DIAGNOSING HIV-ASSOCIATED TUBERCULOSIS

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'The world has made defeating AIDS a top priority. This is a blessing. But TB remains ignored. Today we are calling on the world to recognise that we can't fight AIDS unless we do much more to fight TB as well'. Nelson Mandela: Media briefing on 'Confronting the Joint HIV-TB Epidemics', co-convened by the Bill & Melinda Gates Foundation, Thursday 15 July 2004.

South African health care workers, along with those in other developing countries, are witnessing the disastrous intersection of the HIV and tuberculosis (TB) epidemics. The World Health Organization (WHO) estimates that currently the incidence of TB in South Africa is 536 cases/100 000 annually and that 61% of patients diagnosed with TB are HIV-infected.[1] TB is the commonest HIV-associated opportunistic infection and one of the leading causes of AIDS-related death.[2-4] Ironically, HIV infection is easy to diagnose with highly accurate serological tests but cannot be cured, and TB can often be difficult to diagnose but is curable with freely available and highly effective antituberculosis therapy. There is a pressing need to develop new, cost-effective ways with which to diagnose TB.

Unlike TB in HIV-uninfected patients, which is usually an indolent condition developing over months, HIV-associated TB is aggressive and can render immune-compromised patients moribund within weeks.[5] Health care workers in the public sector have a brief window of opportunity to make the diagnosis before the patient becomes too ill to continue to present to health care facilities. Common TB symptoms are shown in Table I.

TABLE I. SYMPTOMS OF HIV-ASSOCIATED TB

Constitutional

- Weight loss of recent onset and > 2.5% body weight
- Drenching night sweats
- Fatigue
- Asymmetrical lymph node swelling

Pulmonary

- Cough for more than 2 weeks
- Chest pain (usually pleuritic)
- Dyspnoea (parenchymal disease, pleural and pericardial effusions)
- Haemoptysis (unusual in advanced HIV disease)

THE SPUTUM SMEAR

Mycobacterium tuberculosis is primarily a pulmonary pathogen transmitted via droplet nuclei derived from lower respiratory tract secretions. The WHO-sponsored TB control programme[6] has appropriately focused on diagnosing patients with sputum smear-positive disease with a view to interrupting disease transmission. Patients with early HIV disease and pulmonary TB usually have 'typical' upper lobe lung infiltrates and cavitation on the chest radiograph, and sputum smears that are positive for acid-fast bacilli (AFB). The maturing HIV epidemic, however, has challenged the WHO's strategy, as HIV-infected patients with advanced disease are more likely to have negative sputum smears and extrapulmonary infection:[7,8] compromised immune defences are less able to contain infection within the lungs, and are less able to cause the pulmonary cavitation required for optimal proliferation and dissemination of the *M. tuberculosis* bacillus.

However, sputum smear remains the only readily available TB diagnostic test throughout most of the developing world. 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. Experienced laboratory technicians can examine a specimen in a few minutes, but exceptional dedication and concentration is required. Using a fluorescent microscope and an auramine-based stain is less demanding (the mycobacteria are seen as a brilliant green against a dark background) but much more costly.[7] The WHO recommends that all specimens labelled as positive on fluorescent microscopy should be confirmed using the ZN stain.

The South African TB control programme recommends two sputum smears when TB is suspected, followed by a chest radiograph if both specimens are negative. A third sputum smear is recommended if the chest radiograph is suggestive of TB. In practice this means that three sputum smears are necessary, as the chest radiograph is normal in about 10% of

patients with smear-positive TB. If these recommendations are followed TB can be diagnosed rapidly and cost-effectively in a substantial proportion of HIV-infected patients. Clinic and hospital staff need ongoing training on how to obtain a sputum sample correctly. TB programme managers have a substantial challenge to ensure quality control in the laboratory in the face of overwhelming workload and limited funding.

