Rapid Tests and the Diagnosis of Visceral Leishmaniasis and Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Coinfection

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Abstract. After the emergence of the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), the number of visceral leishmaniasis (VL)–HIV/AIDS coinfections has increased worldwide. Herein, we assessed the usefulness of an rK39-based immunochromatographic test (rK39 ICT) (DiaMed-IT LEISH®; DiaMed AG, Cressier-sur-Morat, Switzerland) and a latex agglutination test (KAtex; Kalon Biological, Guildford, United Kingdom) for urinary antigen detection to diagnose VL in 15 HIV/AIDS patients from northeastern Brazil. VL diagnosis was based on clinical findings, cytology, serology, parasite DNA, and/or urinary antigen detection. VL was confirmed in seven out of 15 HIV/AIDS patients. Only three patients were positive in bone marrow cytology, three patients were conventional polymerase chain reaction (PCR) positive, while six were real-time PCR positive. All patients were direct agglutination test (DAT) (Royal Tropical Institute, Amsterdam, The Netherlands) positive; of these, four were positive by rK39 ICT and five by KAtex. Large-scale studies are needed to validate the use of the KAtex in the national public health laboratory network in Brazil, aiming at improving the diagnosis of VL in HIV/AIDS patients in this country.

Visceral leishmaniasis (VL) caused by *Leishmania infantum* is a life-threatening disease that claims the lives of thousands of people annually. After the emergence of the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) during the 1980s, the number of VL–HIV/AIDS coinfections has increased in Europe, Africa, Asia, and Latin America.[1]

Clinical diagnosis of VL–HIV/AIDS coinfection is a difficult task, considering the nonspecific clinical features displayed by some patients, potentially delaying the treatment initiation.[2] Cytological examination of spleen, bone marrow, and/or lymph node stained–smears is considered the gold standard for VL–HIV/AIDS diagnosis.[3] Nonetheless, cytology may require invasive samples (e.g., spleen), which may increase the risk of adverse events, such as fatal hemorrhage. Moreover, cytology may be time consuming and difficult to apply in primary health-care facilities.[4] Polymerase chain reaction (PCR)–based diagnostic tools have been successfully applied for the diagnosis of VL, with high sensitivity, specificity, and reproducibility, though they require an equipped laboratory and may be expensive.[5] Direct agglutination test (DAT) (Royal Tropical Institute, Amsterdam, The Netherlands) is a semiquantitative serological test, and titers ≥ 1:3,200 are confirmatory of VL.[6] Rapid tests, such as immunochromatographic tests (ICT), are point-of-care diagnostic tools that require noninvasive samples and may considerably reduce the time between samples collection and treatment outset.[7]

Despite the recent advances, the diagnosis of VL-HIV/AIDS coinfection is still impaired due to the clinical and laboratory features of HIV patients. Thus, the aim of this study was to assess the usefulness of two rapid tests for diagnosing VL in 15 HIV/AIDS patients from northeastern Brazil.

Patients with suspected VL-HIV/AIDS coinfection referred to the Hospital das Clínicas of the Universidade Federal de Pernambuco, Brazil, from 2008 to 2013, were enrolled. Patients included in this study were males and females, aged > 18 years, with clinical and laboratorial data available in their clinical record. Blood, serum, and urine samples were obtained and stored at −4°C until use. Laboratorial tests used herein were bone marrow cytology, conventional PCR[8] and real-time PCR[9] with primers targeting conserved regions of the kinetoplast minicircle DNA of *L. infantum*, DAT, rK39 ICT (DiaMed-IT LEISH®; DiaMed AG, Cressier-sur-Morat, Switzerland), and KAtex (Kalon Biological, Guildford, United Kingdom). The Ministry of Health of Brazil considers the presence of suggestive clinical signs and a positive result to one or more diagnostic tests for VL as the diagnostic criteria for case confirmation of VL–HIV/AIDS coinfection.[10] The concordance between the rapid tests was evaluated using kappa statistics (κ). The Research Ethics Committee of the Centro de Pesquisas Aggeu Magalhães (CAAE no. 0121.0.095.000-08) approved the study.

Of the patients, 11 were males (Table 1). The mean age was 36.8 years (range: 20–53 years). Four patients had attended school for ≥ 11 years. Anemia, lymphopenia, leucopenia, and neutropenia were the most frequent laboratory alterations. Of the patients, 10 had splenomegaly, eight had hepatomegaly, and eight presented weight loss, while 10 presented asthenia and four had pale mucous membranes. All patients reported fever episodes (4–120 days) and were on highly active antiretroviral therapy. Patients presented variable HIV loads, from undetectable (< 50 copies/mL) to high (> 500,000 copies/mL) viral loads, and the mean CD4+ T-lymphocytes count was 146.85 cells/mm³ (range: 8–458 cells/mm³). All the aforementioned data were available in the patients' clinical record (Table 1).

