CASE REPORT

When smear and molecular diagnostics fail: identification of tuberculosis in advanced HIV infection using the newly developed urine lipoarabinomannan lateral-flow assay

Ashar Vijay Dhana,¹ Pauline Howell,² David Spencer¹

¹Department of Infectious Diseases, Helen Joseph Academic Hospital, Johannesburg, Gauteng, South Africa

²Themba Lethu Clinic, Helen Joseph Academic Hospital, Johannesburg, Gauteng, South Africa

Correspondence to

Dr Ashar Vijay Dhana, ashdha@hotmail.co.za

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SUMMARY

In HIV infection, progression of immunodeficiency is associated with increased risk of paucibacillary and disseminated forms of tuberculosis (TB). As a result, the clinical presentation may be atypical and the conventional diagnostic assays often unreliable, resulting in significant treatment delays. Here, we report a case of HIV-associated immune reconstitution inflammatory syndrome and TB meningitis. Although the smear and molecular assays were negative, *Mycobacterium tuberculosis* was identified in our patient using the new Determine-lipoarabinomannan (LAM) lateral-flow urine 'dip-stick' assay. This case report illustrates the clinical value of this assay for the diagnosis of TB in a subgroup of HIV-infected patients with advanced immunodeficiency. Also, although two recent studies have evaluated the use of the Determine TB-LAM assay in clinical settings, to the best of our knowledge, this is the first case report of TB diagnosed using this novel assay.

BACKGROUND

Tuberculosis (TB) is the leading cause of morbidity and mortality in patients with HIV,¹ particularly in those with the lowest CD4 cell counts.² In these patients, TB often presents as a non-specific illness. Pulmonary findings are frequently absent, whereas extrapulmonary and disseminated forms of the disease are more common.³ Furthermore, many countries still rely on the use of smear microscopy and chest radiography. These tests often have a poor diagnostic accuracy for HIV-associated TB.⁴ While the new Xpert MTB/Rif (Xpert) assay represents a major advance in TB diagnostics, difficulties with implementation have restricted its use in many parts of the developing world.⁵ Also, a negative test is not sufficient to rule out HIV-associated TB in settings where the infection occupies sites beyond access with this test.⁶ The diagnosis of TB is therefore a huge challenge in HIV-infected patients with advanced immunodeficiency.

Lipoarabinomannan (LAM) ELISA assays have shown utility for the diagnosis of TB in this specific subset of patients; however, their use in resource poor settings has been limited.⁴ Recently, the Determine-LAM assay—a lateral flow urine 'dip-stick' format of the LAM ELISA—has been developed and is considered the first bedside test for TB.

We report a case of TB identified with the help of this new assay.

CASE PRESENTATION

A 37-year-old woman was seen at her local clinic with a 2-month history of cough, fever, night sweats and weight loss. Although her sputum was smear negative, chest radiography was abnormal, showing lower lung zone infiltrates bilaterally and hilar adenopathy; subsequently, she was empirically started on the standard four-drug anti-TB regimen: isoniazid, rifampicin, pyrazinamide and ethambutol. Additional tests obtained at the visit confirmed infection with HIV-1 and a CD4 cell count of 15 cells/ μ L. During the next 2 months her symptoms subsided, and antiretroviral therapy (ART)—with tenofovir, lamivudine and efavirenz—was started. At this point, her antituberculous treatment was changed to a two-drug regimen of isoniazid and rifampicin.

She now presented to our hospital 11 weeks after her initial diagnosis and 3 weeks after the initiation of ART. She complained of a 1-week history of gradually worsening headache, photophobia, night sweats and occasional episodes of vomiting. She had no other neurological symptoms. She had no medical history of TB or of any other illnesses. On examination, tachycardia and terminal neck stiffness were present. Neurological examination revealed no focal neurologic deficit, papilloedema or cranial nerve involvement. The remainder of the systemic examination was normal.