SMEAR-NEGATIVE PULMONARY TB

Primary care clinicians frequently see HIV-positive patients with pulmonary and constitutional symptoms and pulmonary infiltrates on the chest radiograph. Some of these patients will have presented to their local TB clinic and been told that their sputum smears are negative, or have been unable to produce sputum. The diagnostic possibilities in this setting are shown in Table II. Importantly, the chest radiograph findings of HIV-associated TB can be entirely nonspecific, although micronodular (miliary) or nodular infiltrates, pleural effusions and mediastinal or hilar lymphadenopathy can be highly suggestive of the diagnosis.

TABLE II. DIAGNOSTIC CONSIDERATIONS IN HIV-INFECTED ADULTS WITH PULMONARY INFILTRATES AND CONSTITUTIONAL SYMPTOMS

HIV related

- Community-acquired pneumonia (usually pneumococcal)
- Pulmonary tuberculosis
- Pneumocystis pneumonia
- Kaposi's sarcoma
- Other opportunistic infections (nocardiosis and cryptococcosis)
- Immune reconstitution disease (often due to previously occult TB)

Non-HIV related

- Post-tuberculous lung disease
- Occupational lung disease
- Bronchial carcinoma
- Sarcoidosis and alveolitis

The recommended response is to treat for community-acquired pneumonia (amoxycillin or doxycycline would be appropriate for outpatients) and to check at least two sputum smears. Response to therapy and the smear results can be reviewed after 2 weeks. If the patient's symptoms remain after antibiotic treatment antituberculosis therapy can be initiated, even if subsequent smears are negative.[6] Detecting and reporting AFB-positive smears remains critically important for the TB control programme, and clinicians should make every effort to obtain smear results.

Dual infections are common,[10] and patients with pulmonary TB may experience partial improvement of their symptoms after antibiotic treatment. Pulmonary TB can also co-exist with *Pneumocystis carinii* pneumonia (PCP), which should be diagnosed and treated first. The WHO's recommendations on how to distinguish between pulmonary TB and PCP are shown in Table III.[6]

TABLE III. WHO GUIDELINES FOR DISTINGUISHING BETWEEN PULMONARY TUBERCULOSIS AND PCP

	Typical of PCP	Typical of TB
Symptoms	Dry cough Sputum mucoid (if any) Dyspnoea	Productive cough Purulent sputum Pleuritic pain Haemoptysis
Signs	May be normal Fine inspiratory crackles	Signs of consolidation Signs of pleural effusion
Chest radiograph	Bilateral diffuse interstitial shadowing (may be normal)	Lobar consolidation Cavitation Pleural effusion Intrathoracic lymphadenopathy

IMPROVING THE YIELD OF AFB FROM RESPIRATORY TRACT SECRETIONS

SPUTUM INDUCTION

Inducing a productive cough using an ultrasonic nebuliser and hypertonic saline has repeatedly been shown to improve the yield of AFB in patients with pulmonary TB, and is at least as effective as bronchoscopy with lavage and much less invasive.[11,12] The ultrasonic nebuliser costs in the region of R1 500 and is simple to use: the technique is described in Table IV. The sputum specimen is usually clear, resembling saliva, and the laboratory needs to be informed not to discard the specimen. Bronchoconstriction in response to the saline can be reversed with two puffs of an inhaled beta-2 agonist. The reusable masks and tubing have to be sterilised between patients using either gas or glutaraldehyde solution.

TABLE IV. 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 and at the end of the procedure
- Mask and tubing must be disinfected for at least 30 minutes with glutaraldehyde solution

Nosocomial transmission of *M. tuberculosis* during sputum induction remains a very real concern, but it is not clear whether the risk is greater than that from a spontaneous cough. The procedure should take place in a well-ventilated room with the operator standing behind the patient, or (preferably) outdoors in the sunshine.

