FREQUENCY OF VISCERAL LEISHMANIASIS RELAPSES IN HUMAN IMMUNODEFICIENCY VIRUS–INFECTED PATIENTS RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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Abstract. There are contradictory data about whether highly active antiretroviral therapy (HAART) prevents visceral leishmaniasis (VL) relapses in human immunodeficiency virus type 1 (HIV-1)–infected patients. The aim of this study was to assess the frequency of VL relapses in individuals receiving HAART. Thirty-one patients who received HAART after developing VL were included in a retrospective cohort study. Ten of them received secondary chemoprophylaxis and the rest did not. Eight (38%) patients without secondary chemoprophylaxis showed a VL relapse. None of the seven subjects with VL relapses and 6 of 11 without recurrence (P = 0.038), in whom all scheduled data were available, showed an increase of more than 100 CD4+ cells/mm³ during the follow-up. Patients with relapse showed higher levels of HIV RNA viral load at their last visit (P = 0.047). The frequency of VL relapses in patients receiving HAART is high. Relapses of VL are observed only in individuals with uncontrolled HIV replication and/or poor immunologic responses.

INTRODUCTION

Leishmania infantum is considered to be the causal agent of all cutaneous and visceral leishmaniasis (VL) cases in Spain. Symptomatic VL is a frequent disease among human immunodeficiency virus type 1 (HIV-1)–infected patients from the Mediterranean basin. Thus, VL is the fourth most frequent major opportunistic event associated with acquired immunodeficiency syndrome in southern Spain. Before highly active antiretroviral therapy (HAART) was extensively used, VL associated with HIV infection relapsed in 27% and 60% of the patients within 6 and 12 months after treatment, respectively.

Since the introduction of HAART, the incidence of opportunistic infections and the mortality of HIV-infected patients has decreased sharply. Since 1997 and non-nucleoside reverse transcriptase inhibitors (NNRTIs) since 1999. Highly active antiretroviral therapy was defined as a combination of at least two nucleoside analogs reverse transcriptase inhibitors and one PI or one NNRTI. Drug compliance was assessed at all follow-up visits. Self-reported compliance to each antiretroviral drug was calculated as the percentage of prescribed doses that the patient indicated were taken during the period of time between visits. A patient was defined as non-compliant with HAART for the present study if an adherence of less than 90% to a drug was reported during at least one visit.

Population and follow-up. From April 1989 to September 2002, 1,715 HIV-infected patients have been followed in two university hospitals in southern Spain, the Hospital Universitario de Valme in Seville and the Hospital Universitario Reina Sofia in Cordoba. All individuals underwent scheduled clinical, hematologic, and immunologic examinations at baseline and every three months thereafter. Eighty-seven HIV-infected patients who received HAART after developing VL were included in a retrospective cohort study. All patients provided written informed consent before participation in the study. The study was reviewed and approved by the Ethics Committee of the Hospital Universitario de Valme and the Ethics Committee of the Hospital Universitario Reina Sofia.

Therapeutic strategies. Patients received antiretroviral drugs depending on the specific drug availability at the time and according to international recommendations. Protease inhibitors (PIs) were extensively available for prescription since 1997 and non-nucleoside reverse transcriptase inhibitors (NNRTIs) since 1999. Highly active antiretroviral therapy

MATERIALS AND METHODS

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Thirteen individuals diagnosed with VL received meglumine antimoniate (20 mg/kg/day) for 28 days, two received pentamidine (4 mg/kg/day) for 28 days, five received amphotericin B desoxycholate (0.7 mg/kg/day) for 28 days, nine received liposomal amphotericin B (2.5–4 mg/kg/day) for 10 days, and two received amphotericin B lipid complex (5 mg/
kg/day) for 14 days. Patients received secondary chemoprophylaxis after the first VL relapse according to the criteria of the clinician responsible for the case. Thus, two groups of patients have been included in this study: those were given secondary prophylaxis and those were not.

