Real-Time PCR Assay for Clinical Management of Human Immunodeficiency Virus-Infected Patients with Visceral Leishmaniasis

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To evaluate the usefulness of a real-time PCR for Leishmania DNA in the diagnosis and follow-up of patients with human immunodeficiency virus type 1 (HIV-1) and Leishmania coinfection, Leishmania DNA levels were measured in whole peripheral blood from 25 HIV-infected patients with clinical features suggestive of visceral leishmaniasis. Leishmania DNA was detected in 10 of 25 patients with microscopically confirmed visceral leishmaniasis and in none of those without this disease. Following treatment with liposomal amphotericin B, a clinical response was observed in 9 of 10 patients, in association with significantly decreased parasite loads. Seven patients relapsed clinically a median of 110 days after the end of treatment, in association with substantial increases in Leishmania DNA levels. Leishmania DNA levels correlated with the clinical course of visceral leishmaniasis, and their measurement at diagnosis and during and after treatment seems to be useful in the clinical management of HIV-infected patients with this disease.

Materials and Methods

Patients. Twenty-five HIV-infected patients with clinical features suggestive of visceral leishmaniasis underwent bone marrow aspiration. Visceral leishmaniasis was diagnosed in 10 of 25 patients (40%) by demonstration of Leishmania amastigotes in Giemsa-stained bone marrow smears. A first diagnosis was made for six patients, while four patients had a relapse. Leukopenia was observed in all of the patients (median, 2,200 cells/mm3; range, 1,300 to 3,200): fever, splenomegaly, and anemia (8.9 g/dL; range, 7.8 to 10.9) each were observed in nine patients; and thrombocytopenia was observed in eight patients (64,000 cells/mm3). CD4+ cell counts, HIV type 1 (HIV-1) RNA levels, and anti-Leishmania antibody titers at the time of diagnosis are shown in Table 1. No other opportunistic disorders were identified in these patients. Of the 15 remaining patients, 3 had non-Hodgkin lymphoma, 1 had atypical mycobacteriosis, and 11 had no opportunistic and/or neoplastic diseases identified. None of these 15 patients had anti-Leishmania antibodies. Their mean CD4+ count and HIV RNA load were 107 cells/ml (range, 48 to 367) and 5.02 log copies/ml (range, 3.14 to 6.11), respectively.

Treatment. Following diagnosis, all of the patients received liposomal amphotericin B (Ambisome; Gilead, San Diego, Calif.) on days 1 to 5 and once weekly thereafter, at a dosage of 3 mg/kg of body weight, except for one patient (patient 10 [Table 1]) who received 1.5 mg/kg because of renal failure. One patient (patient 4 [Table 1]) refused to continue the treatment after day 10. For the other patients, treatment duration was established by the physician caring for the patient, based on clinical response, and varied between 17 and 66 days. Following the end of therapy, five patients received secondary prophylaxis, consisting of...
RESULTS

Leishmania DNA load at the time of diagnosis of visceral leishmaniasis. Leishmania DNA was found in all of the 10 patients with visceral leishmaniasis, with a median of 1,610 parasites/ml (range, 110 to 41,000) (Table 1), and in none of the 15 patients without this disease. Both the diagnostic sensitivity and the specificity of real-time PCR were 100%. Parasite loads at the time of diagnosis were not significantly different for patients with a first diagnosis and those with relapse. No significant correlation was found between the parasite load and CD4+ cell count, HIV RNA viral load, titer of anti-Leishmania antibodies, or HAART administration at the time of diagnosis.

Leishmania DNA load during treatment. Figure 1A shows Leishmania DNA levels during treatment. Following liposomal amphotericin B administration on days 1 to 5, parasite levels decreased significantly from the baseline (median, 14 versus 1,680 parasites/ml; P = 0.004). During subsequent cycles of treatment, Leishmania loads decreased further in all the patients, except for patient 4, who stopped treatment at day 10. At the end of therapy, Leishmania DNA was undetectable (<0.63 parasites/ml) in one patient (patient 10), whereas the median DNA load in the other eight patients was 4 parasites/ml (Table 1). A clinical response was observed in these patients concomitantly with the decrease in parasite concentrations in blood.
FIG. 1. (A) *Leishmania* DNA levels in peripheral blood of patients with visceral leishmaniasis in the course of treatment. Blood samples were drawn prior to weekly drug administration. The horizontal line indicates the detection limit of the PCR assay. Dashed horizontal bars indicate median parasite levels. (B) *Leishmania* DNA levels in three patients (patient 6, patient 7, and patient 8 according to the numbering in Table 1) with visceral leishmaniasis receiving secondary prophylaxis. Blood samples were drawn prior to monthly drug administration. Open symbols indicate the time of clinical relapse.
**DISCUSSION**

*Leishmania* DNA load during posttreatment follow-up and clinical relapse. Following treatment, five patients received secondary prophylaxis (Table 1). The patient with undetectable *Leishmania* DNA in blood after therapy (patient 10) discontinued prophylaxis after 3 months, never relapsed, and had no detectable *Leishmania* DNA for 5 years of follow-up. One patient (patient 9) was clinically stable at the last follow-up after 2 months of prophylaxis, with decreased *Leishmania* DNA levels (median, 5 parasites/ml) but increased progressively during prophylaxis, reaching a median of 364 parasites/ml at the time of clinical relapse (Table 1; Fig. 1B). In these patients, an increase in *Leishmania* load above 10 parasites/ml preceded clinical relapse by 50 to 120 days (Fig. 1B).

Four patients did not receive secondary prophylaxis. All of them relapsed clinically a median of 78 days after the end of the treatment, with a median of 365 parasites/ml (Table 1). Blood samples from the time between the end of treatment and relapse were not available. Patient 4, who refused to continue treatment after day 10, died 250 days after the diagnosis of leishmaniasis.

Upon HAART, two of seven patients showed a virological response (Table 1). For one patient (patient 10), the CD4 count increased to >500 cells/µl, *Leishmania* DNA remained undetectable, and there was no relapse. The CD4 cell count for the other patient (patient 8) remained below 100 cells/µl, the *Leishmania* DNA load increased progressively, and the patient relapsed. No significant response to HAART was observed in the six remaining patients.

REFERENCES

ERRATUM

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Vol. 41, no. 11, p. 5080–5084, 2003. Page 5081, column 1, lines 19 to 21: “5'-AAGGTCAAAGAACAAGGCAAG-3' (LEIF-forward), 5'-GCATCGGAGTCGG-3' (LEIR-reverse), and 5'-AGGAGCGTGTCGCCGGGAGG-3' (LEIP-probe)” should read “5'-TAGACCGCACCAAGACGAACTA-3' (LEIF-forward), 5'-CTAACATCTCTCGACACACCTTT-3' (LEIR-reverse), and 5'-AGGGAAGGCATTCTCAAGGATACCTCC-3' (LEIP-probe).”