INVESTIGATIONS

Laboratory findings confirmed a CD4 cell count of 45 cells/ μ L. The admission cerebrospinal fluid (CSF) was clear in appearance, and further analysis revealed lymphocytes 12 cells/ μ L, neutrophils 0 cells/ μ L, protein 1.75 g/dL (normal 0.15–0.45 g/dL) and glucose 1.9 mmol/L (normal 2.8–4.4 mmol/L). CSF adenosine deaminase (ADA) was not elevated (<6 μ /L). Gram's stain, cryptococcal latex antigen test (CLAT), India ink and syphilis antibodies were all negative. Xpert MTB/Rif assay and TB-smear of the CSF were also negative. However, the urine Determine-LAM assay was positive for TB (figure 1). We collected a fresh urine sample from the patient and applied approximately 60 μ L of the sample to the test strip. After waiting 25 min, which allowed for the urine to

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Figure 1 A Determine-lipoarabinomannan strip of a patient attending the hospital. A band in the patient window can be seen in this photograph and indicates a positive result.

travel up the test strip and interact with colloidal gold labelled antibodies, a purple band developed in the test window, indicating that LAM was present in the sample. In addition, small calcified nodules were present on the chest X-ray, and CT of the brain revealed diffuse leptomeningeal enhancement (figure 2). No hydrocephalus, ischaemic infarcts, cerebral oedema or paraneurymal granuloma formation was evident.

DIFFERENTIAL DIAGNOSIS

The most likely diagnoses based on these findings were TB and pyogenic, viral and fungal meningitis. Cryptococcal meningitis is now the most common cause of meningitis in sub-Saharan Africa.⁷ However, CLAT and India ink of the CSF were both negative. Viral meningitis was unlikely in view of the high CSF protein, low glucose and chronicity of the illness. Pyogenic meningitis enters the differential diagnosis but stains and bacterial cultures were negative. In addition, the white cells were few and were all lymphocytes, unusual in this form of meningitis. The

Figure 2 CT of the brain showing diffuse leptomeningeal enhancement.

absence of CSF antibodies to treponemal antigens makes active neurosyphilis unlikely.

Immune reconstitution inflammatory syndrome (IRIS) should be considered in any patient who has started antiretroviral treatment in the preceding 12 months. The paradoxical progression of disease despite ART, or the development of new symptoms and signs, should raise a suspicion of IRIS.⁸ Up to 43% of patients dually infected with TB and HIV and who are on anti-TB and ART develop IRIS.⁹ Although IRIS is a common diagnosis, it is always one of exclusion.

Identifying TB meningitis (TBM) is a challenge. CSF smear microscopy is too insensitive, and culture positivity too infrequent to reliably rule out the diagnosis.¹⁰ As yet, the new Xpert assay has not been optimised for use with CSF and its current sensitivity may be as low as 27%.¹¹ Evidence to support the role of ADA measurement in TBM is lacking.¹⁰ As a result, the diagnosis of TBM is often presumptive. A combination of clinical, laboratory and radiological findings were used to support the diagnosis in this case. CSF features of TB include lymphocytic pleocytosis, low glucose and high protein. In patients with TB-IRIS and TBM, CSF pleocytosis is typically mild or may even be absent.¹² Compared with other bacterial causes of meningitis, TBM is also more likely if symptoms have been present for more than 5 days.⁹ Abnormalities on chest radiography and meningeal enhancement on CT scan provide additional but non-diagnostic support for TBM. In HIV-associated TBM, extrameningeal TB is common.¹³ Moreover, in patients with lower CD4 cell counts, central nervous system and disseminated forms of extrapulmonary TB are more likely.¹⁴ Thus, the confirmation of TB in non-CNS sites argues for its participation in CNS-disease when alternative causes have been excluded. Even though *Mycobacterium tuberculosis* was not cultured directly from the CSF, and Koch's postulates were not met, the diagnosis of TBM and HIV-associated TB-IRIS is probable in view of the presence of lipoarabinomannan in this patient's urine, that is, the positive LAM test. The patient's subsequent improvement on anti-TB therapy adds weight to this assertion.

TREATMENT

Anti-retroviral and antituberculous therapies were continued in the ward. Intravenous antimicrobial therapy with ceftriaxone was started. In view of the probability of TBM, corticosteroids were added to the regimen. By admission day 10, her condition had improved and she was discharged from hospital.

OUTCOME AND FOLLOW-UP

At the 2-week follow-up visit, the steroid was gradually tapered and no neurological sequelae were apparent. CSF culture was negative for TB after 43 day incubation. So far, she reports no recurrence of her symptoms and is doing well.

DISCUSSION

Early and effective diagnosis of TB remains an important aspect of disease control.³ This case highlights the need for rapid bedside tests for the diagnosis of TB, and while diagnostic accuracy of current assays is often suboptimal, combination testing can substantially improve TB case finding.

