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Serological tests for visceral leishmaniasis

Have high sensitivity, but several limitations

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Visceral leishmaniasis is a parasitic disease transmitted by sandflies, with 0.5 million new cases annually.[1] It is most commonly seen in India, Bangladesh, Brazil, Sudan, and around the Mediterranean. About two cases are seen each year in the United Kingdom, and these usually originate from around the Mediterranean.[2]

Patients with visceral leishmaniasis present with fever, splenomegaly, and weight loss. It can be difficult to diagnose this disease in endemic settings as several causes of febrile splenomegaly exist, notably malaria. In this week's *BMJ*, a meta-analysis by Chappuis and colleagues compares the diagnostic performance of two serological tests in endemic settings, the direct agglutination test (DAT) and rK39 dipstick test.[3] Outside endemic areas visceral leishmaniasis is often only considered after haematological malignancies have been excluded.[2]

In immunocompetent people visceral leishmaniasis can be treated with a 28 day course of a pentavalent antimonial, and the cure rate is 90-95%.[4] In resource rich settings patients are treated with six to 10 days of liposomal amphotericin (an antifungal agent), and the cure rate is higher at 95-98%.[4]

The gold standard for diagnosis of visceral leishmaniasis is to identify parasites in smears of tissue aspirates (spleen, bone marrow, or lymph node). Splenic smears have a sensitivity of >90%, but the procedure carries a small risk of intra-abdominal haemorrhage.[5] Bone marrow aspiration is often done but is painful and less sensitive.

In endemic settings the resources needed to support tissue diagnosis—skilled technicians, good smears, proper stains, appropriately maintained and working microscopes—are often unavailable.[5] Serological diagnosis is safer and two field tests have been developed, DAT and the Ks30 dipstick test.

DAT measures anti-leishmania antibody titres using a freeze dried antigen.[6–8] However, it requires test readings to be standardised, prolonged incubation, and the handling of multiple samples. In Sudan, DAT testing is done in field laboratories on filter paper blood samples. Médecins sans Frontières has developed a management strategy based on DAT titres in which patients with a titre of 1:6400 are treated, and those with titres between 1:400 and 1:6400 have a splenic aspirate. About 10% of suspected cases will need splenic aspirates. The K39 dipstick test is highly specific for visceral leishmaniasis and detects antibodies to a specific 39 amino acid sequence (K39). It has been developed as an immunochromatographic strip test dipstick.[9] The test is easy to perform—a village health worker can be trained in few hours—the kit can be stored at ambient temperature, no equipment is needed, and it can be carried to remote areas.

A meta-analysis published in this week's *BMJ* evaluated the performance of both serological tests.[3] Only studies with the gold standard of parasites seen on splenic aspirate were included, and data from 30 studies on the DAT test and 13 on the K39 dipstick test were analysed. The combined data set relates to 2817 patients with visceral leishmaniasis and 6552 controls. Both tests have high sensitivities, 94.8% for DAT and 93.9% for the K39 test. The authors were rigorous and calculated separate specificities when controls were patients with clinically suspected disease or healthy people; even so the overall specificities were 85.9% and 90.6%, respectively. This confirms that both tests performed well and either could be incorporated into national guidelines for diagnosing visceral leishmaniasis. There are interesting regional differences—the tests are more sensitive in South Asia than in Sudan—perhaps because Sudanese patients produce lower antibody titres. This highlights the importance of validating new diagnostic tests in endemic areas even though this is costly. Visceral leishmaniasis is an important coinfection in HIV positive people, particularly in areas where highly active antiretroviral therapy is not available, and this meta-analysis only considered data from HIV negative patients. Serology may be negative in up to half of HIV positive patients coinfected with visceral leishmaniasis.[1 10] Such coinfections will become common in endemic regions, and studies on the performance of the tests in this group are needed.