Seven patients were coinfected, as confirmed by one or more diagnostic methods used herein. *Leishmania* amastigotes were detected in bone marrow of three patients. Three patients were conventional PCR positive, whereas six were real-time PCR positive. Six patients were positive in one or more rapid

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TABLE 1
Epidemiological, clinical, and laboratory findings of 15 cases of suspected VL-HIV/AIDS coinfection, 2008–2013, Pernambuco, Brazil

Findings	Frequency *a/n* (%)	DT+/*a* (%)
Gender		
Male	11/15 (73.3)	5/11 (45.4)
Female	4/15 (26.7)	2/4 (50)
Mean age (minimum–maximum) years	36.8 (20–53)	38.7 (20–53)
Attended school for 11 or more years	4/15 (26.7)	2/4 (50)
Pernambuco State, Brazil		
Metropolitan Region of Recife	12/15 (80)	4/12 (33.3)
Atlantic Rainforest Region	2/15 (13.3)	2/2 (100)
Middle Scrub Zone	1/15 (6.7)	1/1 (100)
Clinical features		
Cytopenia	14/15 (93.3)	6/14 (42.9)
Anemia	10/15 (66.7)	5/10 (50)
Lymphopenia	10/15 (66.7)	5/10 (50)
Leucopenia	9/15 (60)	4/9 (44.4)
Neutropenia	9/15 (60)	4/9 (44.4)
Irregular fever	15/15 (100)	7/15 (46.7)
Hepatomegaly	8/15 (53.3)	5/8 (62.5)
Splenomegaly	10/15 (66.7)	5/10 (50)
Weight loss > 5 kg	8/15 (53.3)	4/8 (50)
Asthenia	10/15 (66.7)	7/10 (70)
Pale mucous membranes	4/15 (26.7)	2/4 (50)
On HAART	15/15 (100)	7/15 (46.7)
Plasma viral load < 50 copies/mL	5/15 (33.3)	3/5 (60)
50–500,000 copies/mL	5/15 (33.3)	3/5 (60)
> 500,000 copies/mL	2/15 (13.3)	1/2 (50)
Immunological status		
CD4+ T-lymphocytes count		
≥ 200 cells/mm^3	4/15 (26.7)	2/4 (50)
< 200 cells/mm^3	10/15 (66.7)	5/10 (50)

a = number of patients with positive results; DT+ = positive diagnostic tests; HAART = highly active antiretroviral therapy; *n* = number of patients evaluated; VL–HIV/AIDS = visceral leishmaniasis–human immunodeficiency virus/acquired immunodeficiency syndrome.

tests (Table 2). In particular, all patients were DAT positive. Four patients were rK39 ICT positive, whereas five were KAtex positive. A good agreement was found between the results obtained with KAtex and rK39 ICT ($\kappa = 0.526$) or DAT ($\kappa = 0.727$), and also between rK39 ICT and DAT ($\kappa = 0.587$).

This study demonstrates that six out of seven VL–HIV/AIDS coinfected patients were positive by one or more rapid tests. In their meta-analysis, Cota and others[11] concluded that serological tests should not be used to rule out a diagnosis of VL among the HIV-infected patients. They also emphasized that a positive rapid test has diagnostic value only when combined with the clinical data. All coinfected patients displayed clinical signs suggestive of VL, even if two of them did not present splenomegaly (Table 2). Fever and splenomegaly are important for clinical case definition of

VL, and their absence may complicate the diagnosis in HIV/AIDS patients.[12] Physicians dealing with HIV/AIDS patients in areas where VL is endemic should pay attention to any atypical clinical manifestation during the diagnostic workup and request diagnostic tests for VL whenever necessary. Even though the small size of sample weaken the power of the study, our results will pave the way for further studies on the diagnosis and monitoring of patients with VL–HIV/AIDS coinfection.