**Diagnosis of VL.** During the follow-up, a search for Leishmania amastigotes in a bone marrow aspirate was performed when patients showed unexplained fever, spleen enlargement, or a decrease in blood cell counts of unexplained origin. When these symptoms coincided with the finding of Leishmania amastigotes by staining with Giemsa, a diagnosis of symptomatic VL was made. An investigation of the presence of Leishmania amastigotes in blood was done in every case and in other biologic specimens (lymph node or liver) when clinically appropriate.

**Laboratory methods.** Plasma HIV-1 RNA was measured by a polymerase chain reaction (AmpliCord; Hoffman-La Roche, Basel, Switzerland) or nucleic acid sequence–based amplification assays (NucliSens; Organon Teknika, Boxtel, The Netherlands) depending on the availability at each hospital. The limits of detection varied between 20 and 200 copies/mL according to when the analysis was carried out and the procedure used. The CD4 cell counts were measured by standard flow cytometry.

**Statistical analysis.** In this study, we assessed the frequency of VL relapses and the factors associated with recurrences both in patients with VL treated with secondary chemoprophylaxis and in those without secondary chemoprophylaxis. Continuous variables are expressed as median (range) and the categorical variables as number (percentage). Continuous variables were compared using the Mann-Whitney U test. The frequencies were compared using the chi-square test with the Yates’ correction or Fisher’s test if the expected frequency for any cell was five or lower. The Kaplan-Meier method was applied to estimate the time to relapse after the introduction of HAART in the patients without secondary chemoprophylaxis. Data were analyzed with the SPSS statistical software package (SPSS, Inc., Chicago, IL).

**RESULTS**

**Characteristics of the population.** The main characteristics of the population are shown in Table 1. Ten (32.2%) patients with VL underwent secondary chemoprophylaxis and 21 (67.7%) patients did not. Four (12.9%) individuals were lost to the follow-up and 10 (32.2%) patients died. Two patients died due to a symptomatic VL relapse. Plasma HIV RNA load determinations were available in 20 patients during all of the follow-up. This parameter was permanently below the detection threshold in only seven patients. These low numbers were due to failures to comply with the visit schedule and the antiretroviral therapy. The individuals who received secondary chemoprophylaxis and those without secondary chemoprophylaxis were followed after the introduction of HAART for a median of 30 months (range = 4–53 months) and 25 months (range = 2–61 months), respectively.

**Frequency of symptomatic VL relapses in patients without secondary chemoprophylaxis.** Eight (38%) patients without secondary chemoprophylaxis showed a VL relapse after receiving HAART. In this group, five individuals were active intravenous drug users at the time when VL relapse was diagnosed. There was no relationship between the therapy for the primary VL episode and the emergence of relapse. Thus, three individuals had been treated with meglumine antimoniate, three with amphotericin B in lipid formulations, one with amphotericin B desoxycholate, and one with pentamidine. The highest CD4+ cell count observed in a patient with relapse at the visit prior the episode of recurrence was 170 cells/mm³. Thus, no patient without secondary chemoprophylaxis and a CD4+ cell count of 200 cells/mm³ had a relapse. None of the seven patients with VL relapse in whom all scheduled data were available showed an increase of more than 100 CD4+ cells/mm³ over the level found at starting HAART. In contrast, six (55%) of 11 non-relapsing VL patients with available data during all the follow-up show an increase of such a magnitude (P = 0.038) (Table 2). The CD4+ cell counts at the end of the follow-up were higher among patients without VL relapse (P = 0.025). These patients also showed lower levels of HIV RNA viral load at their last visit (P = 0.047) (Table 2). In the actuarial study of the time to the relapse of VL since the start of HAART, we observed that nearly 75% of the patients were free of relapse after 24 months of HAART (Figure 1).

