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# Comparison of an rK39 dipstick rapid test with direct agglutination test and splenic aspiration for the diagnosis of kala-azar in Sudan

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**Summary** We compared an rK39 dipstick rapid test (Amrad ICT, Australia) with a direct agglutination test (DAT) and splenic aspirate for the diagnosis of kala-azar in 77 patients. The study was carried out under field conditions in an endemic area of north-east Sudan. The sensitivity of the rK39 test compared with splenic aspiration was 92% (46/50), the specificity 59% (16/27), and the positive predictive value 81% (46/57). Compared with the diagnostic protocol used by Médecins sans Frontières, the sensitivity of the rK39 test was 93% (50/54), the specificity 70% (16/23), and the positive predictive value 88% (50/57). Compared with splenic aspirates, the sensitivity of a DAT with a titre ≥1:400 was 100% (50/50), but its specificity only 55% (15/27) and the positive predictive value was 80% (50/62). Using a DAT titre ≥1:6400, the sensitivity was 84% (42/50), the specificity 85% (23/27) and the positive predictive value 91% (42/46). All four patients with DAT titre ≥1:6400 but negative splenic aspirate were also rK39 positive; we consider these are probably 'true' cases of kala-azar, i.e. false negative aspirates, rather than false DAT and rK39 seropositives. There were no false negative DATs (DAT titre ≤1:400 and aspirate positive), but there were four false negative rK39 tests (rK39 negative and aspirate positive). The rK39 dipstick is a good screening test for kala-azar; but further development is required before it can replace the DAT as a diagnostic test in endemic areas of the Sudan.

**keywords** rk39, kala-azar, leishmaniasis, diagnosis, Sudan

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## Introduction

Visceral leishmaniasis (VL; kala-azar) is, ideally, diagnosed by demonstration of the parasite in spleen, bone marrow or lymph node aspirates. In the endemic area of north-east Sudan, splenic aspirates are more sensitive (96%) than aspirates of bone marrow (70%) or lymph nodes (58%) (Zijlstra *et al*. 1992). Over the last 13 years, Médecins sans Frontières-Holland (MSF-H) has treated approximately 43 000 cases of VL in Sudan, under harsh field conditions. The use of splenic aspirates in this setting is limited, because of the small but definite risk of internal bleeding, and the time and skill required in reading the slides. Bone marrow and lymph node aspirates are safer but less sensitive. Many patients would be denied life-saving treatment on the basis of false-negative aspirates if bone marrow or lymph node aspirations only were performed. These characteristics make diagnostic aspiration of any tissue unsuitable as a screening test for VL in a field setting.

Serological tests have been developed for VL, and the direct agglutination test (DAT) is used in the Sudan (Harith *et al*. 1986). DAT titres ≥1:3200 have been found to have a sensitivity of 94% and a specificity of 72% (Zijlstra *et al*. 1992) for active disease. Lower titres are less specific because of the high prevalence of antileishmanial antibodies as a result of past infections in this *Leishmania donovani*-endemic area. In 1991–93, an estimated 3.8–4.8% of the population developed kala-azar annually, and a further 2–3% had evidence of subclinical leishmaniasis (Zijlstra *et al*. 1994).

When large numbers of patients are being assessed for kala-azar, e.g. during epidemics, high DAT titres (≥1:6400) plus a typical clinical picture (fever for >2 weeks, and splenomegaly or wasting), may be used as a surrogate method of diagnosing kala-azar without aspirations

(Boelaert *et al.* 1999). In these patients, a clinical response to antimonial treatment within 10 days confirms the diagnosis (WHO 1996). In addition, when the DAT titre is very low ( ≤1:400) this effectively rules out kala-azar (Boelaert *et al.* 1999). Thus the DAT can be used at high titre as a diagnostic test, and at low titre as a screening test. In this situation, if the patient has a DAT titre of >1:400 but <1:6400, aspirations are performed to confirm a diagnosis of leishmaniasis. MSF uses this diagnostic protocol to reduce the number of aspirations (Veeken 1999).

The DAT, although specifically developed for the field, is still difficult to use in remote conditions. First, antigen production in specialized laboratories is laborious and expensive. Secondly, it requires a cold chain of continuous refrigeration; correct titre setting, cross checking with controls, and meticulous implementation. Finally, the method is time-consuming and requires considerable training of the staff. Improvements have been made by the introduction of freeze-dried antigen, replacing the unstable aqueous antigen (Meredith *et al.* 1995). There is an urgent need for a simpler, faster, non-invasive, but reliable test to screen for, and/or to diagnose kala-azar in the field.

Serological tests have been developed, using the cloned antigen of 39 amino acid repeats of a kinesin like gene found in *L. chagasi*, instead of whole Leishmania parasites. Studies using the rK39 antigen, either in an ELISA (Badaro *et al.* 1996) or dipstick form (Sundar *et al.* 1998), performed well in Brazil, India and Europe (Badaro *et al.* 1996; Medrano *et al.* 1998; Ozensoy *et al.* 1998; Sundar *et al.* 1998; Houghton *et al.* 1998). In Sudanese kala-azar patients, a rK39-based ELISA was 93% sensitive, but only 80% specific (Zijlstra *et al.* 1998). We evaluated an rK39 dipstick test in the diagnosis of kala-azar, under epidemic field conditions in Sudan.