Bone marrow cytology was positive in three VL cases (42.9%). The sensitivity of bone marrow cytology may range from 60% to 85%.[5] In particular, Cota and others[13] reported a > 90% sensitivity for bone marrow cytology in HIV-infected patients. The higher sensitivity in HIV-infected patients is attributed to the higher *Leishmania* parasitemia in this patient group. However, the low number of *Leishmania*-infected cells in patients with pancytopenia as well as previous treatments may lead to false-negative results.[2]

Three VL patients (42.9%) were conventional PCR positive. Patients 1 and 2 were bone marrow cytology negative and conventional PCR negative, which suggested a low parasite load in these patients. Six out of seven patients (85.7%) were real-time PCR positive, confirming that this technique provides a highly sensitive and noninvasive diagnosis for VL.[14]

Even if all patients were DAT positive, only four (57.1%) were positive by rK39 ICT. Remarkably, patient 6 was bone marrow cytology positive and rK39 ICT negative. Anti-rK39 antibodies usually correlate with active VL, but the use of DAT after negative rK39 ICT results for HIV patients with clinical suspicion of VL may be advisable.[15,16] The sensitivity of this rapid test ranges from 90% to 96% and specificity from 93% to 100%.[17] The reduced sensitivity in VL–HIV/AIDS coinfected patients is because anti-*Leishmania* antibodies are detectable in only 40–50% of the cases, which increases the probability of false-negative results.[2,13] Besides, cross-reactions in patients with other diseases may also lead to false-positive results.[5] Although the rK39 ICT does not distinguish past and subclinical infection, it has been recommended by public health authorities as a screening test, considering that it correlates with clinical disease.[18] This test has already been validated and is currently available at the national public health laboratory network in Brazil.[17] Nonetheless, our results indicate that further studies are needed to assess the usefulness of rK39 ICT in VL–HIV/AIDS coinfected patients.

The mean CD4+ T-lymphocyte counts in the seven VL–HIV/AIDS coinfected patients included in this study was 162.21 cells/mm^3 (range: 8–392 cells/mm^3). It is acknowledged

TABLE 2
Clinical and laboratorial findings of seven VL-HIV/AIDS coinfection patients, 2008–2013, Pernambuco, Brazil

Patient no.	Bone marrow cytology	PCR cPCR	qPCR	rK39 ICT	DAT	KAtex	Splenomegaly	Hepatomegaly	T CD4+ cells/mm^3	Viral load copies/mL
1	Neg	Neg	Pos	Neg	Pos (1/3,200)	Pos (+)	Present	Present	255	543
2	Neg	Neg	Pos	Neg	Pos (1/3,200)	Pos (+)	Absent	Present	8	> 500,000
3	Neg	Pos	Pos	Pos	Pos (1/240,800)	Pos (+++)	Present	Present	49	99,383
4	Neg	Pos	Pos	Pos	Pos (1/102,400)	Pos (+++)	Present	Present	187	60
5	Pos	Neg	Pos	Pos	Pos (1/240,800)	Pos (++)	Present	Present	56	< 50
6	Pos	Neg	Pos	Neg	Pos (1/3,200)	Neg	Absent	Present	392	< 50
7	Pos	Pos	Neg	Pos	Pos (1/102,400)	Neg	Present	Absent	150	< 50

cPCR = conventional polymerase chain reaction[8]; DAT = direct agglutination test; KAtex = a latex agglutination test; Neg = negative; Pos = positive; qPCR = real-time PCR[9]; rK39 ICT = rK39-based immunochromatographic test; T CD4+ = CD4+ T lymphocytes; VL–HIV/AIDS = visceral leishmaniasis–human immunodeficiency virus/acquired immunodeficiency syndrome.

that low CD4+ T-lymphocyte counts may affect antibodies production, by impairing antigen presentation in HIV-infected patients.[3] Furthermore, a low CD4+ T-lymphocyte count is considered as a predictor for VL relapses in HIV patients.[2]

Although Vilaplana and others[19] reported 100% sensitivity for KAtex in HIV-infected patients, two patients reported herein were KAtex negative even though they were cytology positive. World Health Organization[18] recommends the KAtex for diagnosing VL in HIV-infected patients, because it shows high sensitivity in this patient group. The association between urinary antigen and active infection makes it advantageous over serological tests for VL.[20] Indeed, KAtex can detect subclinical infections and may be useful for treatment follow-up, as it becomes negative rapidly after successful treatment.[2,5] In contrast to KAtex, serological tests may remain positive for weeks or months after successful therapy and clinical cure.

Real-time PCR and DAT detected most of the coinfections, being therefore effective tools for screening VL in HIV-infected patients. Although DAT, rK39 ICT, and KAtex tests represent different diagnostic methodologies, they showed a good level of agreement in the cases reported herein. Despite its limitations in terms of sensitivity for diagnosing VL in HIV/AIDS patients, rK39 ICT is a fast and very simple test that can be applied under field conditions. Nonetheless, this study reinforces the usefulness of KAtex for diagnosing VL in HIV patients. Because the World Health Organization recommends this test for suspected cases of VL–HIV coinfection, further studies are needed to validate the KAtex for the diagnosis of VL in HIV/AIDS patients in Brazil. The inclusion of this test to the national public health laboratory network would increase the quality of the VL diagnosis in this patient group in Brazil.

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