DIAGNOSING TUBERCULOSIS IN ADULTS: OPPORTUNITIES AND CHALLENGES

Cough, haemoptysis, pleuritic pain, weight loss, fever, drenching sweats and fatigue are all important symptoms pointing to the diagnosis of tuberculosis.

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Mycobacterium tuberculosis is superbly adapted to infect humans, and tuberculosis remains one of the most lethal infections to afflict our species. Tuberculosis has been described in the writings of many ancient civilisations and an estimated one-third of people alive today have latent infection. The global human immunodeficiency virus (HIV) epidemic has driven the incidence of active tuberculous disease to unprecedented heights: in some impoverished urban areas in South Africa with high HIV prevalence the incidence of tuberculosis exceeds 1 000 cases per 100 000. It is one of the conundrums of our time that HIV is simple to diagnose but cannot yet be cured, whereas tuberculosis can be exceptionally difficult to diagnose but can be cured easily with combinations of potent antimycobacterial drugs.

THE SPUTUM SMEAR

M. tuberculosis is primarily a pulmonary pathogen, and is most likely to be found in sputum. Examining expectorated sputum specimens for acid-fast bacilli (AFB) remains the cornerstone of tuberculosis diagnostics. The World Health Organization (WHO) recommends that two to three sputum specimens are examined in all adults who have been coughing for more than 2 weeks. The easiest way to obtain these specimens is to ask the patient for one 'on the spot' specimen in the clinic and to give two containers to be brought back to the clinic the following day with one 'early morning' specimen and a subsequent specimen. The sputum should be produced after the client takes a few deep breaths, and the cough should be performed in private in a well-ventilated room, or outside to prevent transmission to bystanders.

The least expensive way to examine the specimen for AFB is by using the

Ziehl-Neelsen (ZN) stain, where the mycobacteria are seen using the light microscope as pink/red beaded rods against a blue background (Fig. 1).

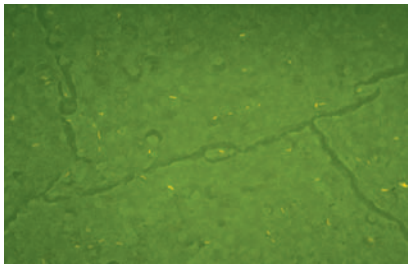

Fig. 1. Acid-fast bacilli seen on sputum Ziehl-Neelsen stain.

Experienced laboratory technicians can examine a specimen in about 15 minutes, but exceptional dedication and concentration is required. Using a fluorescent microscope and an auramine-based stain is faster and less demanding (the mycobacteria are seen as a brilliant green against a dark background) but the test is more costly (Fig. 2). The WHO recommends that all specimens labelled as positive on fluorescent microscopy should be confirmed using the ZN stain.

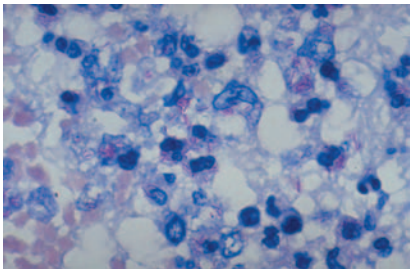

Fig. 2. Acid-fast bacilli seen using auramine-based staining and fluorescent microscopy.

Laboratory technicians can try to improve on the yield of AFB by initially liquefying the sputum in household bleach (sodium hypochlorite) and then centrifuging the specimen or allowing it to stand overnight (importantly, this technique kills mycobacteria, so the specimen cannot be cultured). Clinicians who make regular use of a tuberculosis laboratory should ask the manager whether quality control measures are in place according to WHO guidelines: this may help to motivate and reward dedicated technicians.

Table I. **WHO guidelines for the classification of smear-positive pulmonary TB**

Smear-positive	Indeterminate	Smear-negative
At least 2 smears examined and both positive, i.e. reported 1 - 9 per 100 fields (scanty) or greater	Several possibilities e.g. • only one smear examined (whatever the grading) • 3 smears examined but only one reported positive In either of these situations either further sputum smears or a chest X-ray are required before a patient can be classified	At least 2 smears reported 0 (negative)

Table II. **Technique for sputum induction**

- The patient fasts for 8 hours and rinses out the mouth well with tap water
- The hypertonic saline is made by mixing 6 ml of a 5% sodium chloride solution (available from the hospital pharmacy) with 4 ml of sterile water for injection
- Up to 60 ml of the saline solution is administered over 20 minutes using an ultrasonic nebuliser machine (**not** a gas-driven nebuliser as the particles are too large)
- Sputum samples are collected halfway through the procedure, and at the end of the procedure
- The mask and tubing must be disinfected using glutaraldehyde solution for 30 minutes between patients

Table I shows the WHO recommendations for diagnosing tuberculosis using the results of sputum smears.