**Frequency of symptomatic VL relapses in patients with secondary chemoprophylaxis.** One (10%) patient with secondary

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group with secondary chemoprophylaxis (n = 10)</th>
<th>Group without secondary chemoprophylaxis (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (range)</td>
<td>34 (26–37)</td>
<td>33 (24–57)</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>10 (100)</td>
<td>19 (90)</td>
</tr>
<tr>
<td>IDU, no. (%)</td>
<td>10 (100)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>CDC clinical category C, no. (%)</td>
<td>8 (80)</td>
<td>16 (76)</td>
</tr>
<tr>
<td>Self-reported compliance with HAART &gt;90% during the follow-up† (%)</td>
<td>3/10 (30)</td>
<td>6/19 (31)</td>
</tr>
<tr>
<td>Median (range) CD4+ cells/mm³ at the beginning of HAART</td>
<td>33 (2–199)</td>
<td>35 (2–407)</td>
</tr>
<tr>
<td>Median (range) CD4+ cells/mm³ at the end of the follow-up</td>
<td>161 (126–282)</td>
<td>158 (2–616)</td>
</tr>
<tr>
<td>Patients with a CD4+ &gt;100 cells/mm³ increase during the follow-up (%)</td>
<td>2/8 (25)</td>
<td>6/18 (30)</td>
</tr>
<tr>
<td>Median (range) log HIV RNA copies/mm³ at the beginning of HAART</td>
<td>5.4 (3.8–6.35)§</td>
<td>5 (3.8–6)§</td>
</tr>
<tr>
<td>Median (range) log HIV RNA copies/mm³ at the end of the follow-up</td>
<td>1.9 (1.7–7.75)#</td>
<td>3.5 (1.7–6)##</td>
</tr>
<tr>
<td>Patients with undetectable viral load during the entire follow-up period†† (%)</td>
<td>3/5 (60)</td>
<td>4/15 (26)</td>
</tr>
</tbody>
</table>

* IDU = intravenous drug users; CDC = Centers for Disease Control and Prevention; HAART = highly active retroviral therapy; HIV = human immunodeficiency virus.
† Number/total available cases.
‡ Number/total patients who attended all visits and in whom all CD4+ cell count measurements were scheduled.
§ Nine available cases.
‖ Twelve available cases.
¶ Five available cases.
** Fifteen available cases.
†† Number/total patients who attended all visits and in whom all plasma HIV RNA load measurements were scheduled.

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**Table 1**

**Characteristics of the population**
chemoprophylaxis had three relapses of VL after the beginning of HAART. This patient maintained undetectable viral load during the entire follow-up period and experienced a maximum increase of 93 CD4+ cells/mm³. He was completely compliant with the different chemoprophylaxis regimens prescribed, which were given as directly observed therapy in our outpatient clinic. This patient had four relapses of symptomatic VL undergoing secondary chemoprophylaxis before starting HAART.

### DISCUSSION

These results show that the frequency of VL relapses in HIV-infected patients receiving HAART is high. However, relapses have been observed only in subjects showing poor control of viral replication and/or a low grade of CD4+ cell repopulation. These facts were attributable to a lack of adherence to HAART.

Our study is limited because of the relatively small sample size. This is due to the fact that the incidence of symptomatic VL is low since the introduction of effective antiretroviral therapy. It is possible that additional cases of relapsing VL would have been found in patients with good recovery of immune function if a larger sample had been studied. However, our series is the largest in which this issue has been evaluated. Thus, this study clearly shows that relapses of VL in patients receiving HAART are mainly seen in cases with poor or no response to therapy. In these patients, VL is observed as it was before the introduction of HAART, i.e., as a chronic, relapsing opportunistic disease that emerges in patients with uncontrolled viral replication and poor immune reconstitution.

The results obtained in this survey differ from those found in other studies dealing with the same issue. In the study carried out by Villanueva and others,12 there were no differences in HIV viral load at the end of the follow-up period and in the increase in CD4+ cell counts after HAART in patients with or without VL relapse. Casado and others11 found that patients with relapsing VL showed lower increases in CD4+ cell counts than those with limited disease. However, they did not find any differences in plasma viremia between these groups. Conversely, in the study of Tortajada and others,13 there was a decrease in the frequency of VL relapses in patients treated with HAART in comparison with individuals receiving monotherapy or bitherapy, although the difference was not statistically significant.

