CHALLENGES OF TB DIAGNOSIS AND TREATMENT IN SOUTH AFRICA

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It is estimated that 2 billion of the world's population are latently infected with *Mycobacterium tuberculosis* (Mtb) with a resultant 8 - 9 million cases of active tuberculosis (TB) and 1.6 million deaths annually.[1] The tools used for diagnosis of TB have remained largely unchanged since the 1880s when sputum microscopy, Mtb culture on solid media, tuberculin skin testing and chest radiology were initially developed. In 1991 the World Health Assembly set targets to be reached in 2005 for 70% case finding of smear-positive TB, which represents 6 million cases to be identified per annum.[2] A second target was that 80% (5 million) of those identified cases should complete anti-TB treatment.[2] Subsequently the millennium development goals of 2000 set a target of halving the prevalence of TB disease from 300/100 000 to 150/100 000 and deaths from 30/100 000 to 15/100 000 by 2015.[3] While progress toward these targets was being made in countries with established market economies there was a quadrupling of TB incidence between 1990 and 2005 in most African countries. In 2005 the World Health Organization Regional Committee for Africa declared TB an emergency for the African region.[4]

In South Africa in 2005 the WHO estimated that of 284 592 TB cases 270 360 were notified to the national TB control programme, representing a somewhat ambitious reported case finding proportion of 95%.[5] The proportion treated under the directly observed treatment (DOTS) programme is 94%, and HIV prevalence among notified cases was 58% (97.5% confidence interval (CI) 49 - 65%). South Africa is a middle-income country and is relatively well provided with 143 laboratories performing sputum smears, and 18 culture laboratories also capable of performing drug sensitivity testing.[5] Multidrug resistance (MDR) in new TB cases varies between provinces from 0.9% to 3.6%, while MDR is higher among retreatment cases, with prevalence rates varying between 1.8% to 13.7% in different provincial surveys.

THE CHALLENGE OF HIV IN TB CONTROL

The HIV epidemic in South Africa has been associated with a similar increase in TB case load as reported in other sub-Saharan countries. The incidence of TB has increased markedly among HIV-infected individuals, with a proportional increase in smear-negative pulmonary disease, extrapulmonary involvement, frequent atypical clinical presentations, an increased mortality and a strong association with multidrug and extreme drug-resistant TB. The impact of HIV on a TB clinic is illustrated by the changes in TB notification data from a Cape Town peri-urban township over the last 10 years as HIV adult seroprevalence has increased from 8% in 1996 to 23% in 2005.[6] During this period TB incidence rates have increased 4.75-fold from 400/100 000 in 1996 to 190 000/100 000 in 2005, with the highest increase occurring in 20 - 40-year-olds.[6] In 2005 the overall TB notification rate was 5.4-fold higher among HIV-positive individuals (5 140/100 000) than HIV-negative individuals (953/100 000) (Table I). Smear-negative disease notification was 8-fold higher among HIV-positive individuals (1 891/100 000) than HIV-negative individuals (238/100 000). Despite a plateauing of HIV seroprevalence, TB notifications have continued to increase.[6]

THE CHALLENGE OF TB IN AN ART PROGRAMME

The diagnosis of TB is particularly challenging in patients accessing antiretroviral therapy (ART) when their HIV infection is advanced. The Hannan Crusaid clinic was the first dedicated public sector ART facility in South Africa and

TABLE I. ADULT PULMONARY TB NOTIFICATION RATES PER 100 000 IN A CAPE TOWN PERI-URBAN TOWNSHIP 2005

TB type	Total adult population	HIV-positive adults	HIV-negative adults	HIV+ve/HIV -ve ratio
(PTB)	1 931	5 140	953	5.4
Smear-positive PTB	1 307	3 248	715	4.5
Smear-negative PTB	624	1 891	238	7.9

PTB = pulmonary tuberculosis.