Sputum induction using hypertonic saline and an ultrasonic nebuliser is an effective and inexpensive way of obtaining high-yield sputum specimens from patients with non-productive cough. The technique is described in Table II. Several important points should be noted:

- there is a high risk of nosocomial transmission and the procedure should be conducted in a well-ventilated room with an open window, or (preferably) outside
- the induced sputum is usually transparent and resembles saliva – the laboratory must be asked not to discard the specimen
- bronchoconstriction occasionally occurs in response to the hypertonic saline, but is usually readily reversed by two puffs of salbutamol.

Low-cost techniques if patients are too weak to cough include early morning gastric lavage using a nasogastric tube: 30 ml of gastric fluid is aspirated and sent to the laboratory to be examined for AFB; if no gastric fluid can be obtained a 50 ml lavage with normal saline is performed first.

Advanced HIV infection, while making patients exceptionally vulnerable to TB, also greatly reduces the yield of AFB in the sputum. This means that many HIV-infected patients living in poor communities will die from TB despite presenting repeatedly with TB symptoms to their local clinic. The reason for this phenomenon is that an intact immune system is necessary to cause lung cavitation and the optimal conditions required for proliferation and dissemination by coughing. The smear will usually only be positive if there are >10 000 organisms/ml of sputum.

Patients with pulmonary TB and negative sputum smears will usually have positive mycobacterial sputum cultures. A sputum smear cannot distinguish between *M. tuberculosis* and other mycobacterial species but culture techniques allow speciation. In most laboratories a positive niacin test is used to confirm that the isolate is *M. tuberculosis*. Polymerase chain reaction (PCR) assays can also be used (see below). Culture is also required before drug sensitivity testing. However, mycobacterial culture on the Lowenstein-Jensen medium is very slow (taking up to 6 weeks) and the culture is vulnerable to contamination. Automated liquid culture media systems (e.g. the Bactec MGIT system; Becton-Dickinson, Baltimore) approximately halve the time to positive culture and are less labour-intensive, but are also prone to contamination, are very expensive and are usually only available in large urban laboratories. The additional workload and administration required to maintain a mycobacterial culture system may easily overwhelm under-resourced laboratories struggling to keep pace with large volumes of new specimens.

USING CLINICAL CASE DEFINITIONS TO DIAGNOSE SPUTUM SMEAR-NEGATIVE TB

The WHO guidelines for the diagnosis of smear-negative pulmonary tuberculosis are:

- three sputum smears negative for AFB
- failure to improve after a course of antibiotics (amoxycillin would be an appropriate choice in South Africa)
- a chest radiograph compatible with active TB
- the decision made by a doctor to commit the patient to a full course of antituberculous therapy.

These guidelines rely on interpretation of a chest radiograph, which can appear normal or non-specifically abnormal in HIV-infected patients with pulmonary TB. Typical chest radiograph features of smear-negative pulmonary TB include:

- pulmonary infiltrates including nodules, interstitial patterns, air-space opacification with or without cavitation, and miliary/micronodular patterns (it can be very difficult to distinguish active TB from healed fibrocystic disease)
- hilar and mediastinal lymphadenopathy
- pleural effusions.

Fig. 3 illustrates many of the features of HIV-associated smear-negative pulmonary TB.

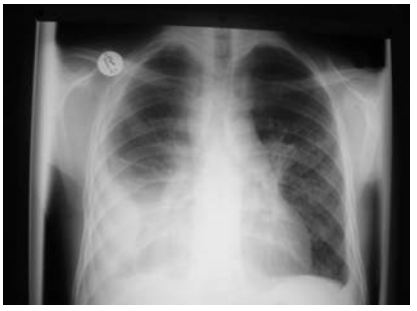

Fig. 3. Chest radiograph of immunocompromised HIV-infected patient with smear-negative TB showing pleural effusions, right middle lobe consolidation, left mid-zone infiltrates and mediastinal lymphadenopathy.

Several other HIV-associated conditions can mimic pulmonary TB, and district laboratories often do not have the facilities to diagnose these conditions. The WHO has published a useful guide, shown in Table III, on how to clinically distinguish *Pneumocystis* pneumonia (PCP) from pulmonary TB. Pulmonary Kaposi's sarcoma is readily diagnosed by examining the skin and mouth for purple nodules and plaques. Pulmonary cryptococcosis can be diagnosed by requesting a serum cryptococcal latex agglutination test. Pulmonary nocardiosis produces branching Gram-positive rods in the sputum that are also weakly positive using acid-fast stains, but this is a rare disease. Lymphocytic interstitial pneumonitis is usually seen in children with HIV infection, and looks like miliary TB on a chest radiograph. Finger clubbing accompanying diffuse late fine inspiratory crackles on auscultation are clues to the diagnosis but active TB can only be ruled out with confidence if sputum cultures are negative.