GASTRIC LAVAGE AND THE STRING TEST

Early morning lavage of stomach contents through a nasogastric tube is an effective technique in hospitalised patients.[13] The procedure is intended to detect AFB in respiratory secretions swallowed during the night, although the AFB isolated may be environmental non-tuberculous mycobacteria. Thirty millilitres of gastric fluid is aspirated; if no gastric fluid can be obtained a 50 ml lavage with normal saline is performed. The string test is a recently described variation,[14] where the patient swallows a string originally designed to detect *Giardia lamblia* and *Salmonella typhi*. Further research will be needed to validate the string test, but the approach is promising and may be less uncomfortable than lavage.

EXTRAPULMONARY TUBERCULOSIS

Many HIV-infected patients with pulmonary tuberculosis will also have extrapulmonary disease, often in lymph nodes, serous spaces, or the liver, spleen or bone marrow. The diagnostic strategy should initially always focus on obtaining sputum specimens. WHO guidelines for extrapulmonary tuberculosis are very broadly defined,[6] 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 antituberculosis therapy. Biopsies of pleura, lymph nodes, bone marrow and liver can be very useful in diagnosing extrapulmonary tuberculosis, but are invasive and require a degree of operator expertise. Fine-needle aspiration biopsy[15] and wide-needle aspiration biopsy[16] of cervical, axillary and inguinal lymph nodes are useful techniques to obtain specimens for cytology and ZN staining. Importantly, specimens submitted for cytology should be fixed, and those submitted for staining for AFB should be air-dried and not fixed. If these investigations are unhelpful a needle core ('Trucut') biopsy of the node may be diagnostic.[17] Classic cytological/histological features include caseation (sometimes reported as tissue necrosis), granuloma formation, giant cells or epithelioid macrophages, and AFB.

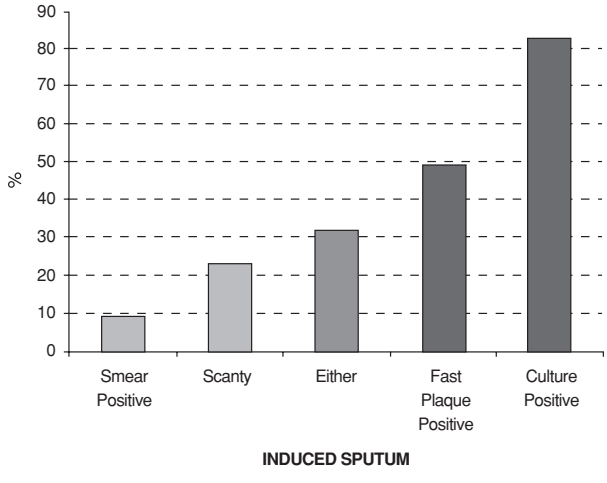
MYCOBACTERIAL CULTURE

In the developed world mycobacterial culture remains the gold standard for diagnosing tuberculosis.[18] However, mycobacterial culture on the Lowenstein-Jensen medium, although inexpensive, is very slow (taking up to 6 weeks) and the culture is vulnerable to contamination. Automated liquid culture media systems (for example the Bactec MGIT system; Becton-Dickinson, Baltimore) may return a positive culture result more rapidly (within 2 - 3 weeks) and be less labour-intensive, but are also prone to contamination, are very expensive, and are usually only available in large urban laboratories.

Importantly, a culture system is essential before multidrug-resistant tuberculosis (MDRTB) can be diagnosed. Performing sensitivity testing on MDR isolates is a lengthy process, and the TB control programme is reluctant to initiate MDR therapy without a sensitivity testing. Given the relatively rapid pace of HIV-associated TB and the paucity of TB culture facilities in the country, many MDRTB suspects may die before cultures are requested or the results become available.

