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First Case of Visceral Leishmaniasis Caused by *Leishmania martiniquensis*

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Abstract. We report the first case of visceral leishmaniasis (VL) caused by *Leishmania martiniquensis* in the Caribbean, which until now, was known only to cause cutaneous leishmaniasis. The disease presented with fatigue, anemia, and hepatosplenomegaly in a 61-year-old man with human immunodeficiency virus (HIV) infection who was receiving antiretroviral therapy. Diagnosis was made by bone marrow biopsy. VL is life-threatening, and its emergence in the Caribbean is of concern.

INTRODUCTION

Intracellular protozoan of the *Leishmania* genus, mainly transmitted by sandflies, are the causative agents of leishmaniasis. More than 12 million people are currently infected worldwide.[1] Among its different clinical presentations, visceral leishmaniasis (VL) is life-threatening. Human immunodeficiency virus (HIV) infection is one of the major risk factor for developing VL and reported in 2–12% of all cases.[1,2]

The new autochthonous and divergent *L. (L.) martiniquensis* n. sp. was first isolated in 1995; its taxonomical position was established in 2002, and it was named in 2014.[3–5] This species is up to now restricted to Martinique and has been only reported in patients with cutaneous lesions. We report the first case of VL caused by this parasite in an immunocompromised HIV-infected patient.

CASE REPORT

A 61-year-old heterosexual Caribbean male was diagnosed with acquired immunodeficiency syndrome (AIDS) prurigo in 2006.[6] He worked as a painter and a coconut picker and seller. He was born and had always lived in Martinique, except from 1994 to 2001, when he had lived in Guadeloupe. He had traveled to northern Europe and Haiti. His sole medical history was hypertension. He had never used intravenous drugs. At the time of HIV diagnosis, no opportunistic infection was found. Immunological, virological, and therapeutic data are summarized in Table 1.

Combination antiretroviral therapy (cART) was introduced in May of 2007, with significant reduction of HIV viral load and increased CD4+ cell count 1 month later. However, at 1 year, although there was clinical improvement of prurigo and continued viral suppression, CD4 counts were decreasing. Genotypic testing for HIV-1 drug resistance on an initial sample had shown no transmitted resistance, and drug monitoring revealed normal absorption. Despite cART regimen switching in January of 2010, the CD4 counts continued to decline, and the patient remained virally suppressed (Table 1).

He progressively developed hepatosplenomegaly and a normochromic normocytic aregenerative anemia of < 10 g/dL. The patient had no fever but reported permanent fatigue. Sulfamethoxazole/trimethropin (SMX-TMP) toxicity was hypothesized because of a reduced serum folate level, but folinic acid supplementation failed to correct it. A bone marrow biopsy performed in November of 2011 revealed intrahistiocytic parasites consistent with the amastigote forms of *Leishmania* spp. (Figure 1).

The diagnosis was confirmed by *Leishmania* polymerase chain reaction (PCR) realized on whole blood with RV1/RV2 probes targeting a kinetoplastic DNA locus (145 bp).[7] The molecular identification based on the ribosomal 18S RNA locus analysis gave a 100% identity with the sequence of the MHOM/MQ/92/MAR1 strain (GenBank accession number AF303938.1), a divergent *Leishmania* strain described for the first time in Martinique in 1995 and recently named *L. martiniquensis*.[4,5] A retrospective analysis of several sera from our patient stored since 2007 detected high levels of antileishmanial antibodies by indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) using *L. infantum* antigen (Table 1). Immunoblot revealed two specific bands of 14 and 16 kDa, confirming the specificity of these antibodies.

The patient was treated with liposomal amphotericin B (4 mg/kg equal to 300 mg/day from day 1 to 5 and on days 10, 17, 24, 31, and 38 for a cumulative dose of 3 g) and also required a blood transfusion. Symptoms rapidly improved, with decreasing hepatosplenomegaly and disappearance of fatigue, and the patient remained asymptomatic more than 1 year later. Significant increases of hemoglobin level and CD4+ cell count above 350/mm^3 followed as well as a negative blood *Leishmania* PCR 20 months later. The patient did not receive secondary prophylaxis.

