Use of the Kala-Azar Detect® and IT-LEISH® rapid tests for the diagnosis of visceral leishmaniasis in Brazil

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The performances of two rapid tests and a standard serological test for the diagnosis of visceral leishmaniasis (VL) were compared using sera from 193 patients with VL and 85 controls. The Kala-Azar Detect®, IT-LEISH® and IFI-LH® assays showed sensitivities of 88.1%, 93.3% and 88.6%, respectively, and specificities of 90.6%, 96.5% and 80%, respectively. The sensitivity values were similar for both rapid tests, but the specificity and positive predictive values of IT-LEISH® were higher than the corresponding values for IFI-LH®. Both rapid tests showed satisfactory performances and can be used in primary health care settings; however, IT-LEISH® permits the use of whole blood, making this assay more suitable for bedside diagnosis.

Key words: visceral leishmaniasis - diagnosis - rapid test

The development of rapid tests using a recombinant K39 antigen (rK39) represented an important advance in the diagnosis of human visceral leishmaniasis (VL). The adequate performance of these tests combined with their ease of use provided greater access to diagnostic services in remote endemic areas. Since then, rapid tests have been evaluated and validated in different endemic areas (Ritmeijer et al. 2006, Cañavate et al. 2011). In Brazil, the rapid test Kala-Azar Detect® (InBios International, Seattle, WA) was first evaluated in 2003 in 128 VL cases and 50 controls, showing a sensitivity of 90% and a specificity of 100% (Carvalho et al. 2003). In 2008, another rapid test, IT-LEISH® (DiaMed Latino-America SA, Switzerland), was validated through a multicentre study supported by the Brazilian Ministry of Health (MoH). That study included 213 VL cases and 119 controls and the IT-LEISH® assay was found to have a sensitivity of 93% and a specificity of 97%. Based on these results, de Assis et al. (2011) recommended the use of IT-LEISH® to increase access to VL diagnostic testing in primary health care centres in this country (de Assis et al. 2011).

In 2010, the MoH purchased Kala-Azar Detect® kits and made them available to the National System of Laboratories and hospitals. To assess the performance of this kit, part of the purchased lot was used to evaluate the same biological samples that were used to validate the IT-LEISH® assay. Therefore, the purpose of the present study was to compare the performance of the Kala-Azar Detect® assay to the validated IT-LEISH® assay and the immunofluorescence test (IFI-LH®), which is the method routinely used by the National System of Laboratories (IFI-Leishmaniose Humana - Biomanguinhos, Fiocruz, Brazil) for the diagnosis of VL in Brazil.

Stored sera from 278 patients were tested. These patients included 193 VL patients with parasitologically confirmed disease using bone marrow aspirates and 85 controls with a clinical suspicion of VL, but negative parasitological results and another confirmed aetiology (de Assis et al. 2011). The Kala-Azar Detect®, IT-LEISH® and IFI-LH® assays were performed according to the manufacturers' instructions. The rapid tests were positive when two red lines appeared on the nitrocellulose membrane, negative when only one red line appeared and invalid when no line was evident. The results of the IFI-LH® assay were considered positive when sera diluted 1:80 or more yielded membranous promastigote fluorescence. A database was constructed using SPSS 12.0 software (SPSS Inc, Chicago, IL, USA) and the performance characteristics of the laboratory tests were determined using Open Source Epidemiologic Statistics for Public Health (OpenEpi) version 2.3 (Dean et al 2009). Epi Info 6.04 software (Centres for Disease Control, Atlanta, GA, USA) was used to calculate the confidence intervals. The chi-square test was applied to compare the proportions. The significance level was fixed at $p < 0.05$.

This study was approved by the Research Ethical Committee of the René Rachou Research Centre (protocol 15/2010).