Both serological tests can remain positive for several years after cure and so cannot be used to detect relapse or reinfection. Furthermore, in areas with high transmission of visceral leishmaniasis, many people will be infected but only a minority will develop clinical illness. People who carry the infection test positive serologically and sometimes form up to 32% of the healthy population.[8 11] Tests are therefore needed that identify only active disease. A limitation of the dipstick test is that it is only positive or negative and the titre based screening strategy used by Médecins sans Frontières cannot be applied.

Other tests are being developed. The latex agglutination test (KAtex) detects a leishmanial antigen in boiled urine that disappears after cure. It has been tested in Asia and Africa, and specificity has ranged

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from 47% to 95%[12 13]; it may be developed as an immunochromatographic strip test for use with unboiled urine. Another potential test detects erythrocyte sialic acid, which is positive in patients with active disease but becomes negative after cure.[14] The test warrants further clinical evaluation.

The World Health Organization could help in ensuring that these tests remain available for the people who need them. Two manufacturers have already stopped producing dipsticks (Arista Biological and Amrad). Academic studies are needed for evaluation, and public health action is needed to incorporate good tests into national policies while recognising the commercial needs of manufacturers. Progress in neglected diseases like leishmaniasis is often hampered by lack of commitment from industry, and diagnostics and drugs are abandoned when profits are insufficient.

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Combining aspirin with antithrombotic agents

Risk of bleeding is increased but can be minimised

The use of aspirin and other antiplatelet agents has sky rocketed in the past decade as the indications have widened to include primary and secondary prevention of myocardial and cerebrovascular ischaemia. In the United States, an estimated 34.8% of men and 26.2% of women over 40 years use aspirin every day or on alternate days.[1] Half of these patients are classified as at high risk of cardiovascular disease. With the development of safer antiplatelet agents such as thienopyridines and the publication of major randomised studies, the combination of aspirin and clopidogrel has become a class I recommendation (that is, it is considered to be beneficial, useful, and effective) after percutaneous coronary interventions with stenting in the US and in Europe.[2 3] Combined antithrombotic therapy is recommended for up to 12 months "in patients who are not at high risk of bleeding."[2] The problem for clinicians is to balance the benefits of combined antiplatelet therapy with the potentially increased risk of gastrointestinal bleeding. In this week's *BMJ* a case control study by Hallas and colleagues goes some way to measuring this risk.[4]

Aspirin is widely known to cause mucosal ulceration that can lead to upper gastrointestinal bleeding. Lower doses and slow release formulations of aspirin have been used in the hope that they carry a reduced risk of bleeding. In a meta-analysis of almost 66 000 people, Derry and colleagues found no relation between gastrointestinal bleeding and aspirin dose or formulation.[5] They also showed that risk of bleeding was not lower with prolonged use of aspirin, so the notion of mucosal tolerance to aspirin remains a myth. These findings were confirmed in a later study from Spain, which focused on patients taking low dose aspirin to prevent cardiovascular diseases.[6]

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Clopidogrel has been recommended as the treatment of choice for patients with coronary heart disease who cannot tolerate aspirin.[7] This is based primarily on the CAPRIE study, which compared the efficacy of clopidogrel with aspirin in preventing myocardial ischaemia.[8] Gastrointestinal bleeding was reported in 1.99% of patients taking clopidogrel and 2.66% of those taking aspirin ($p = 0.05$). It has been shown repeatedly that combining aspirin with clopidogrel increases the risk of gastrointestinal bleeding compared with using aspirin[9] or clopidogrel alone.[10]

Hallas and colleagues found that upper gastrointestinal bleeding was associated with the use of low dose aspirin, dipyridamole, and vitamin K antagonists but not with clopidogrel.[4] The finding that clopidogrel did not increase bleeding risk contradicts other reports.[11] This discrepancy could be related to the relatively small sample size of the current study (only 1443 participants, with 30 cases of bleeding). Importantly, the authors found that combining aspirin with any of the antithrombotic agents (clopidogrel, dipyridamole,

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