Clinical survey of *Leishmania*/HIV co-infection in Catania, Italy: the impact of highly active antiretroviral therapy (HAART)

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Received and accepted 30 May 2003

The clinical and parasitological features of visceral leishmaniasis (VL) were investigated, retrospectively, in 27 HIV-infected patients who attended the out-patient clinic of Catania University’s Department of Infectious Diseases between 1990 and 1998. The aim was to evaluate the epidemiological, clinical, therapeutic and prognostic characteristics of the co-infection, to determine if there were any interactions between the two infections, and to see if the use of highly active antiretroviral therapy (HAART) had any impact on the leishmaniasis. The most dramatic observation was a marked, HAART-attributable reduction in the annual incidence of VL relapses among the patients.

In humans, *Leishmania* parasites are the causative agents of a wide spectrum of diseases of variable severity, ranging from a localised cutaneous lesion, often benign and self-healing, to a visceral dissemination that may be fatal. Wherever parasites, vectors and vertebrate hosts (as reservoirs) combine to create the required ecological conditions, human leishmaniasis may become endemic. Over the last 20 years, visceral leishmaniasis (VL) has become increasingly common in the Mediterranean basin, largely as the result of the HIV pandemic spreading to areas where VL already occurred. In southern Europe, the annual number of cases of VL in HIV-positive individuals gradually increased through the 1990s, although recently the routine treatment of HIV-positives with highly active antiretroviral therapy (HAART) seems to have reversed this trend. It remains unclear whether HIV infection increases the probability of a newly acquired leishmanial infection becoming symptomatic or allows the re-activation of a previous asymptomatic, latent infection, or both.

In Europe, VL is generally caused by *Leishmania infantum* and differs according to the HIV status of the patient. There is greater heterogeneity in the *L. infantum* zymodemes isolated from HIV-positives (Nigro et al., 1996) and those zymodemes that always dermatotropic in the immuno-competent may visceralize in those infected with HIV (Jiménez et al., 1991; Nigro et al., 1996). Among HIV-positives, the clinical onset of the VL is often unusual (Condom et al., 1989; Matheron et al., 1992), and oligosymptomatic disease and atypical localisations are common (Casado et al., 1998). The serodiagnosis of VL is often complicated by HIV infection (López-Vélez et al., 1998) and relapses of VL, after a first episode has been clinically cured by treatment with antileishmanials, tend to be much more common if the patient is HIV-positive (Medrano et al., 1992; Rosenthal et al., 1995).

The aim of the present study was to review the clinical course of *Leishmania*/HIV
co-infection in patients attending an outpatient clinic in Catania, Italy. As some of the patients presented after HAART — based on protease inhibitors (PI) — was introduced for the routine treatment of HIV infection, and some before, the impact of the antiretroviral therapy on VL could be explored.

RESULTS

VL was detected in 27 of the 620 HIV-infected patients investigated — 22 males and five females, with a mean (s.d.) age of 33.5 (3.7) years. According to the standard criteria of the Centers for Disease Control and Prevention in Atlanta (CDC; Anon. 1992), 19 of the 27 co-infected cases had ‘full-blown’ AIDS when their leishmaniasis was diagnosed. The co-infected cases were HIV-positive probably because they were intravenous-drug users (16 cases), because of their homosexual or heterosexual behaviour (seven cases), because of the products they had received in the treatment of haemophilia (one case), or because of unknown risk factors (three cases).

Of the eight cases who did not have AIDS when their VL was diagnosed, four were found to be HIV-positive when they were suffering from their first episode of VL. Another eight of the cases first met the CDC criteria for the clinical diagnosis of AIDS at the time their VL was diagnosed. Twelve of the co-infected cases who had AIDS when their VL was diagnosed were found to have other opportunistic diseases at the same time: oesophageal candidiasis (five cases), urinary tuberculosis (two), pulmonary tuberculosis (two), Pneumocystis carinii pneumonia (two), cryptosporidiosis (one) and cerebral neoplasm (one). At the time their leishmaniasis was diagnosed, all 27 cases appeared to be very immunodeficient, 26 having <200 CD4+ cells/μl; the CD4+ counts for those with AIDS were not significantly different from the other cases, with medians of 62 and 73 cells/μl, respectively.