## Methods

The evaluation was carried out in the MSF-Holland kala-azar treatment centre at Um el Kher, Gedaref State, during the months January and February, 1999. During these 2 months, 1093 patients were treated, and more than 2000 patients were screened for kala-azar. Patients with fever and splenomegaly or wasting, were evaluated for kala-azar. Alternative diagnoses in this region include malaria, typhoid, and brucellosis. Patients were included in the study if all the following results were available: frozen serum stored at −20 °C; splenic aspiration yielding material of good quality; DAT performed. Suspects with a positive spleen aspirate or a positive DAT (≥1:6400) were treated for kala-azar, according to a protocol (WHO 1996). The spleen aspirates were examined by direct microscopy after staining with Giemsa. One of the authors (JS) checked all splenic aspirates and DAT titres, and performed rK39 dipstick tests as specified by the manufacturer (Amrad ICT, PO Box 228, Brookdale, NSW 2100, Australia).

We used the following definitions. Sensitivity: the percentage of diseased with a positive test result (True Positive/True Positive + False Negative). Specificity: the percentage non-diseased with a negative test result (True Negative/False Positive + True Negative). Positive Predictive Value: the percentage of persons with a positive test result, who truly have the disease (True Positive/True Positive + False Positive). The protocol was internally reviewed at MSF-H for ethical and scientific validity.

## Results

The 77 patients being evaluated for kala-azar had a median age of 11 years (range: 4–66); 41 were male; they had a median spleen size of 2 cm below the costal margin (range: 1–4) and median haemoglobin of 8.7 g/dl (range: 4.5–13). Of the 77 patients, 54 were treated for kala-azar according to the MSF diagnostic protocol: 50/54 had a positive splenic aspirate and 46/54 had a DAT titre ≥1:6400. The results of the DAT compared with spleen aspiration are shown in Table 1. The results of the rK39 dipstick test are compared with spleen aspiration, DAT and the MSF protocol in Tables 2–4, respectively.

The sensitivity of the rK39 test compared with splenic aspiration was 92% (46/50; 95% CI: 81–98%), the specificity 59% (16/27; 95% CI: 39–78%), the positive predictive value 81% (46/57; 95% CI: 68–90%). Compared with the diagnostic protocol used by MSF, the sensitivity of the rK39 test was 93% (50/54; 95% CI: 82–98%), the specificity 70% (16/23; 95% CI: 47–87%), and the positive predictive value 88% (50/57; 95% CI: 76–95%).

Compared with splenic aspirates, the sensitivity of a DAT with a titre ≥1:400 was 100% (50/50; 95% CI: 93–100%), but its specificity only 55% (15/27; 95% CI: 35–75%) and the positive predictive value was 80% (50/62; 95% CI: 69–90%) (see Table 1). Using a DAT titre

**Table 1** Results of the DAT compared with spleen aspirates of the 77 kala-azar suspects

| DAT | Aspirate+ | Aspirate– | Total |
|---|---|---|---|
| ≥1:6400 | 42 | 4* | 46 |
| >1:400 and <1:6400 | 8 | 8 | 16 |
| ≤ 1:400 | 0 | 15 | 15 |
| Total | 50* | 27 | 77 |

* These 54 patients (50 + 4) were treated for kala-azar.

**Table 2** Results of rK39 test compared with spleen aspirates of the 77 kala-azar suspects

| | Aspirate+ | Aspirate– | Total |
|---|---|---|---|
| rK39+ | 46 | 11 | 57 |
| rK39– | 4 | 16 | 20 |
| Total | 50 | 27 | 77 |

**Table 3** Results of the rK39 test compared with the DAT of the 77 kala-azar suspects

| | DAT | | | |
|---|---|---|---|---|
| | ≥1:6400 | >1:400 and <1:6400 | ≤ 1:400 | Total |
| rK39+ | 43 (39)* | 11 (7) | 3 (0) | 57 (46) |
| rK39– | 3 (3) | 5 (1) | 12 (0) | 20 (4) |
| Total | 46 (42) | 16 (8) | 15 (0) | 77 (50) |

* Numbers in brackets are the number of positive spleen aspirates.

**Table 4** Results of the rK39 test compared with the MSF protocol (aspiration+, or DAT ≥ 1:6400)

| | MSF protocol+ | MSF protocol– | Total |
|---|---|---|---|
| rK39+ | 50 [(46 aspirate+) and (four aspirate–, but DAT ≥ 1:6400)] | 7 | 57 |
| rK39– | 4 | 16 | 20 |
| Total | 54 | 23 | 77 |

≥1:6400, the sensitivity was 84% (42/50; 95% CI: 71–93%), the specificity 85% (23/27; 95% CI: 66–96%) and the positive predictive value 91% (42/46; 95% CI: 79–98%). All four patients with DAT titre ≥1:6400 but negative splenic aspirate were also rK39 positive; we consider these are probably 'true' cases of kala-azar, i.e. false negative aspirates, rather than false DAT and rK39 seropositives. There were no false negative DATs (DAT titre ≤1:400 and aspirate positive), but there were four false negative rK39 tests (rK39 negative and aspirate positive).