currently provides treatment to 3 000 patients. Eighty-nine per cent of those accessing ART have symptomatic HIV disease (WHO clinical stage 3 and 4) with a median CD4 cell count of 95 cells/µl. More than 50% have a history of prior completed TB treatment, 15% are on current TB treatment, 11% are diagnosed with previously undiagnosed TB, and a further 10% develop new incident TB after initiation of ART.[7] Multivariate analysis identified risk factors for development of incident TB to be WHO stage 3 and 4 disease (relative risk (RR) 5.9, 95% CI 3.2 - 10.9 and 8.9 95% CI 4.6 - 17.3 respectively), baseline CD4 cell count (RR 1.41, 95% CI 1.2 - 3.1 for each 50 CD4 cell count decline) and baseline viral load (RR 1.4, 95% CI 1.1 - 1.8). A history of completed TB treatment within the previous 2 years was associated with significant protection against incident TB (RR 0.21, 95% CI 0.2 - 0.7).

THE CHALLENGE OF HIV/TB IN A COMMUNITY

The high case finding proportion (close to 100%)[2] reported for the South African TB control programme is based on an estimate of the TB burden. The programme is based on passive case finding together with directly observed therapy of those cases identified. Active case finding enables a direct assessment of TB burden and can identify differing case finding proportions for either HIV-negative or HIV-positive individuals. Active TB case finding and HIV testing of a randomly selected sample of 762 individuals living in Masiphumelele, a peri-urban township outside Cape Town, was performed in 2005 and identified 23% of adults to be seropositive for HIV, 11 individuals with prevalent treated TB and a further 12 individuals with previously unrecognised smear-positive ($N = 6$) and culture-positive ($N = 6$) pulmonary TB.[8] Both HIV infection and a history of recent incarceration were strongly associated with TB. The TB prevalence among HIV-infected individuals was 7.6%, of which 4.4% was smear-positive disease. The case finding proportion for HIV-negative individuals (ratio of prevalence of treated to prevalence of treated and untreated with smear-positive disease) was 67% (95% CI 41 - 100), while that for HIV-positive individuals with smear-positive disease was 37% (95% CI 25 - 53) (Table II). In this community, with a single TB clinic providing care to the whole community, the TB control programme appeared to perform less well for those with HIV infection than for those who were HIV negative.

THE CHALLENGE OF MULTI- AND EXTREMELY DRUG-RESISTANT TB

In 2005 an outbreak of extremely drug-resistant TB (XDR) was recognised in Tugela Ferry, situated in a rural area of northern KwaZulu-Natal. A report of the first 53 cases was published in 2006.[9] The epidemic was recognised in predominantly HIV-positive individuals and was characterised by an extremely high early mortality rate. Over 50% of cases of XDR died within 30 days of presentation. Eighty per cent of the identified cases had positive sputum smears and 25% had evidence of extrapulmonary involvement. Analysis of risk factors for XDR identified that 55% had no history of prior treatment for TB, indicating that these cases had primary rather than acquired resistance. Only 15% had a history of treatment default or failure. The one factor which was of concern was that 67% gave a history of admission to the local health facility, raising the concern that nosocomial transmission may have played a significant role in the epidemic. XDR cases have continued to be recognised, and 266 cases have been confirmed of which 264 were HIV-positive. This epidemic illustrates the potential problems associated with integration of HIV and TB programmes and the increasing need for rapid diagnosis of initial TB infection in both HIV-positive and negative individuals and recognition of early treatment failure and development of resistance within a treatment programme.

TABLE II. PREVALENCE OF TREATED AND UNTREATED SMEAR-POSITIVE PULMONARY TB WITH ACTIVE CASE FINDING AMONG HIV-SEROPOSITIVE AND HIV-SERONEGATIVE INDIVIDUALS

	HIV-positive adults	HIV-negative adults
Prevalence of treated smear-positive PTB	1 563 (1 108 - 2 138)	352 (233 - 507)
Prevalence of treated and untreated smear-positive PTB	4 400 (3 619 - 5 299)	527 (280 - 711)
Case finding proportion	0.37 (0.25 - 0.53)	0.67 (0.41 - 1.0)

PTB = pulmonary tuberculosis.