The recurring symptomatic VL cases diagnosed in this study could be theoretically due to actual relapses or reinfec tions. The fact that most of the patients were active intravenous drug users at the time when infection was diagnosed could lead us to consider these as newly acquired infections. In this regard, it is known that *L. infantum* infection can be spread among intravenous drug users.14 However, recent studies have demonstrated that the majority of VL recurrences are true relapses.15

In our study, VL probably relapsed because of a failure of immune reconstitution due to low adherence to HAART. Thus, no patients in whom CD4+ cell counts either reached the level of 200 cells/mm³ or increased 100 cells/mm³ over the

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**TABLE 2**

Characteristics of 21 patients with visceral leishmaniasis without chemoprophylaxis treated with highly active antiretroviral therapy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with VL relapse (n = 8)</th>
<th>Patients without VL relapse (n = 13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, no. (%)</td>
<td>8 (100)</td>
<td>11 (84)</td>
<td>0.5</td>
</tr>
<tr>
<td>IDU, no. (%)</td>
<td>8 (100)</td>
<td>10 (77)</td>
<td>0.5</td>
</tr>
<tr>
<td>CDC clinical category C, no. (%)</td>
<td>7 (87)</td>
<td>9 (69)</td>
<td>0.5</td>
</tr>
<tr>
<td>HAART self-reported compliance &gt;90% during the follow-up †</td>
<td>1/8 (12)</td>
<td>5/11 (45)</td>
<td>0.2</td>
</tr>
<tr>
<td>Median (range) CD4+ cells/mm³ at beginning of HAART</td>
<td>32 (2–176)</td>
<td>35 (2–407)</td>
<td>0.5</td>
</tr>
<tr>
<td>Median (range) CD4+ cells/mm³ in the first VL episode</td>
<td>31 (2–277)</td>
<td>26 (2–200)</td>
<td>0.9</td>
</tr>
<tr>
<td>Median (range) CD4+ cells/mm³ at the end of the follow-up</td>
<td>33 (2–162)</td>
<td>188 (15–616)</td>
<td>0.025</td>
</tr>
<tr>
<td>Patients with an increase of 100 CD4+ cells/mm³ during the follow-up †</td>
<td>0/7</td>
<td>6/11 (55)</td>
<td>0.038</td>
</tr>
<tr>
<td>Median (range) log HIV RNA copies/mm³ at beginning of HAART</td>
<td>5.2 (5–6)§</td>
<td>4.8 (3.8–5.7)¶</td>
<td>0.12</td>
</tr>
<tr>
<td>Median (range) log HIV RNA copies/mm³ at the end of the follow-up</td>
<td>5.4 (1.9–6)§</td>
<td>3.2 (1.7–4.6)**</td>
<td>0.047</td>
</tr>
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</table>

* VL = visceral leishmaniasis; IDU = intravenous drug users; CDC = Centers for Disease Control and Prevention; HAART = highly active retroviral therapy; HIV = human immunodeficiency virus.
† Number/total available cases.
‡ Number/total patients who attended all visits and in whom all CD4+ cell count measurements scheduled were available.
§ Five available cases.
¶ Seven available cases.
** Eight available cases.
†† Number/total patients who attended all visits and in whom all plasma HIV RNA load measurements scheduled were available.
baseline value had recurrences. This finding suggests that in HIV-infected patients with previous VL, secondary prophylaxis should be given until a safe degree of immunoreconstitution is reached. A CD4+ cell count of 350 cells/mm³ has been said to be high enough to discontinue safely secondary prophylaxis against VL. According to the results of this and other surveys, a CD4+ cell count greater than 200/mm³ may prove to be a safe level at which chemoprophylaxis can be ended. However, such recommendations must be based on further randomized studies with larger populations.

More than one relapse of VL was observed in a patient who received anti-Leishmania chemoprophylaxis after the first VL episode. This individual took HAART correctly, but he showed a poor increase in CD4+ cells counts. This fact underlines the importance of the immune response for the control of relapsing VL stated earlier, and conversely shows that secondary prophylaxis is not entirely effective against this disease.

Based on the results of this study, antiretroviral therapy compliance programs, and improvement of the efficacy of these drugs should be a priority to prevent VL relapses. In addition, a more adequate secondary chemoprophylaxis for the control of infection with this parasite should be investigated.

References


