

The use of K39 test in the diagnosis of visceral leishmaniasis

Robert Sciberras

Abstract

From time to time patients admitted with fever of unknown origin prove to be a diagnostic dilemma. While textbooks describe typical symptoms and signs, and also diagnostic tests, these are not always helpful. The following are two case reports of patients who were eventually diagnosed to be suffering from visceral leishmaniasis. It is apparent that the usual diagnostic tools at our disposal were not enough to identify the disease and new tests were needed. The case reports have some similarities; both patients are male, diabetic, resident in Gozo but not of Gozitan stock. These factors could have reduced their immunity to visceral leishmaniasis, making them more susceptible to the disease.

Case history 1

CB is a 60 year old male British resident in Gozo. He presented in March 2002 with a 2 month history of lethargy, reduced appetite, weight loss and fever. He is a diabetic on metformin and gliclazide, non-smoker and social drinker.

Examination revealed an ill-looking man with an oral temperature of 101.2 degrees Fahrenheit. He was very pale, and lethargic. His abdomen revealed hepatomegaly, palpable 7cm below right costal margin and splenomegaly, palpable 2cm below left costal margin. Rest of the examination was unremarkable; there was no rash and no lymph nodes were palpable. Initial investigations revealed blood glucose of 23 mmol/L, haemoglobin of 5.1 g/dL, white blood count of $2.5 \times 10^3/\mu\text{L}$, platelets of $58 \times 10^3/\mu\text{L}$. The patient was admitted to Gozo General Hospital and reverse barrier nursing was instituted. Several blood cultures were taken all of which eventually proved to have no growth. Imipenem 1g 8 hourly intravenously was started, with poor effect. Bone marrow aspirate was carried out, specifically asking for evidence of visceral leishmaniasis. This was also negative. Gastroscopy revealed small, non-bleeding varices with an *H. pylori* negative duodenitis. Abdominal ultrasound confirmed hepatosplenomegaly. The liver was coarse and had irregular echostructure. The splenic veins were tortuous. Ascites was present in the perihepatic and perisplenic regions. The patient was therefore also suffering from portal hypertertension. At this point the patient admitted to alcohol abuse especially in the past. Computerised Axial Tomography (CAT scan) of the chest was normal and that of the abdomen confirmed the ultrasound findings. Full infective screen including Brucella, Rickettsiae, Ebstein Barr, Hepatitis, HIV, and Leishmania antibodies were all negative. Liver function tests were deranged, with a mildly high bilirubin, and ALT, and with a high gamma GT. Over a period of 10 days the WBC fell to $1.4 \times 10^3/\mu\text{L}$, the platelets went down to $18 \times 10^3/\mu\text{L}$ and his haemoglobin was 8.5g/dL. He was transfused with blood and platelets. At this point the patient expressed his wish to return back to the UK, which he did. While in his home country all the tests which were done locally were repeated including several bone marrow aspirates and trephine biopsies, all drawing a blank regarding diagnosis. His fever did not settle, and he remained undiagnosed for a further 12 weeks.

However the breakthrough came when the patient consulted another consultant in the UK who was convinced that, in spite of lack of evidence, this was a case of visceral leishmaniasis. A blood sample was sent to the Hospital for Tropical Diseases in

Key words

Visceral leishmaniasis, diabetes, immunity

Robert Sciberras MD, FRCP
Consultant Physician, Gozo General Hospital
Email: robscib@maltanet.net

London for assay of K39 antigen. This was found to be positive. The patient was started on liposomal amphotericin for 21 days, made a full recovery and came back to Gozo. He was reviewed last December and was well and asymptomatic with normal complete blood count.

Case history 2

JG is a 50 year old male, diabetic Maltese living in Gozo for the past 10 years. He was admitted to Gozo General Hospital in June 2004 complaining of a 3 week history of fever (up to 104 degrees Fahrenheit), rigors, and sweating. There was no obvious focus of infection i.e. no respiratory, urinary, or bowel symptoms. He did complain of mild frontal headache especially when febrile, but did not complain of weight loss or loss of appetite. He was on insulin, never traveled to exotic places, and kept pigeons at home. He worked as ground crew with a local shipping line.