THE FUTURE

Mycobacteriophage assays are promising novel diagnostic techniques[19] where clinical specimens are incubated overnight with a mycobacteria-specific phage (viruses that parasitise mycobacteria). The next morning a virocidal 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 a 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. 1 shows the results of a mycobacteriophage assay (FASTPlaqueTB assay, Ipswitch) on induced sputum specimens from HIV-infected tuberculosis suspects in Cape Town. **Nucleic acid amplification** tests using polymerase chain reaction technology were widely hyped in the late 1990s, but are very expensive and no more sensitive than sputum smear.[20] In the USA these assays are only licensed to differentiate between *M. tuberculosis* and other mycobacteria on smear-positive



Smear pos. vs. FASTPlaqueTB $p < 0.001$
Either vs. FASTPlaqueTB $p = < 0.01$
FASTPlaqueTB vs. culture $p < 0.001$
Difference between proportions test (two tailed)

Fig. 1. Diagnostic yield of a mycobacteriophage assay (FASTPlaque™) in 103 HIV-infected patients with pulmonary tuberculosis (adapted from: Wilson D, Nachega J, Chaisson R, Maartens G. Identifying sputum smear-negative TB in HIV-infected adults using a bacteriophage assay. South African AIDS conference, Durban, 2003).

sputum specimens. Both nucleic amplification tests and mycobacteriophage assays hold promise for rapidly screening isolates for drug resistance.

The interferon-γ assay tests the *in vitro* response of patients' blood mononuclear cells against purified protein derivatives of *M. tuberculosis*. The technique may not be able to distinguish between latent infection and active disease, and may not perform well in patients who are severely immunocompromised from HIV infection. The test holds promise for diagnosing paediatric TB. *Antibody tests* have proved to be disappointing and to date have been unable to reliably distinguish between latent infection and active disease.

Expanded clinical case definitions based on WHO guidelines may be useful in high HIV/TB burden settings. Table V shows the results of a recent study that enrolled HIV-infected adults with tuberculosis symptoms in Cape Town who met one or more case definitions based on the results of clinical examination, chest radiograph, and abdominal and pericardial ultrasound scan. 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 tuberculosis.

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 tuberculosis.[21]

TABLE V. POSITIVE PREDICTIVE VALUE OF CLINICAL CASE DEFINITIONS FOR THE DIAGNOSIS OF PULMONARY AND EXTRAPULMONARY TUBERCULOSIS IN 149 HIV-INFECTED PATIENTS WITH TUBERCULOSIS SYMPTOMS AND NEGATIVE SPUTUM SMEARS

		TB diagnosis		Positive predictive value
Case definition	Number	Confirmed	Response to therapy	
Pulmonary*	83	64	10	89%
Lymphadenitis†	118	102	9	94%
Serositis‡	36	25	9	94%
Constitutional§	11	4	0	36%

*Cough > 21 days; pulmonary opacification or nodular infiltrate on chest radiograph; PCP excluded; no resolution after treatment with a broad-spectrum antibiotic (except for patients with diffuse micronodular (miliary) infiltrate on chest radiograph who should be treated for TB immediately).

†Significant peripheral nodes (long axis ≥ 3 cm) **plus** fever ≥ 38°C OR drenching sweats for > 2 weeks; visceral nodes (mediastinal or abdominal nodes seen on imaging) **plus** fever ≥ 38°C on 2 occasions OR drenching sweats for > 2 weeks.

‡Pleural effusion (lymphocytic exudate); pericardial effusion (effusion on ultrasound, **plus** fever ≥ 38°C on 2 occasions OR drenching sweats for > 2 weeks (aspiration reserved for patients with haemodynamic compromise)); ascites (lymphocytic exudate **plus** fever ≥ 38°C on 2 occasions OR drenching sweats for > 2 weeks).

§Wasting (body mass index of < 18.5 or documented weight loss of ≥ 10% body weight in a month) with fever ≥ 38°C on 2 occasions OR drenching sweats for > 2 weeks.

Adapted from: Wilson D, Morroni C, Nachega J, Chaisson R, Maartens G. Validation of expanded case definitions smear-negative tuberculosis in HIV-infected South African adults (Abstract MoPeB3229). 15th International AIDS Conference, 2004.

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