The mean age of the 278 patients was 12 years (range, 1 month-77 years) and 58% (161/278) were male. The Kala-Azar Detect®, IT-LEISH® and IFI-LH® assays showed sensitivities of 88.1% [confidence interval(CI) 95%: 83.0-92.3)], 93.3% (CI 95%: 89.0-96.4) and 88.6% (CI 95%: 83.393.0) ($p \geq 0.05$), respectively, and specificities of 90.6% (CI 95%: 82.3-96.0), 96.5% (CI 95%: 90.0-99.3) and 80% (CI 95%: 70.0-88.0) (IT-LEISH® vs. IFI-LH® = p = 0.0009), respectively. The positive predictive values (PPVs) for the Kala-Azar Detect®, IT-LEISH® and IFI-LH® assays were 95.5% (CI 95%: 91.3-98.0), 98.4% (CI 95%: 95.3-99.6) and 91% (CI 95%: 86.0-95.0), respectively, and the negative predictive values were 77% (CI 95%: 67.5-85.0), 86.3% (CI 95%: 78.0-92.5) and 75.5% (CI 95%: 65.4-84.0), respectively (PPV IT-LEISH® vs. IFI-LH® = p = 0.001) (Table).

TABLE

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)
of diagnostic methods for the diagnosis of visceral leishmaniasis

Methods	Sensitivity (%) (CI 95%)	Specificity (%) (CI 95%)	PPV (%) (CI 95%)	NPV (%) (CI 95%)
Kala-Azar Detect®	88.1 (83.0-92.3)	90.6 (82.3-96.0)	95.5 (91.3-98.0)	77 (67.5-85.0)
IT-LEISH®	93.3 (89.0-96.4)	96.5[a] (90.0-99.3)	98.4[a] (95.3-99.6)	86.3 (78.0-92.5)
IFI-LH®	88.6 (83.3-93.0)	80 (70.0-88.0)	91 (86.0-95.0)	75.5 (65.4-84.0)

[a]: statistical significant differences between IFI-LH® and rapid test IT-LEISH®; CI: confidence interval.

In Brazil, rK39-based rapid tests can be used for the bedside diagnosis of VL in primary health care centres because these assays have adequate performance, require minimal laboratory infrastructure and skilled labour, provide results in 30 min and allow the use of blood or serum. These tests represent a major advance in the diagnosis of disease, particularly in peripheral health settings, by reducing the time between diagnosis and treatment.

A recent multicentre study organised by the Special Program for Research and Training in Tropical Diseases (TDR) reported considerable differences in the performance of rapid tests in different endemic areas (WHO/TDR 2011). To elucidate the regional differences, we compared the performance of two of the kits, Kala-Azar Detect® and IT-LEISH® (chi-square test for comparison of proportions), using the data provided in the TDR/World Health Organization report (available in table 4). The sensitivities of Kala-Azar Detect® and IT-LEISH® were 67.6% and 87.2% in East Africa (p = 0.00), 84.7% and 92% in Brazil (p = 0.01) and 99.6% and 98.8% in India (p = 0.62). The specificities were 90.8% and 96.4% in East Africa (p = 0.01), 96.8% and 95.6% in Brazil (p = 0.46) and 96% and 97.6% in India (p = 0.31). The TDR study showed that it is crucial to perform regional validation before the purchase and introduction of any non-validated test.

In the current study, the sensitivity was statistically similar between the three methods evaluated (88.1% Kala-Azar Detect®, 93.3% IT-LEISH® and 88.6% IFI-LH®). IT-LEISH®, but not Kala-Azar Detect®, had a higher specificity than IFI-LH® (90.6% Kala-Azar Detect®, 96.5% IT-LEISH® and 80% IFI-LH®) and a higher PPV (95.5% Kala-Azar Detect®, 98.4% IT-LEISH® and 91% IFI-LH®).

The high PPV observed in this study is influenced by the high frequency of VL among patients referred to the reference centres. Therefore, the data must be interpreted with caution and should not be extrapolated to primary health care settings or epidemiological analyses. Moreover, the present data are not valid for patients who do not show clinical signs consistent with VL.

In the current study, Kala-Azar Detect® and IT-LEISH® showed satisfactory performance, justifying their use in primary health care settings using an appropriate protocol and with close supervision and monitoring. It is worth noting that rapid tests that permit the use of whole blood obtained by a finger prick, such as the IT-LEISH® assay, are more convenient than tests that require the use of serum. The use of whole blood enhances the ease of use under field conditions. Unquestionably, cost-effectiveness studies should be performed for these tests. A strong policy should be implemented to sustain the availability and affordability of these tests and should include a plan for national production.

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