CURRENT TB DIAGNOSTICS

Current TB diagnostics, TB skin testing (TST), sputum smear and culture and radiology have remained the mainstay of TB diagnostics since 1882. TST has been used to support the diagnosis of TB in populations where TB infection is low. In South Africa a positive TST can be used to support the diagnosis in paediatric TB but is of limited use in adult diagnosis. TST is however still a useful tool for epidemiological studies of the annual risk of infection in children but by adulthood the majority of South Africans have been exposed to TB. In industrialised countries the TST identifies a minority of individuals who are at increased risk of disease progression, to whom diagnostic and preventive resources can be targeted. In contrast, in South Africa the majority of adults have already been exposed to TB and in those with advanced HIV infection, who are at highest risk of disease progression, the test has low sensitivity with up to 50% false negatives.

Sputum smear microscopy has high specificity in high TB prevalence settings. Sputum microscopy has been the mainstay of TB control programmes as it is able to identify the most infectious cases. The test is relatively inexpensive and widely available. Despite its wide availability in the field it is dependent on highly motivated technicians. The overall sensitivity for identifying TB infection is 35 - 70%, but the sensitivity in HIV-infected cases can be as low as 20%.

Diagnosis of TB with a chest X-ray is fast, convenient and has high sensitivity in HIV-negative individuals. In HIV infection the radiological findings of active TB disease decrease with the level of immune suppression, resulting in low sensitivity in advanced disease.[10] The occurrence of opportunistic diseases that can also mimic the radiological changes of TB results in lowered specificity in AIDS cases. The combination of relative expense, restricted availability and low sensitivity and specificity in HIV infection limits the role of radiology for TB control in the high HIV prevalence setting.

Mtb culture is sensitive and specific for pulmonary TB in both HIV-positive and HIV-negative individuals. Solid media culture, however, is limited by the prolonged time required for positive and negative results, which can be delayed for 6 - 8 weeks. Culture of sputum has low sensitivity for extrapulmonary TB, which occurs more commonly in HIV-infection. TB culture also requires a high level of laboratory bio-safety, which consequently restricts its availability to sophisticated centralised reference laboratories.

NEW TECHNOLOGIES

Serological diagnosis of TB is a relatively simple and inexpensive technology which could be a potentially attractive strategy for paediatric and adult extrapulmonary TB diagnosis. A large number of commercially marketed antibody detection tests are available. In 2005 the WHO performed an evaluation of available TB rapid diagnostic antibody tests. Nineteen of 27 invited manufacturers agreed to submit their products for evaluation against a panel of known specimens. The evaluation study found that the performance of antibody tests varied widely with high 'lot to lot' and 'reader to reader' variability. The specificity was less than 80% in the majority of products. Those tests with higher sensitivity lacked specificity and detected fewer than 40% of TB cases. The conclusion of the study was that none of the antibody assays performed well enough to replace microscopy.[11]

Liporabinomannan (LAM) is a component of Mtb cell walls, which is excreted unchanged in urine. A quantitative enzyme-linked immunoassay (ELISA) has been used to demonstrate a correlation between urinary LAM concentrations and the level of Mtb organism load in the sputum of pulmonary TB cases.[12] Urinary LAM concentrations may therefore be a reflection of infecting Mtb organism load, able to diagnose TB in both HIV-positive and HIV-negative individuals. The quantitative thresholds of urinary LAM for diagnosis and sensitivity, and specificity for identifying pulmonary and extra-pulmonary TB, are still to be defined. The LAM ELISA format is suited for peripheral laboratory use; however, a simpler 'tube format' test has also been shown to be robust and does not require a cold chain. A dipstick format of the test is under development, which could possibly prove be the first TB diagnostic suitable for use in 'point of care' clinics.

