



YOU ARE HERE:

- > news

Blood and urine tests for TB: still great hope for antigen detection

Theo Smart, Friday, March 23, 2007

“At the most peripheral level of the health system [the health post/primary care level], there is currently no [TB lab] test available,” said Dr. Vinand Nantuyla of FIND. “FIND is evaluating a urinary antigen test and we expect that the demonstration phase will be completed by 2009, at which point the test will be available.”

In fact, FIND appears to be working (or has at least signed letters of intent) with a number of competing teams who are racing to develop a rapid point-of-care lateral flow or dipstick TB antigen test (similar to a home-pregnancy test). Thus far the approach seems quite promising — particularly in people with HIV and smear negative or extrapulmonary TB.

To some extent, though, the TB antigen tests may suffer from guilt by association with TB antibody tests, which looked good in preclinical development, but failed miserably in the field. This, and the fact that FIND is focused on the eventual point-of-care test, seems to have delayed anyone looking seriously at formats of the test that are already available (and which could improve the diagnosis of diagnosis of TB in people with HIV today) — but which could only be performed at better equipped peripheral laboratories.

Early disappointment with serological tests

Serological tests, which look for antibodies, and sometimes antigens, in the blood serum or other bodily fluids, are commonly used to diagnose infections, including HIV. For years, the hopes of the TB community were pinned on developing a rapid antibody test for TB. But despite years of work, the leading tests failed to be sensitive or specific enough once they were tested in the field in high burden countries — particularly in people with HIV).

Perhaps the final nail in the coffin was hammered in at last year’s World Union Conference, when researchers from WHO/TDR announced that screening of 19 different tests on clinical specimens from high burden settings had shown that the tests were either highly variable, not specific to TB or very insensitive — again, especially in people with HIV (Cunningham). The researchers concluded that none of these assays were reliable enough to replace smear microscopy (for more see the MSF report).

Part of the problem could have been the nature of what was being screened for. An antibody response might be detectable in some people even while they are only latently infected with TB; and exposure to non-tuberculosis mycobacteria could possibly elicit similar responses. Conversely, in the case of people with advanced HIV, antibody responses to TB might be too weak to be reliably detected. Also, people developing active TB may have an inherently inadequate antibody response to it, and what antibodies are produced may be already bound in a complex with the antigen (parts of the TB microbe) they are trying to eliminate.

But what about screening for the antigen itself? Antigen tests are used to diagnose a variety of other active infections such as hepatitis A & B, helicobacter pylori, giardia, and strep throat, to name just a few. Why not TB?

In fact, as far back as the late 1980s, several teams of researchers published papers describing how ELISA-type tests they developed in-house could indeed detect TB antigens in blood serum, cerebral spinal fluid and sputum from people with active disease (Yáñez, Sada 1988, Cho, Sada 1992). The

sensitivity rates in these studies ranged from 45% to 88% and the specificity rates were between 91% to 100%. But there was no commercial development, possibly because antibody screening looked easier at the time.

LAM

Of those early papers, the highest sensitivity and specificity were reported with an ELISA test that screened for one particular TB antigen called lipoarabinomannan (LAM), which is part of TB's cell wall, in sputum specimens from people with smear-positive TB (Sada 1992). The sensitivity was decreased somewhat in smear negatives (67%) and people coinfecting with HIV and TB (57%) but it was still better than smear microscopy in those patients. Even so, such a technique would only be suitable for diagnosing pulmonary TB.

Other researchers reported that LAM could be detected in serum, but was often bound in immune complexes that decreased ELISA sensitivity or made specimen processing somewhat laborious (Pereira Arias-Bouda).

Then in 2001 and 2002, researchers from the Swedish Institute for Infectious Disease Control and the University of Bergen, Norway published a couple of papers showing that LAM could be detected in urine (Hamasur; Tessema).

Urine is a much better biological material to use in a lab test, as its collection is safer and far less invasive. It could also dramatically improve diagnosis in children, the majority of whom have trouble producing sputum (smear microscopy is even less sensitive in children than in people with HIV).

The Scandinavian researchers very quickly took an ELISA that they developed into the field to screen a large cohort of 1050 Ethiopians (200 with TB, 800 with other illnesses, and 50 who were healthy), and 150 Norwegians (50 healthy, and 100 non-TB patients).

Their enthusiasm may have gotten the better of them because they wound up with results that were a bit difficult to interpret. The test was only slightly more sensitive than smear microscopy (74% vs. 69%) in the culture-proven TB patients. However, the test detected LAM in 57.4% of the smear negative TB patients.

The problem is it also detected LAM in 105 (13.1%) of the Ethiopian non-TB patients and 5 (10%) of the Ethiopian healthy controls. Everyone in Norwegian groups was LAM negative however. It's not clear what the explanation is for the difference in the high specificity in Norway versus Ethiopia. It could have been that this ELISA methodology was difficult to perform in that setting. This particular technique has been criticised because the urine had to be processed (concentrated and frozen), which is not really practical for field use in Ethiopia (Boehme).

It's also possible that some of those patients were developing TB, or had extrapulmonary TB, acute TB infection, or they had another mycobacterial infections (all of which produce LAM) such as leprosy, which isn't so common in Norway.

However, the researchers have continued to work on this test and at the World Union Conference in Paris this year, they presented some data using a modified dipstick version of this test in 35 culture proven Estonian TB patients and 15 healthy Swedish controls. 77% of the TB patients were correctly identified by the dipstick test and all controls were negative (Svenson). They are now working with industrial partners on a lateral flow strip test.