LAM, a glycolipid constituent of the mycobacterial cell wall, has shown promise as a TB-antigen test in extrapulmonary and pulmonary TB.¹⁵ Unlike tests that detect whole organisms or genetic material, antigen tests have a greater potential to identify TB in locations remote from the disease site and are therefore an attractive diagnostic modality.⁵ Sputum and urine LAM ELISA tests have been available on the market for a few years.

Although these assays perform poorly in HIV non-infected patients, they have shown acceptable diagnostic accuracy in those with HIV-associated TB.¹⁵ A systematic review of studies published before October 2010 provided a pooled sensitivity of 56% and a pooled specificity of 95% for diagnosis of HIV-associated TB.¹⁶ Interestingly, among patients with CD4 cell counts less than 50 cells/ μ L, sensitivity was much higher and ranged from 67% to 85%.¹⁶ However, as LAM ELISAs require sophisticated laboratory facilities and technique, their use in resource poor settings has been limited.²

A new point-of-care, lateral-flow version of this test has become available: the Determine TB-LAM urine assay. This test is easy to use, provides results in 15–25 min, and requires no prior preparation.¹ Two retrospective studies have assessed the use of this assay in HIV-infected inpatients and outpatients. Sensitivity ranged between 28% and 45% and specificity was 96–98.2%.^{17,18} 'Agreement' was high among investigators, suggesting reliability of the dip-stick assay in field conditions.² As seen with the LAM ELISA, the dip-stick assay showed improved diagnostic accuracy in patients with low CD4 cell counts. In one of these studies, sensitivities were 67% and 52% at CD4 cell counts of less than 50 and 100 cells/ μ L, respectively.¹⁸

The topic of assay specificity deserves further mention, as some evaluations of the older LAM ELISAs have reported a greater proportion of false-positive results.⁴ The reasons for low specificity are not well understood, but non-tuberculous mycobacteria may play an important role. In one study, for instance, 29% of patients with false-positive results grew non-tuberculous mycobacteria on culture.⁴ As non-tuberculous mycobacteria are common at CD4 cell counts below 50 cells/ μ L, these findings suggest that it would be prudent to carefully monitor clinical response to treatment in these patients.

An important characteristic of the Determine-LAM assay is that it can be used in combination with other diagnostic tests. In HIV-infected patients with CD4 cell counts of 50 cells/ μ L, smear microscopy has a low sensitivity of approximately 30%, but this figure rises to 72% when smear is combined with the new assay.² Furthermore, the Determine-LAM assay increases specificity of chest radiography and accelerates diagnosis in patients waiting for culture results.² Although the assay provides little benefit for the diagnosis of pulmonary TB when combined with the Xpert assay,⁴ until now no study has specifically evaluated its use in extrapulmonary disease. Sensitivities of the Xpert assay on CSF samples, for example, have been variable, and lower diagnostic accuracy has also been observed with pleural and ascitic fluids.¹⁹ If combined with the Xpert assay, this dip-stick test may thus provide improved sensitivity for the diagnosis of extrapulmonary TB.

Although our patient was started on empiric treatment for TB, the Determine-LAM assay proved a valuable adjunct to existing tests. This allowed the rapid confirmation of a microbiological diagnosis and the avoidance of a prolonged hospital stay. The assay has other additional advantages. First, it can be used as a point-of-care test in HIV-infected patients with low CD4 cell counts,²⁰ making diagnosis and rapid initiation of treatment at one outpatient visit a reality. Second, at a cost of US\$3.50, this assay is an economical alternative to other diagnostic tests such as the Xpert assay and culture, which cost around US\$18.⁴ Given its low cost, the Determine-LAM assay could be used as a first-line diagnostic test for TB in patients with advanced immunodeficiency. Finally, it poses a minimal biohazard risk and requires no special processing or storage.⁴

In summary, the Determine-LAM assay adds to the clinical decision making processes either alone or in combination with

other diagnostic tests in patients with low CD4 cell counts. These patients are often the most ill; consequently, they stand to benefit from rapid diagnosis and timely treatment. While the Determine-LAM assay shows promise as a diagnostic tool, further research is needed to outline how best to integrate this novel test into existing TB diagnostic guidelines.

Learning points

- Diagnosis of tuberculosis (TB) is a challenge in patients with advanced HIV infection.
- The Determine TB-LAM (lipoarabinomannan) assay, a rapid new 'dip-stick' test, shows diagnostic potential in these patients.
- It can be used as a point-of-care test, making diagnosis and treatment in one visit a reality.
- Diagnostic accuracy is enhanced when the Determine-LAM assay is combined with other diagnostic tools.

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Competing interests None.

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