patients were lost to follow-up and the two patients who suffered from a relapse and were not followed could have suffered from an initial treatment failure. The absence of an ART (p = 0.036) and a CD_{4+} T-cell count under 100 cells at the time of the first infection (p = 0.006) were considered as predictive factors for relapse, as well as maintained CD_{4+} T-cell counts under 100 throughout the follow-up (p = 0.005).[8] In addition, a systematic revision of 18 studies by Cota *et al.* on predictive factors for relapse confirmed CD_{4+} T-cell count as the main predictive factor and have not confirmed the adherence to an ART or any other factors, namely viral load.[16] It is well known that HIV, as well as VL have an impact on T-cell mediated immunity and these are currently considered as having a synergistic action on the reduction of an effective host immune response.[17] Therefore, initial low CD_{4+} T-cell count allows for the dissemination of the parasite through mononuclear phagocytic system at infection onset, increasing the number of locations with quiescent parasites and perpetuating their cycle (called 'sanctuaries')[16] where exposure to drugs is lower[18] and which may have explained for the highly prevalent relapse in this subgroup of patients.

Adherence to therapy or prophylaxis seems to clearly reduce the relapse rate, with 31% rates found in patients on prophylaxis and 67% without it.[16] These data are in line with those found in our study, as only four (28.6%) from the 14 patients having completed chemoprophylaxis suffered from any relapse episode. Different prophylactic approaches have been recommended, with different regimens in Mediterranean strains and the use of liposomal amphotericin B (intravenous 3 mg/kg, each 21 days) is the most consensual.[7] Nevertheless, a Portuguese study by Marques *et al.* showed that miltefosine was effective for relapse and maintenance treatment (50 mg, 3 times a week), with a 20-month free-of-disease period.[19] Prophylaxis can be safely withdrawn with a negative PCR for *Leishmania* in blood and ART- controlled CD_{4+} T-cell count (> 200 cells/μL) for at least six months or whenever CD_{4+} T-cell count remains under 200 cells/μL with negative PCR for more than 18 months.[8] However, in line with our study, in which two patients having relapsed had 281 and 362 CD_{4+} T-cells/μL, Villanueva *et al.* have identified two relapsing VL patients with CD_{4+} T-cell counts of 274 and 302,[20] leading to the recommendation of a CD_{4+} T-cell count of 350 as the limit for prophylaxis withdrawal.[21] Even though primary prophylaxis is strictly contraindicated, PI can be an alternative for the ART of infected patients or in high risk for the development of VL, as these can have a direct anti-parasitic action (parasite protease can become a non-specific target for PI).[22]

Treatment failure may refer to the initial failed removal of the parasite upon the first treatment regimen or to a relapse with reappearance of the parasites in follow-up, usually a six-month follow-up, upon a confirmed initial cure.[2] Resistance mechanisms have been described against all anti-leishmania drugs and resistance mechanisms to amphotericin B have been recently described [S-adenosyl-l-methionine:C-24-Δ-sterol methyltransferase (SCMT) gene mutation – an enzyme acting on the pathway for biosynthesis of ergosterol in the membrane of the resistant parasite, decreasing drug's affinity and over-expressing the efflux of amphotericin B, reducing its intracellular concentration].[23] Therefore, the use of a combined therapy in these patients has been increasingly described in literature, as it seems more cost-effective, preventing or delaying the occurrence of resistance, preserving sensitivity and increasing drug's utilization, reducing treatment duration and total dose of each drug, with lower toxicity and higher compliance with treatment.[2]

In our study, relapses were challenging as upon the first relapse all the patients relapsed again or showed a confirmed treatment failure on a second episode at least once (on average three times). Upon different monotherapy treatment cycles (liposomal amphotericin B, miltefosine), these could have a strong indication for a combined therapy. In a 2015 revision on VL, Makala *et al.* have described six phase-II or III studies on VL combined therapy, all with non-coinfected patients.[24] Also Sundar *et al.* have confirmed non-inferiority of combined treatment regimens in VL in India by adapting 2010 WHO recommendations in endemic areas, even though used in non coinfected patients and suggesting combined single doses of liposomal amphotericin B (5 mg/kg) with 7-day miltefosine, with 10-day paromomycin and 10–day miltefosine + paromomycin.[25] As regards VL-HIV coinfected Mediterranean patients, there were still not enough data in 2010 to support any combined treatment regimen.[7] An Indian study carried out in 2015 with a group of 102 VL/HIV coinfected patients analysed the use of a combined therapy with liposomal amphotericin B, 30 mg/kg, divided into six similar doses every other day + miltefosine, 50 mg, 2xd, for 14 days. A 46% percentage of patients presented with a first *Leishmania* infection and 54% presented with a relapse and were on a monotherapy treatment regimen. Two patients died during treatment and 100 have completed the recommended regimen, with a 6% 12-month relapse and a 14.5% mortality rate. Even though

mortality and relapse rates were always higher in coinfected patients when compared with the remaining patients, the combined therapy showed a lower (6%) relapse rate when compared to the one obtained with amphotericin B in monotherapy (16.2%).[26]

Current dose-sparing approaches reducing treatment duration seem an alternative with great potential in leishmaniasis. Immune modulation should also have an important role in this area, leading to an enhanced response and to a synergistic action with antibiotics. The use of interferon has been only confirmed in VL by three studies carried out in the nineties, in association with amphotericin B and has been abandoned thereafter.[12]

CONCLUSION

This study supports the need for considering VL diagnosis in immunosuppressed patients (mainly in endemic areas, such as in Portugal and in the whole Mediterranean region). The approach to these patients involves a complex diagnosis and mainly the cure, and a significantly high relapse rate associated with VL-HIV coinfection is still obtained with currently available monotherapy regimens. The combined therapy has already emerged in literature as an alternative with multiple advantages and seems more cost-effective and inducing lower relapse rates, particularly in this population.

HUMAN AND ANIMAL PROTECTION

The authors declare that the followed procedures were according to regulations established by the Ethics and Clinical Research Committee and according to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in writing this manuscript.

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