Cytokine detection assays are based on the observation that lymphocytes with immunological memory produce interferon-gamma (INF-γ) when re-exposed to a specific antigenic challenge. Two commercially available assays have been developed; the QuantiFERON®-TB GOLD, which uses whole blood as its substrate, and the T-SPOT TB assay, which uses isolated peripheral blood mononuclear cells. Both assays use Mtb specific antigens and therefore should not be subject to cross-reactions due to exposure to other environmental mycobacteria or exposure to the *M. bovis* strain used for BCG vaccination. The performance criteria of these tests for identifying latently infected individuals in published studies is in the range of 0.75 - 0.95 for sensitivity and 0.9 - 1.0 for specificity; however, the sensitivity in HIV-infected individuals may be reduced.[13] The advantage of INF-γ assays over TST, of a single visit with a result not subject to observer error, is offset by the requirement of venepuncture, the cost and the need for laboratory infrastructure. Although cytokine assays appear to be more sophisticated and sensitive versions of the TST, these tests may be measuring differing aspects of the immune responses to Mtb infection. INF-γ secretion by cells incubated with mycobacterial antigens over a week may reflect long-term immunological memory, while the shorter 3-day incubation may reflect more recent immunological memory. We have shown that the 3-day INF-γ secretion from PBMCs co-incubated with Mtb antigens is similar in both HIV-negative and HIV-positive controls, but the secretion from cells of those with a history of recent TB treatment was significantly lower than controls (S D Lawn – personal communication).

CULTURE TECHNIQUES

Culture of Mtb remains the gold standard for both diagnosis and drug sensitivity testing. The characteristics of culture and media and growth detection are shown in Fig. 1. Culture in liquid media is faster, with results available as soon as 7 days compared with 42 - 56 days' required growth on solid media. A variety of growth detection methodologies have been utilised. Early detection of growth may be based on mycobacterial metabolism, identification of microscopic

Culture

- More sensitive than direct sputum smear, requires between 5 and 100 organisms/ml v. 5 000 - 10 000 for positive smear
- Allows species identification and concomitant drug sensitivity testing (DST)

Media

- Solid media requires 6 - 8 weeks for confirmed diagnosis and further 4 - 6 weeks for DST
- Liquid culture is faster with results of diagnosis and DST as soon as 7 days

Growth detection methods

- Radioactivity – BACTEC 460-TB®
- Fluorescence – BACTEC MGIT 960®
- Phage-based tests – FASTPlaque TB-RIF™
- Inverted microscopy – MODS

Fig. 1. Characteristics of Mycobacterium tuberculosis *culture.*

colonies or macroscopic plaques on secondary organisms. The BACTEC 460-TB® commercial assay incorporates a radioactive marker in the liquid media which is detected when growth occurs. A more recent development, the BACTEC MGIT 960® assay, utilises a plastic tube containing a broth with a fluorescence quenching-based oxygen sensor. Consumption of oxygen by growth of Mtb produces fluorescence when illuminated by a UV lamp. The non-commercial microscopic optical detection system (MODS) uses an inverted microscope to detect characteristic tangles of developing Mtb colonies in 96 well pates. The FAST-plaque™ assay identifies viable organisms which have been infected with bacteriophages by the development of macroscopically visible plaques on a lawn of fast-growing *M. smegmatis*.[14]

Drug sensitivity testing (DST) conventionally takes 4 - 6 weeks after confirmation of primary detection on solid media, a process which takes 6 - 8 weeks. Liquid culture media can incorporate antibiotics at the time of initial inoculation, with the inference that surviving organisms are phenotypically resistant to the specifically incorporated antibiotic. DST results can therefore be available within the same time frame as mycobacterial diagnosis.

NUCLEIC ACID AMPLIFICATION TESTS

Nucleic acid amplification (NAA) is a rapidly evolving improvement in the detection and identification of Mtb which requires strong laboratory capacity and good quality control procedures and is relatively expensive. Bacterial or ribosomal RNA transcribed into DNA is amplified, followed by an appropriate reading system using a signal generating probe. The inclusion of internal positive controls reduces the incidence of false negatives, and use of a single tube format can reduce potential for contamination. NAA tests can be used for TB diagnosis but cannot be used for evaluation of patients receiving therapy as the technology cannot distinguish between live and dead organisms. The outstanding feature of NAA tests is that a positive result together with a high degree of specificity can be achieved within hours.

NAA tests usually have high specificity but variable sensitivity, so a positive test is good evidence of infection but a negative test is less informative. It is considered that current NAA tests cannot replace microscopy or culture, are unsuitable for smear-negative disease, and should be used only in conjunction with these tests and clinical data.[15,16] Drug resistance can be identified by identifying sequences in the rpoB and katG genes which encode for rifampicin and isoniazid resistance or by hybridisation of this region with specific DNA probes. Recent advances in NAA technology include the ability to amplify directly from clinical samples, isothermal amplification of DNA and improved amplification product detection.