Twenty-five (93%) of the co-infected cases with leishmaniasis had at least one of the symptoms considered typical of VL in the immunocompetent: splenomegaly (19 cases), hepatomegaly (17), fever (15), and/or a constitutional syndrome comprised of fatigue, asthenia and weight loss (three). There were, however, some atypical symptoms; five cases had diarrhoea, two had dry coughs, and

PATIENTS AND METHODS

The subjects of the present, retrospective study were the HIV-infected patients who attended the outpatient clinic of Catania University’s Department of Infectious Diseases between January 1990 and August 1998. All of these patients had been checked, routinely, for Leishmania co-infection. Their sera had been investigated, in a commercial IFAT (bioMérieux Laboratories, Marcy l’Etoile, France), for antileishmanial antibodies, and smears of buffy coats produced from peripheral blood and of biopsies (of bone-marrow and sometimes other tissues) had been Giemsa-stained and checked for amastigotes, under the microscope. Their HIV status had been determined serologically, in a commercial ELISA designed to detect antibodies to HIV-1 or HIV-2 (Abbot Laboratories, Abbott Park, IL), and confirmed using another commercial assay that was based on western blotting (DuPont, Geneva). The patients’ CD4+ cells had been counted, their concomitant opportunistic diseases had been identified, the clinical presentation and evolution of any VL episode had been followed and recorded, and the diagnosis methods used and their results had been noted. The clinical efficacy of treatment had been evaluated by determining the clinical and, where possible, parasitological responses. Each patient’s survival, in relation to the treatment of the HIV and, if present, the VL, had also been recorded.

The Kaplan–Meier method was used to compare survivals, from the date of the diagnosis of AIDS, in patients who did and did not have VL.
two had signs of cutaneous leishmaniasis as well as VL.

Each first episode of VL was suspected as the result of the IFAT test and confirmed, parasitologically, when amastigotes were found in smears (of bone-marrow in 26 of the 27 cases, and of a lymph-node biopsy in the other one). Amastigotes were also seen in some of the smears of buffy-coats, including 34% of the those prepared during the subsequent VL relapses.

Most of the VL cases were given parenteral antileishmanial therapy, based on pentavalent antimonial compounds or, more rarely, on free amphotericin B deoxycholate (two cases) or its lipidic formulations (one case was given Ambisome® and another Abelcet®). Such treatment generally led to full clinical cure of the VL but 12 of the cases (including 10 of those who had AIDS when their VL was first diagnosed) are known to have had VL relapses. Although VL relapses were more likely to have been recorded for the AIDS cases than for the other HIV-positives (53% v. 25%), the mean number of relapses recorded for each relapsing AIDS case was lower than that for each of the other relapsing cases. Part of this difference could be attributed to the difference in the interval between relapses, which was generally longer for the AIDS cases than for the other cases (with means of 7 and 5 months, respectively). For the treatment of the relapses, amphotericin B deoxycholate and its lipidic formulations (used in 28% and 37% of cases, respectively) were preferred to the antimonials (17%).

The date of death was known for 13 of the co-infected cases: they died a mean of 16 months from the first diagnosis of their VL. The survival of the AIDS cases presenting in Catania did not appear to be adversely affected by symptomatic leishmanial infection (Fig. 1).

The treatment of HIV in Catania changed and became more effective in 1996, when

![FIG. 1. Survival in AIDS patients with (—) or without (→) visceral leishmaniasis, in Catania, Italy.](image-url)
HAART was introduced and protease inhibitors (PI) were added to the treatment regimens of most of the HIV-positives. In the period of presentation considered in the present study, from 1990 to 1998, the annual number of new cases of VL in HIV-infected patients remained stable. However, the annual incidence of VL relapses, per 100 co-infected cases, fell from a maximum of 140 relapses in 1992 to just 10 relapses in 1998 (Fig. 2). All the data from Catania were re-analysed, splitting the HIV-positive patients into those who presented in or before June 1996 (taken as the date when PI treatment of local HIV-positives became common) and those who presented after June 1996. Although the incidence of new cases of VL was then found to be similar for the ‘pre-PI’ and ‘post-PI’ patients, the percentage of post-PI patients who had post-treatment relapses of VL during follow-up was markedly lower than the percentage of the pre-PI patients, whether the patients had AIDS (36% v. 64%) or not (0% v. 50%). Only two of the 12 relapsing cases of VL identified in the present study had responded to PI treatment; the others had either not been given PI (seven cases) or failed to tolerate their treatment with PI (three cases). Among the co-infected cases followed after June 1996, recorded VL relapses were notably rarer in those who responded to PI treatment (no relapsing cases amongst eight patients) than in those who failed (one relapsing case amongst three cases) or were not given PI treatment (three relapsing cases amongst four patients).

DISCUSSION

The clinical presentation and evolution of VL is the result of the complex interplay between the parasite and its host’s immune

FIG. 2. The annual incidence of relapses of visceral leishmaniasis among cases of *Leishmania infantum*/HIV co-infection in Catania, Italy.
system. As the outcome of human infection with *Leishmania* is largely determined by how effectively the host’s Th1 response can destroy the intracellular parasites, it is not surprising that those made immunodeficient by HIV appear particularly susceptible to symptomatic and relapsing leishmaniasis. As the HIV pandemic has spread around the globe, the incidence of *Leishmania/HIV* co-infection has gradually increased, especially in the Mediterranean basin. In southern Europe, 3%–7% of HIV-infected patients develop VL (Tremblay *et al.*, 1996).