On examination he was pale, weak, and had 104 degrees Fahrenheit temperature. He had long standing extensive vitiligo but no rashes, poor dental hygiene but a clear throat. His abdomen revealed splenomegaly, felt 5 cm below left costal margin. Initial investigation showed pancytopenia: white blood count $2.3 \times 10^3/\mu\text{L}$, haemoglobin 8.6g/dL, platelets $92 \times 10^3/\mu\text{L}$. The ALT and GGT were also elevated. Full infection screen was negative. Bone marrow aspirate and trephine biopsies did not show evidence of Leishmania Donovanii bodies and were reported as normal. CAT scan of the abdomen was normal except for splenomegaly with no focal lesions. Repeated blood cultures did not show any growth. Leishmania antibodies were negative. The platelet count fell to $65 \times 10^3/\mu\text{L}$. At this point a diagnosis of lymphoproliferative/neoplastic disorder was suggested and the thought of splenectomy was entertained. However, based on experience of Case History 1 (above), K39 assay was requested and the patient's blood samples were sent to the same hospital in London. The report came back as 'Antibody to Leishmania K39 antigen POSITIVE.' The patient was started on liposomal amphotericin intravenously for 21 days. The fever settled after 4 days and the patient eventually left hospital healthy and asymptomatic. Subsequent follow-ups revealed a healthy patient with normal blood indices.

Discussion

The diagnosis of visceral leishmaniasis (VL), a serious and often fatal parasitic disease caused by members of *Leishmania donovani* complex, remains problematic. Current methods rely on clinical criteria, parasite identification in aspirate material and serology. The latter methods use crude antigen preparations lacking in specificity. In fact in the case histories illustrated above, all the above-mentioned methods failed to clinch the diagnosis. The patient in Case 2 had a more rapid diagnosis because of our experience with the first patient.

The test which gave the diagnoses in both cases was the K39 assay. The blood samples of both patients were tested at the Hospital for Tropical Diseases, London. K39 represents 39

amino acid repeats encoded by a kinesin-like gene of visceral leishmaniasis. There are 2 methods how to test for K39:

1. Recombinant K39 dipstick method: One drop of the patient's peripheral blood is applied to a nitrocellulose strip. 3 drops of test buffer (phosphate-buffered saline plus bovine serum albumin) are added to the dried blood. 10 minutes later the test area is observed. Two visible bands confirm the presence of IgG anti-K39. This test is much like the usual home pregnancy test kit, here a single band indicates a valid but negative test while 2 bands indicate a valid and positive test.
2. Recombinant K39 ELISA: This is an enzyme linked immunosorbent assay for IgM and IgG for K39.

The method used by the Hospital for Tropical Diseases in both patients was the dipstick method (*John Bligh, Senior Biomedical Scientist, Hospital for Tropical Diseases, personal communication*).

Comparison of K39 dipstick test to other methods

Several studies have been carried out to assess sensitivity and specificity of this test, and especially how it compares to other methods. Sundar *et al*¹ and Goswami *et al*² found 100 percent sensitivity and 98 percent specificity in 2 separate and independent studies. When compared to the ELISA method, rK39 dipstick had 90% sensitivity compared to 89% for ELISA. The latter was 98% specific while rK39 was 100%.³ ELISA was found to be valuable in monitoring drug therapy and detecting relapse of the disease.⁴

The dipstick method for rK39 was also compared to Direct Agglutinin Titration (DAT). While DAT had a sensitivity of 99% and specificity of 82%, rK39 was 97% sensitive and 71% specific.⁵ This was a study in Nepal and the authors suggested that the dipstick test in this area should be used for screening suspect patients in endemic areas. The use of the dipstick tests as confirmatory methods should be restricted to situations where the proportion of Kala-azar among clinically suspect patients is high. This was confirmed by Igbal *et al*.⁶ Another study comparing DAT with rK39 carried out in the Netherlands⁷ confirmed that DAT was superior and more specific than the dipstick rK39 test but the latter was more rapid, more user friendly and cheaper. The advantageous practical and economic aspects of the rK39 dipstick method were confirmed by another study in Nepal.⁸ Burns *et al*⁹ even suggest using rK39 instead of all other serological tests for VL.

