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Parasitic Culture of Buffy Coat for Diagnosis of Visceral Leishmaniasis in Human Immunodeficiency Virus-Infected Patients

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Two samples of buffy coat from the peripheral blood of 25 human immunodeficiency virus-positive patients with proven visceral leishmaniasis, as determined with a bone marrow aspirate (stain and culture), were cultured onto Schneider's and Novy-McNeal-Nicolle media. Hemoculture positivity was 67%. The average growing time was 10 days. This is an easy, noninvasive, and sensitive technique.

Leishmaniasis is endemic in more than 80 countries. About 600,000 new cases are diagnosed every year, and it is calculated that about 12 million people could be infected (6). At present, the areas where leishmaniasis and human immunodeficiency virus are endemic and (HIV) overlap each other are in South America, Africa, and the Mediterranean Basin. Increases in the number of cases of visceral leishmaniasis (VL) and HIV coinfection have been observed during the last few years (5, 7, 18, 19). In those patients, VL is an opportunistic disease, with the outcome being recurrent infection, and patients with VL are subjected to aggressive diagnostic techniques, which results in much suffering and great expense. The occurrence of HIV-*Leishmania* coinfection in southern Europe has been estimated to be 1 to 3% (23).

Amastigotes of *Leishmania* spp. can be observed in the peripheral blood of HIV-positive patients (14, 15). Moreover, it has been proved that viable parasites circulate in peripheral blood, as demonstrated by xenodiagnosis, and were recovered from 100% of the 10 tested patients (13, 19).

The objective of the study described here was to evaluate the parasitic culture of the buffy coat from peripheral blood as a nonaggressive tool for diagnosing VL in patients infected with HIV.

MATERIALS AND METHODS

From January 1993 to June 1994, leishmanial cultures of the buffy coat from the peripheral blood of 25 HIV-positive patients with VL were performed. Testing for HIV antibodies was done by an enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories, North Chicago, Ill.) and was confirmed by Western blotting (immunoblotting; Diagnostic Biotechnology Ltd., Singapore).

A buffy coat from 10 ml of anticoagulated (with heparin) peripheral blood was obtained and a bone marrow biopsy specimen from the iliac crest was obtained from every patient with clinical features of fever and liver and spleen enlargement or pancytopenia, or both. Biopsy specimens were submitted for histopathologic study and for bacterial, fungal, and mycobacterial cultures.

Leishmanial cultures were done by inoculating the bone marrow tissue and the buffy coat layer onto Schneider's Drosophila Medium supplemented with 30% fetal calf serum (FCS; Gibco Ltd., Paisley, Scotland) or Novy-McNeal-Nicolle (NNN) medium (22). All cultures were incubated at 25°C and were considered negative after 4 weeks if no flagellates were observed. Isolated parasites were subcultured in NNN medium and were sequentially passed into RPMI–12% FCS. Parasites were identified as *Leishmania infantum* by DNA probing (21). Antileishmanial antibodies were measured by indirect immunofluorescence antibody test (IFAT) (BIOS GmbH, Munich, Germany). Titers greater than or equal to 1:80 were considered positive.

In 10 patients a concomitant indirect xenodianosis was performed by using sand flies, as reported previously (16, 17).

RESULTS

Twenty-five patients were studied (Table 1). In one of the patients (patient 13) the bone marrow aspirate was not available for direct examination or culture. By risk factor, 21 of the 25 patients (84%) were intravenous drug users (IVDU), 3 patients (12%) were male homosexuals, and 1 patient was a hemophiliac. The CD4 lymphocytes count was less than 150/mm^3 in all patients. Almost all patients (24 of 25) were male. Of the 25 patients, 17 (67%) had a positive hemoculture. In one patient (patient 18) with a negative hemoculture the xenodiagnosis was positive, which demonstrated the existence of viable circulating parasites in the blood. The average culturing time when the patient was hemoculture positive was 10 days. Serology was positive in 8 of 24 patients (33%).

DISCUSSION

An increase in the number of cases of VL has been observed in Spain during the last few years. More than 50% of these patients are coinfected with HIV (1, 2). A similar observation has been made in the countries of southern Europe (9), and this increase represents a severe health problem in that region. In our experience, the prevalence of HIV-VL coinfection is about 3%, and VL is the fourth most common opportunistic parasitic disease after pneumocystosis, toxoplasmosis, and cryptosporidiosis.