As seen in the present study, VL in the HIV-positive may often produce symptoms similar to those seen in the HIV-negative, but the immunodeficiency caused by the viral infection may also lead to atypical manifestations. The dry cough, diarrhoea and concomitant cutaneous lesions seen in some of the present HIV-positive cases are not typical of the parasite involved (i.e. *L. infantum*). Unusual symptoms reflect the failure of the host’s immune system to prevent the spread of amastigotes into sites (e.g. the respiratory tract, gastro-intestinal tube and peripheral blood) where they are rarely if ever observed in the immunocompetent (Russell *et al.*, 1992).

In the present study, curiously, the clinical presentation, clinical and parasitological outcomes and response to therapy of the VL in the AIDS patients were roughly similar to those of the VL in the other, less severely ill, HIV-positives. The two groups were however, similar in immunostatus, at least in terms of CD4+ counts.

In general, the most reliable way to diagnose VL is by the direct detection of leishmanial amastigotes in bone-marrow aspirates. The culture of such aspirates may be advisable when the suspected case is infected with HIV, however, as the hypoplasia frequently seen in the bone-marrow of HIV-positive patients reduces the sensitivity of the microscopical examination of bone-marrow smears (Berenguer *et al.*, 1989). The serodiagnosis of VL in the HIV-positive may be unreliable because of unusually low titres of anti-*Leishmania* antibodies or because polyclonal lymphocyte-B stimulation may lead to false-positive results (Montalbán *et al.*, 1990; WHO, 1997).

The treatment of VL in the HIV-positive patients investigated in the present study did not vary over the study period. The usual therapy, especially for a first episode of VL, was based on a pentavalent antimonial drug, whether the patient had AIDS at the time of the treatment or not.

In some regions of Italy, as elsewhere in southern Europe, VL is a relatively common opportunistic disease of the HIV-positive wherever it is endemic. VL in those co-infected with HIV is almost always complicated by a great number of post-treatment relapses. Some of these relapses probably result from re-infection and some from the recrudescence of parasites that have not been killed by previous treatment. The severe immunodeficiency induced by HIV infection may prevent the host from mounting a cell-mediated immune response of the type that might, in an immunocompetent host, clear all parasites (perhaps aided by antileishmanial treatment) or at least prevent a leishmanial infection from becoming symptomatic.

The frequent VL relapses seen in the HIV-positive each require a cycle of antileishmanial treatment. If the same drug is used on each occasion (and the relapses result from recrudescences) then clinical efficacy of each treatment may gradually fall as the parasites develop resistance to the drug. It is then necessary to resort to alternative treatment regimes. The use of secondary prophylaxis, to prevent or at least slow the development of VL relapses, has been considered (Ribera *et al.*, 1996). The choice of drugs for such prophylaxis is, however, limited. Most antileishmanial compounds cause adverse effects that many clinicians would consider more dangerous, if the drugs were used for regular maintenance therapy, than the risks associated with the relapses and their treatment. Most of the antileishmanial drugs available for parenteral
or oral administration have been tested as secondary prophylactics but long-term benefits, in terms of relapse prevention or the survival of the patients, have not always been observed (Russo et al., 1996; López-Vélez, 2003).

It is unclear why, among the present cases of Leishmania/HIV co-infection, those with AIDS should appear more than twice as likely to have at least one VL relapse than the other cases, yet have relatively long inter-relapse intervals. Patient survival was seen to be significantly related not to the clinical or parasitological course of the VL but to the clinical development of the HIV infection. Deaths occurred more frequently and rapidly in the AIDS patients with VL than in the other co-infected patients, and the survival of an AIDS case appeared largely unaffected by leishmanial infection (Fig. 1).

Although the present results indicate that widespread use of PI in the treatment of HIV-positives in Catania failed to prevent a significant number of first episodes of VL, most new cases of VL in the HIV-positive residents of the city now occur in patients who have either not been given PI or been given PI but failed to respond (in terms of CD4+ counts; unpubl. obs.). PI-based HAART does seem to have a significant impact on the occurrence of VL relapses and/or the inter-relapse interval. It is unclear if this impact reflects the development of more effective immune responses in the PI-treated patient or the direct effect of PI on the leishmanial parasites. These and many other factors that might affect the morbidity and mortality associated with Leishmania/ HIV co-infection require long-term, multi-centre investigations with many more patients.

REFERENCES