Apart from its use in diagnosing VL, the dipstick rK39 test has also been found to be effective to differentiate American VL from other endemic diseases in Venezuela.¹⁰

Canine infections with *Leishmania infantum* are important as a cause of serious disease in the dog, and as a reservoir for human VL. Accurate infection is essential to the veterinary community and for VL surveillance programs. Testing for rK39 in the field has been proposed as a relatively fast, reliable and simple test for assessing infection of dogs.¹¹

Conclusion

As illustrated by the case histories above, the rK39 test should be considered in any patient who fits the clinical criteria for visceral leishmaniasis, but where the usual tests have been repeatedly negative. To date, the simple dipstick test for rK39 is not available in the Maltese islands, neither in the public laboratories nor in the private sector. Given the above information, maybe it is time to change this state of affairs.

References

1. Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW. Rapid accurate field diagnosis of Indian Visceral Leishmaniasis. *Lancet* 1998;351:563-5.
2. Goswami RP, Bairagi B, Kundu PK. K39 strip test - easy, reliable and cost-effective field diagnosis for visceral leishmaniasis in India. *J Assoc Physicians India*. 2003;51:759-61.
3. Carvalho SF, Lemos EM, Corey R, Dietze R. Performance of recombinant K39 antigen in the diagnosis of Brazilian visceral leishmaniasis. *Am J Trop Med Hyg*. 2003;68:321-4.
4. Kumar R, Pai K, Pathak K, Sundar S. Enzyme-linked immunosorbent assay for recombinant K39 antigen in diagnosis and prognosis of Indian visceral Leishmaniasis. *Clin Diagn Lab Immunol*. 2001;8:1220-4.
5. Chappuis F, Rijal S, Singh R, Acharya P, Karki BM, Das ML, *et al*. Prospective evaluation and comparison of the direct agglutination test and an rK39 antigen-based dipstick test for the diagnosis of suspected kala-azar in Nepal. *Trop Med Int Health*. 2003;8:277-85.
6. Iqbal J, Hira PR, Saroj G, Philip R, Al-Ali F, Mada PJ, *et al*. Imported visceral leishmaniasis: diagnostic dilemmas and comparative analysis of three assays. *J Clin Microbiol*. 2002;40:475-9.
7. Scallig HD, Canto-Cavalheiro M, da Silva ES. Evaluation of the direct agglutination test and the rK39 dipstick test for the serodiagnosis of visceral leishmaniasis. *Mem Inst Oswaldo Cruz*. 2002;97:1015-8.
8. Bern C, Jha SN, Joshi AB, Thakur GD, Bista MB. Use of recombinant K39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. *Am J Trop Med Hyg*. 2000;63:153-7.
9. Burns JM Jr, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed J. Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. *Proc Natl Acad Sci USA* 1993;90:775-9.
10. Delgado O, Feliciangeli MD, Coraspe V, Silva S, Perez A, Arias J. Value of a dipstick based on recombinant rK39 antigen for differential diagnosis of American visceral leishmaniasis for other sympatric endemic diseases in Venezuela. *Parasite* 2001;8:355-7.
11. Scalone A, De Luna R, Oliva G, Baldi L, Satta G, Vesco G, *et al*. Evaluation of the *Leishmania* recombinant K39 antigen as a diagnostic marker for canine leishmaniasis and validation of a standardized enzyme-linked immunosorbent assay. *Vet Parasitol*. 2002;104:275-85.