Chemogen's MTB LAM urine tests

They could be in direct competition with another company from the US, Chemogen (www.chemogen.com), which claims to have a strip test currently being "optimised" that they anticipate moving into the clinic sometime around the middle of 2007. In the meantime, they have a 96 well ELISA plate format of the test (which can be performed at any lab with an ELISA reader) and an ELISA tube

format, which packages individual tests in a tube and uses a portable ELISA reader.

Chemogen's ELISA has at least one advantage over the Scandinavian process — the urine doesn't have to be processed (or refrigerated) if tested within one to two days (although Chemogen says that it is most sensitive if the urine is first boiled — particularly if it needs to be transported for more than a day). However, in the only large study to date, there was no specimen processing whatsoever. Of course, once the sample is placed onto the ELISA plate, there is some fairly standard ELISA processing with reagents and incubation periods so that the whole process takes at least a few hours — though once a batch is ready, the ELISA reader is fast. With the tube format, however, all these manipulations need to be performed with each individual tube — so the process might be more tedious and the throughput obviously much lower and a trained lab technician would still be required.

Chemogen's LAM-ELISA plate format has been field tested in a study in Tanzania in 231 patients with suspected pulmonary TB and 103 health volunteers (Boehme). Of 132 culture-proven TB cases, 106 were positive for LAM (80.3% sensitivity) compared to 82 who were smear-positive (62.1% sensitivity). The LAM-ELISA detected 76% of the smear-negative culture-positive patients. 17 of the 231 TB suspects were both culture and smear negative, but had typical x-rays suggestive of TB (military or enlarged hilar lymph nodes) and had failed cotrimoxazole antibiotic treatment — 13 of these 17 were positive for LAM. This is a reminder that even culture itself isn't always 100% sensitive (particularly for extrapulmonary disease). The 82 remaining TB suspects remained undiagnosed by culture or chest x-ray. LAM was detected in 8 (9.7%) of these but since this study was cross-sectional (without longitudinal follow-up) it is impossible to say whether they had TB or not.

Among the healthy volunteers, all but one had LAM results below the test cut-off — for a specificity of 99% (if you exclude the undiagnosed TB suspects). Of note, there was also a correlation between the number of bacilli of smear microscopy and LAM concentration in the urine (this suggests that LAM measurements could potentially be useful for monitoring treatment effect or for early relapse).

And perhaps most importantly, HIV prevalence was high (69.01%) in the 213 TB suspects who agreed to be tested — but it didn't appear to negatively impact the sensitivity of this test at all.

Which raises this question of why aren't more clinical teams exploring using the plate reader format of this test to detect smear negative TB in people with HIV? While ELISA readers are too complex to introduce into lesser-resourced laboratories, many reference laboratories and some district hospitals already have them. Where infrastructure permits they could also be purchased for about \$5000 (around the price of 3 light microscopes). This is not to say that the LAM-ELISA is at a place where it could replace smear microscopy but even a team of three full time lab technicians working round the clock wouldn't be able to diagnose the majority of smear negative cases that this test picks up quite readily.

And if the HIV community is clamouring for universal access to culturing (which takes at least a week to reach a result), why isn't it at the very least calling for more research into this technique which could diagnose three fourths of those people on the same day — and which may even pick up some patients with extrapulmonary TB that culture misses. Although it is not point-of-care, the same sort of transport systems that are used to transport specimens for culture could be used to transport urine specimens to a facility that can perform the test.

True, if that transportation is expected to take more than a day, the urine sample would be more stable if it is first boiled — and not every remote site has access to reliable electricity. Still if the remote site can't use a heating plate, how difficult could it be to procure a bunsen burner?

Although the next generation format, the strip test, is coming, it may take some time to refine, and will take at least a couple of years to evaluate in the field. There will be a lot of operational questions about how to best use a TB test that can truly be performed anywhere without a technician (for example, at a VCT clinic). But in the meantime, programmes could be using the ELISA plate format to learn how to integrate LAM detection into patient management today — especially for HIV.

References

Boehme C et al. *Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis*. *Trans R Soc Trop Med Hyg.* 99 (12):893-900, 2005.

Tessema TA et al. *Diagnostic evaluation of urinary lipoarabinomannan at an Ethiopian tuberculosis centre*. *Scand J Infect Dis.* 33(4):279-84, 2001.

Cunningham J et al. *Global survey of tuberculosis laboratory services*. 36th Union World Conference on Lung Health, Paris, 2005.

Guillerm M, Usdin M, Arkininstall. *Tuberculosis diagnosis and Drug Sensitivity Testing. An overview of the current diagnostic pipeline*. *Médecins Sans Frontieres* 2006.

Hamasur B et al. *Rapid diagnosis of tuberculosis by detection of mycobacterial lipoarabinomannan in urine*. *J Microbiol Methods*;45(1):41-52, 2001.

Pereira Arias-Bouda LM et al. *Development of antigen detection assay for diagnosis of tuberculosis using sputum samples*. *J Clin Microbiol*;38(6):2278-83, 2000.

Svenson SB et al. *Towards development of new point of patient care tuberculosis diagnostics*. 37th Union World Conference on Lung Health, Paris, abstract PS-61862-02, 2006.

Sada E, Aguilar D, Torres M, Herrera T. *Detection of lipoarabinomannan as a diagnostic test for tuberculosis*. *J Clin Microbiol.* 30:2415–2418, 1992.

Sada E et al. *Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay*. *Lancet.* 651–652, 1988.

Yanez MA et al. *Determination of mycobacterial antigens in sputum by enzyme immunoassay*. *J Clin Microbiol.*;23(5):822-5, 1986.

aidsmap resources

- Tuberculosis

Tuberculosis news

- Transformation of TB diagnostics on horizon: special report
- Sniffing out tuberculosis - TB breath tests under investigation
- Interferon Gamma Release Assays (IGRAs) — a complicated and expensive way to answer a simple question?

subscribe to aidsmap email bulletins