The WHO guidelines for extrapulmonary TB are very broadly defined, requiring strong evidence for a disease process compatible with TB (such as tissue histology) and the decision by a clinician to commit the patient to a full course of antituberculous therapy. Biopsies of pleura, lymph nodes, bone marrow and liver can be very useful in diagnosing extrapulmonary TB. Classic histological features include caseation (sometimes reported as tissue necrosis), granuloma formation, giant cells or epithelioid macrophages, and AFB. However, biopsies are invasive, and require a degree of operator expertise. There is a need for detailed case definitions for use when diagnosing extrapulmonary TB in resource-limited settings. However, the clinical case definitions rely on clinical and radiological acumen, and should not be seen as a long-term solution to the problem of diagnosing smear-negative TB. Fine- and wide-needle aspiration biopsies and Trucut biopsies of cervical, axillary and inguinal nodes have a high yield for AFB and TB histology and cytology.

TUBERCULOUS MENINGITIS

Clinicians have been trying for decades to find accurate ways to diagnose tuberculous meningitis. AFB

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are rarely seen in the cerebrospinal fluid (CSF). Classic pointers to the diagnosis include:

- raised CSF protein
- ratio of CSF/blood glucose < 50%
- CSF lymphocyte predominance
- no bacterial pathogens isolated on CSF culture.

Other clues have also been identified in recent research conducted in Vietnam, but need to be validated in populations with a high background incidence of HIV. These include:

- blood white cell count < 15 x 10^9/l
- history of illness > 6 days (but seldom longer than 30 days)
- CSF white cell count < 900 x 10^3/ml
- CSF neutrophils < 75% of total white cell count
- CT brain scan showing hydrocephalus, oedema or basal meningeal enhancement.

When the distinction between bacterial and tuberculous meningitis is unclear it is acceptable to treat for bacterial meningitis for 48 hours (e g. ceftriaxone 2 g daily or cefotaxime 2 g 8 hourly) and then to repeat the lumbar puncture. If the patient has bacterial meningitis the CSF opening pressure, white cell count and protein should fall, and the CSF glucose should rise. Failure of the 48-hour CSF parameters to improve should raise the possibility of tuberculous meningitis, but cryptococcal meningitis should be excluded (the cryptococcal latex agglutination is the most sensitive test).

TB AND IMMUNE RECONSTITUTION DISEASE

With the advent of the widespread use of highly active antiretroviral therapy (HAART), South African clinicians are increasingly seeing smear-negative TB manifesting as immune reconstitution inflammatory syndrome (IRIS) — a paradoxical clinical deterioration in spite of starting treatment. IRIS occurs in patients with undiagnosed or recently diagnosed TB who start antiretroviral therapy. Immune reconstitution secondary to HIV suppression allows the immune system to mount a vigorous response against *M. tuberculosis* (or other opportunist infections). Patients who start HAART with a low CD4 count (< 100) are more likely to develop IRIS, which occurs in the first 2 weeks, before the CD4 count starts to rise significantly. Diagnosing IRIS TB is an evolving art. Other reasons for the deterioration include poor adherence to antituberculous therapy, and multidrug-resistant infection.

Features compatible with IRIS due to TB include:

- new persistent fever (temperature ≥ 38.5°C) that develops after the initiation of antiretroviral therapy
- marked worsening or emergence of intrathoracic lymphadenopathy or pulmonary infiltrates
- worsening or emergence of cervical adenopathy
- worsening of other tuberculous lesions or manifestations, such as cutaneous, peritoneal or central nervous system inflammatory pathology
- symptoms of an inflammatory condition that cannot be explained by a newly acquired infection, the expected clinical course of a previously recognised infectious agent, or the side-effects of treatment.

It is important to exclude acute bacterial infections with blood, sputum and urine cultures, and to consider obtaining tissue for histology. Non-steroidal anti-inflammatory drugs or prednisone (usually 60 mg daily) can be used to control the symptoms of IRIS once the patient has been started on antituberculous therapy.

NOVEL DIAGNOSTIC TESTS

TB diagnostics is a field of intense research and several new avenues are being pursued.