DISCUSSION

Leishmaniasis is endemic in Central and South America but rarely occurs in the Caribbean.[8]

The first cases of presumed autochthonous cutaneous leishmaniasis (CL) in Martinique were diagnosed based on direct

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TABLE 1
Summary of biological and clinical data and history of treatment of a patient coinfected by HIV and autochthonous *Leishmania* in Martinique

	January of 2007	May of 2007	January of 2008	August of 2008	February of 2009	June of 2009	January of 2010	August of 2010	January of 2011	November of 2011	February of 2012	June of 2012	January of 2013	July of 2013
HIV-1 viral load (copies/mL)	1.6×10^6	485	49	40	< 40	< 40	< 20	< 20	< 20	27	< 20	< 20	< 20	< 20
CD4 count (/μL)	186	663	290	218	187	211	244	151	109	133	287	381	412	362
Lymphocytes (/μL)	1,280	2,210	1,285	1,830	1,367	949	833	939	1,624	2,409	2,613	2,061		
Total leukocytes (/μL)	4,580	5,530	4,200	3,650	5,350	4,130	3,110	2,730	5,230	3,610	5,920	7,250		
Hemoglobin (g/dL)	13	11	11	11	10	11	9	9	9	6	9	10	12	11
Platelet count ($\times 10^9$/L)	166	214	206	220	250	216	201	152	241	111	273	300		
Clinical manifestations										HSM and WL				
Others investigations										BM+, bPCR+	bPCR–	bPCR–		bPCR–
Serology IFA (retrospective)	1/640	1/640	1/320	1/320	1/320									
Serology ELISA (retrospective)	+	+				+		+		+				
Red blood cells transfusion														
Antibiotic	SMX-TMP										SMX-TMP			
cART	cART 1: ABC/3TC/LPV/r									cART 2: ABC/3TC/EFV				
Amphotericin B										Yes				

ABC = abacavir; BM = bone marrow biopsy and aspiration; bPCR = blood *Leishmania* PCR; EFV = efavirenz; HSM = hepatosplenomegaly; LPV/r = lopinavir plus ritonavir; WL = weight lost; 3TC = lamivudine.

FIGURE 1. Intrahistiocytic amastigote forms of *Leishmania* on a bone marrow specimen from the patient (May Grunwald Giemsa ×1,000).

examination of skin smears and consisted of localized CL in immunocompetent patients, except for one case of diffuse CL in an HIV-infected patient.[3,4] More recently, seven additional CL cases (six of them were unpublished) were found to be caused by a new *Leishmania* species. None of these cases presented visceral dissemination. This parasite was identified by both molecular and isoenzymatic techniques and found to be a member of the *Leishmania* subgenus at the base of the phylogenetic tree.[4] The new *Leishmania* taxon was recently described and named *L. (L.) martiniquensis* n. sp.[5] Capacity of visceralization and dissemination has been shown in a murine model.[9] This is the first reported case of VL caused by *L. martiniquensis*, which highlights the possibility of dissemination in immunocompromized patients of this new species as has been reported for other dermotropic leishmania.

VL is uncommon in the Caribbean. In Guadeloupe, three cases of possibly autochthonous VL have been observed. Two cases were presumed to be autochthonous based on epidemiological data but without species identification.[10,11] In 2008, a third case of VL was diagnosed in Guadeloupe in an immunocompetent patient, but it was caused by *L. infantum* (unpublished data). No other autochthonous VL cases have been reported elsewhere in the Caribbean.[8] Our patient most probably acquired leishmaniasis in Martinique, because *L. martiniquensis* has only been reported on this island, but late reactivation of an infection acquired in Guadeloupe or Haiti cannot be ruled out.

Although patients coinfected with VL and HIV commonly are very symptomatic,[12] our patient was clinically well for a long time, but his immunological pattern mimicked that of failure of cART.[13] Cases of VL initially presenting with isolated CD4+ cell count drops are uncommon.

VL/HIV coinfection is usually characterized by significantly lower cure rates of leishmaniasis and higher drug toxicity, relapse, and mortality rates than infection with VL in HIV-seronegative individuals.[2,14] Although post-treatment increase in CD4+ cells is considered greatly predictive of relapse-free evolution,[15] relapses have been reported in well-controlled HIV patients on cART, thus mandating prolonged follow-up.[1,2] The patient has continued to do well with no relapse 1 year post-VL treatment.[2]

Research tracks have been paved to identify the parasitic cycle, sandfly vectors, and host reservoirs of *Leishmania*

species in the Caribbean. *Lutzomyia atroclavata* has been identified in Martinique, Guadeloupe, and the Virgin Islands; black rats (*Rattus rattus*), mongooses (*Herpestes auropunctatus*), marsupials (*Didelphis marsupialis)*, and canids are all potential animal reservoirs that should be investigated.[8,16]

The emergence of *L. martiniquensis* infection with the possibility of visceral extension could be of concern in the Caribbean region, where the prevalence of HIV infection is high.

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