The AMPLICOR® MTB test can give a result within 6 - 7 hours and is a US Federal Drug Administration (FDA) approved test for confirming smear-positive pulmonary TB. LAMP (loop mediated isothermal amplification) is able to amplify TB DNA directly from clinical samples and does not require a thermocycling device, and a positive result, confirmed by a colour reaction visible with the naked eye, can be achieved within 2 hours. With further development a version of the LAMP test may be suitable for use in peripheral laboratories. A commercial assay (Hain Lifesciences) allows a specific Mtb diagnosis together with detection of rifampicin and isoniazid resistance achieved by PCR amplification of the 16S-23S ribosomal DNA spacer region followed by hybridisation of the amplified DNA product with specific oligonucleotide probes. The probes are immobilised as parallel lines on membrane strip; however the test format is suited to reference laboratories rather than lower resourced peripheral laboratories.

DIAGNOSTIC PIPELINE

There is now more interest and financial investment in the development of new TB diagnostics than has occurred over the prior decades (Fig. 2). The development pipeline is active; however, most of the immediate advances in diagnosis and drug sensitivity testing will be applicable only to the reference laboratory.[17] Advances in peripheral laboratory capacity are 2 - 5 years away and are characterised by improved microscopy techniques and development of simplified nucleic acid amplification methodologies. There is little immediate hope of improved 'point of care' TB diagnostics, where the need is greatest. The time frame for new tests in the peripheral clinic is 3 - 7 years and is dependent on formulating simplified antigen and antibody testing such as dipstick tests. The role of newer diagnostics with potential to address the spectrum of clinical scenarios posed by TB and HIV infection are shown in Table III.

TABLE III. TESTS WITH POTENTIAL TO ADDRESS DIFFERENT CLINICAL SCENARIOS

Clinical scenario	Potentially useful tests
TB infection	
HIV negative	TST, INF-γ whole blood assays
HIV positive	TST, INF-γ isolated PBMC assays
TB disease	
Smear-positive	Direct smear, rapid Mtb culture, phage, NAT
Smear-negative	Rapid Mtb culture, urinary LAM
TB treatment failure	Rapid Mtb culture with DST
Drug sensitivity testing	Rapid Mtb culture, phage assay, NAAT, hybridisation assays

CONCLUSIONS

The HIV epidemic has reversed the advances made in global TB control. The increase in smear-negative disease has exposed the inadequacies of existing diagnostics which have remained essentially unchanged since the 1890s. HIV infection has been associated with decreased sensitivity of all present diagnostic modalities of TST, sputum microscopy, TB culture and radiology. Active community case finding indicates that the present diagnostic algorithm used in the national TB

Target timetable	2007	2008	2009	2010	2011	2012
Reference Laboratory  "Faster than culture"	MGIT-960 diagnosis & DST Phage tests for rifampin resistance	Manual molecular DST	Manual NAAT resistance screen	Automated NAAT Real time PCR		
Peripheral Laboratory  "More sensitive than sputum smear"		Fluorescent microsopy	First generation LAMP	Urinary NAAT		
Clinic Based  "As simple as a dipstick"			Urinary antigen dipstick		Dipstick antibody test test	Second generation LAMP

*FIND = Foundation for Innovative New Diagnostics, NAAT = nucleic acid amplification test; DST = drug sensitivity test; LAMP = loop-mediated isothermal amplification.

Fig. 2. FIND timetable for availability of new Mtb diagnostics.*[17]

control programme fails to identify a large proportion of HIV-associated TB. There is therefore an urgent need for point of care tests with increased sensitivity for screening of HIV-positive individuals.

The association of HIV-infection with MDR and XDR has also driven the need for tests which can be used to recognise early treatment failure and rapid identification of drug resistance. The rapid progression of TB in HIV-infected individuals together with increased mortality has also emphasised a need for much faster identification of infection and failure of therapy. The development of rapid liquid culture assays and NAA assays offer significant advances over conventional solid media culture and drug sensitivity testing.

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