Mycobacteriophage assays are promising novel diagnostic techniques where clinical specimens are incubated overnight with a mycobacteria-specific phage (phages are viruses that parasitise bacteria). The next morning a virucidal agent is added, neutralising all free phage virions, so that only phages that have entered and infected mycobacteria in the specimen survive. The specimen is then plated onto a Petri dish covered with a lawn of rapidly growing mycobacteria and is incubated for 24 - 48 hours. If mycobacteria were present in the original specimen the phage will be released into the Petri dish culture and destroy areas of the mycobacterial lawn (Fig. 4). Fig. 5 shows the results of a mycobacteriophage assay (FastPlaque assay, Ipswitch) on induced sputum specimens from HIV-infected TB suspects in Cape Town.

The interferon assay tests the *in vitro* response of patients' blood

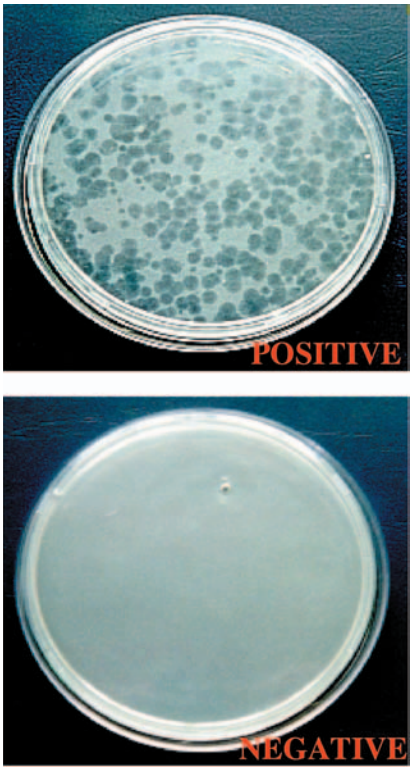

Fig. 4. Example of a positive and negative result using a mycobacteriophage assay (FastPlaque).

mononuclear cells against purified protein derivatives of *M. tuberculosis*. The technique is promising, but cannot distinguish between latent infection and active disease, and may not perform well in patients who are severely immunocompromised from HIV infection.

Antibody tests have proved to be disappointing and, to date, have been unable to reliably distinguish between latent infection and active disease.

Nucleic acid amplification tests using polymerase chain reaction technology were widely hyped in the late 1990s, but are very expensive and their utility is not well studied in settings with a high tuberculosis prevalence. However, the FDA in the US has licensed two tests for smear-negative TB.

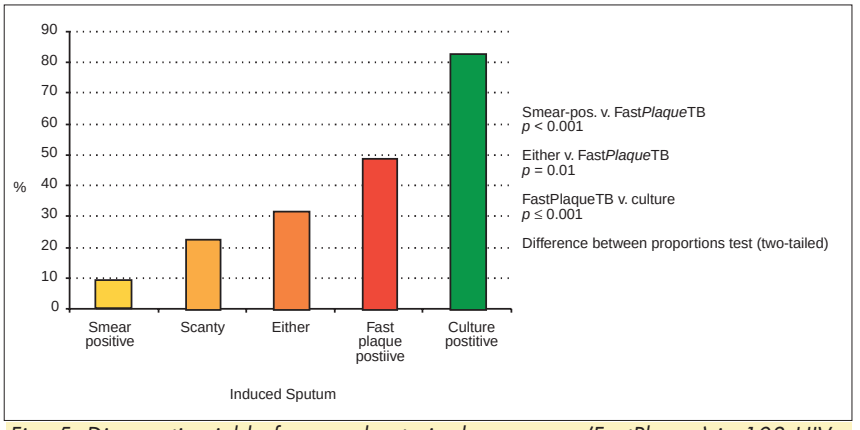

Fig. 5. Diagnostic yield of a mycobacteriophage assay (FastPlaque) in 103 HIV-infected patients with pulmonary TB. (Adapted from Wilson D, Nachega J, Chaisson R, Maartens G. Identifying sputum smear-negative TB in HIV-infected adults using a bacteriophage assay. Paper presented at the South African AIDS Conference, Durban 2003.)

Proteomic systems hold the most promise for the future. This experimental technique concentrates and fractionates secreted mycobacterial proteins, potentially allowing these proteins to be identified in the body fluids of patients with active TB.

Further reading

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IN A NUTSHELL

Sputum smear for AFB remains the cornerstone of TB diagnostics in South Africa. Culture techniques are slow and can be expensive.

The HIV epidemic has substantially increased the number of patients with sputum smear-negative TB. Implementing clinical case definitions may allow these patients to be more readily identified and treated.