Of the eight borderline DATs with a positive splenic aspirate, seven also tested positive for rK39, whereas of the eight borderline DATs with a negative splenic aspirate, four tested positive for rK39. Of the 11 false positive rK39 tests, four had a borderline DAT test.

## Discussion

The high sensitivity of the rK39 compared with MSF diagnostic protocol (93%) means that it is a good screening test for kala-azar in our hands. However, the advantage of the DAT over the rK39 test is that titres ≥1:6400 also have high specificity (85%) for kala-azar, so DAT can be used for diagnosis as well as screening. It is worth emphasizing that the positive predictive value (PPV) of DAT (or any serological test for kala azar) is only high in circumstances of high prevalence. If the prevalence of kala azar among patients being tested were 15.6%, the PPV of DAT would be only 29.5% and at a prevalence of 8.5%, the PPV of DAT would be only 17.3%.

Unlike the DAT, the rK39 dipstick test is either positive or negative – it lacks information on the strength of the immune reaction. We consider it is important not to lose this information by substituting the DAT with a dipstick. False positive readings with the rK39 test were common in this highly endemic area of Sudan, a finding not seen in India (Sundar *et al.* 1998). Whether these false positive tests could be caused by other endemic diseases such as brucellosis and malaria remains to be investigated. Our main interest was to see whether the test was useful under the field circumstances, where we have no means to perform further serology. Its low specificity in relation to splenic aspiration (59%) and to the diagnostic protocol for kala-azar (70%) makes the rK39 test in its current form unsuitable as a diagnostic test to replace the DAT, or aspiration (or a combination of the two), under field conditions in Sudan. One should be cautious to extrapolate the results of the study to other regions in the world, given the different epidemic conditions. We suggest two ways in which positive/negative rapid tests like the rK39 dipstick might be used in a population with a high background prevalence of antibodies to *Leishmania*. The first method would be to develop an rK39 test strip that gives a positive reaction at low titre (for screening) and a separate test indicating a high titre (for diagnosis). The second method would be to use a highly sensitive dipstick test on neat and serially diluted blood or serum samples, to determine the titre of antibody in much the same way as is carried out with the DAT at present.

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## References

Badaro R, Benson D, Eulalio MC *et al.* (1996) rK39: a cloned antigen of *Leishmania chagasi* that predicts active visceral leishmaniasis. *Journal of Infectious Diseases* **173**, 758–761.

Boelaert M, El Safi S, Jacquet D, De Muynk A, van der Stuyft P & Le Ray D (1999) Operational validation of the DAT for diagnosis of visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene* **60**, 129–134.

Harith AE, Kolk AH, Kager PA *et al.* (1986) A simple and economical direct agglutination test for the serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 583–586.

Houghton RL, Petrescu M, Benson DR *et al.* (1998) A cloned antigen (recombinant K39) of *Leishmania chagasi* diagnostic for visceral leishmaniasis in human immunodeficiency virus type 1 patients and a prognostic indicator for monitoring patients undergoing drug therapy. *Journal of Infectious Diseases* **177**, 1339–1344.

Medrano FJ, Canavate C, Leal M, Rey C, Lissen E & Alvar J (1998) The role of serology in the diagnosis and prognosis of visceral leishmaniasis in patients co-infected with human immunodeficiency virus type 1. *American Journal of Tropical Medicine and Hygiene* **59**, 155–162.

Meredith SE, Kroon NC, Sondorp E *et al.* (1995) Leish-KIT, a stable direct agglutination test based on freeze dried antigen for serodiagnosis of visceral leishmaniasis. *Journal of Clinical Microbiology* **33**, 1742–1745.

Ozensoy S, Ozbel Y, Turgay N *et al.* (1998) Serodiagnosis and epidemiology of visceral leishmaniasis in Turkey. *American Journal of Tropical Medicine and Hygiene* **59**, 363–369.

Sundar S, Reed SG, Singh VP, Kumar PC & Murray HW (1998) Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* **351**, 563–565.

Veeken H (1999) *Manual for the Diagnosis and Treatment of Kala-Azar Under Field Conditions*. MSF, Amsterdam.

WHO (1996) *Manual on Visceral Leishmaniasis Control*. (Report No. WHO/Leish /96.40) WHO, Geneva.

Zijlstra EE, Daifalla NS & Kager PA (1998) rK39 enzyme-linked immunosorbent assay for diagnosis of Leishmania donovani infection. *Clinical Diagnostic Laboratory Immunology* **5**, 717–720.

Zijlstra EE, El-Hassan AM, Ismael A & Ghalib HW (1994) Endemic kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post kala-azar dermal leishmaniasis. *American Journal of Tropical Medicine and Hygiene* **51**, 826–836.

Zijlstra EE, Siddig Ali M & El-Hassan AM (1992) Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 505–507.

 