In patients coinfected with HIV-*Leishmania* spp., atypical clinical pictures have been seen because of the spread of the parasite, like effects on the gastrointestinal tract (13) or skin lessions which ultimately produce VL (11). All of this is the result of the failure of cellular immunity, which controls the number of parasites (10, 12). All of our patients had high parasite counts in their bone marrow and showed CD4 lymphocyte counts of less than 150/mm^3 (average, 45/mm^3), which favors the hematogenous spread of the parasite. The efficiency

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TABLE 1. Sex, risk factor, and CD4 counts of the patients and hemoculture, bone marrow, and serology results[a]

Patient no.	Sex	Risk factor	CD_4 count (no./mm^3)	Hemoculture result	Bone marrow result	Serology result (titer)
1	M	IVDU	60	+	+	−
2	M	IVDU	33	+	+	+ (1/320)
3	M	Homosexual	14	+	+	−
4	M	IVDU	100	−	+	+ (1/80)
5	M	IVDU	10	−	+	−
6	M	Homosexual	150	−	+	+ (1/640)
7	M	IVDU	80	+	+	+ (1/160)
8	M	IVDU	90	−	+	−
9	M	IVDU	20	−	+	−
10	M	IVDU	30	−	+	+ (1/128)
11	F	IVDU	37	+	+	−
12	M	IVDU	144	+	+	−
13	M	IVDU	85	+	NA	+ (1/1,024)
14	M	Hemophiliac	100	+	+	−
15	M	IVDU	3	+	+	−
16	M	IVDU	12	+	+	−
17	M	IVDU	10	+	+	+ (1/256)
18	M	Homosexual	27	−	+	−
19	M	IVDU	12	+	+	−
20	M	IVDU	30	+	+	−
21	M	IVDU	20	+	+	−
22	M	IVDU	33	+	+	+ (1/620)
23	M	IVDU	66	+	+	−
24	M	IVDU	50	+	+	−
25	M	IVDU	14	+	+	−

[a] Xenodiagnosis was performed for patients 11 to 16, 18, and 21. The result was positive for all patients except patient 13. Abbreviations: NA, not available; IVDU, intravenous drug user; M, male; F, female; +, positive; −, negative.

of hemoculture would probably be less for patients with higher CD4 counts. The outcome of VL in HIV-positive patients is chronic infection. The patients suffer multiple relapses, which requires the use of repeated aggressive diagnostic techniques, like bone marrow sampling (5, 18, 19), which leads to unnecessary suffering and extra cost.

Unlike what happens with immunocompetent patients, serology (IFAT and indirect hemagglutination) in HIV-positive patients is of little value considering its low sensitivity (0 to 35%). In our experience only 33% of the blood samples had positive titers when the IFAT technique was used. Serology results can be improved when dot blot ELISA or Western blot techniques are performed (8).

The parasitologic diagnosis of leishmaniasis is carried out by identification of the *Leishmania* organism after staining or culture, or both. The most useful samples (sensitivities are in parentheses) are those obtained from spleen aspirations (>94%), liver biopsies (76 to 90%), and bone marrow biopsies (76 to 90%) (24). However, retrieval of liver and spleen biopsy specimens sometimes results in severe complications. The detection of amastigotes can be improved by the use of monoclonal antibodies (4) or PCR (20).

Several investigators have demonstrated that *Leishmania* organisms circulate in the peripheral blood; in 53% of the patients it has been detected by its appearance in the buffy coat (14), and up to 100% of the organisms are recovered by xenodiagnostic feeding laboratory *Phlebotomus* (sand fly) colonies the patient's blood (16, 17). The fact that viable parasites can be isolated in peripheral blood at such a high percentage makes us believe that this illness can be transmitted hematogenously among IVDU while they are sharing needles and that HIV-positive patients can be human reservoirs of the organism that causes this disease (3). These two facts could modify the epidemiology of VL in southern Europe.

In our experience the usefulness of hemoculture is 67%. We recommend its use because of its high degree sensitivity, easy means of operation, and low level of invasiveness to the patient.

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