Published Online April 30, 2007

Diagnosing tuberculosis in HIV-infected patients: challenges and future prospects

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Introduction: The lack of an effective diagnostic test for smear-negative tuberculosis (TB) is a major contributor to the death of HIV patients in countries with high burden of HIV/*Mycobacterium tuberculosis* co-infection.

The problem: The prolonged incubation time of traditional culture techniques delays time to diagnosis and instigation of effective antituberculous therapy in those who are smear negative. In addition, the increased prevalence of extrapulmonary TB in HIV patients presents a particular diagnostic challenge in resource-poor settings

Discussion: This review highlights the challenges of diagnosing TB in patients with HIV and the recent advances in development of commonly used and novel diagnostic tests for TB.

Keywords: Tuberculosis HIV diagnosis

Introduction

The convergence of HIV and tuberculosis (TB) pandemics in developing countries has been a disaster practically unequalled in medical history. Sub-Saharan Africa bears the brunt of the 8 million annual new cases of active TB worldwide.[1] Thirteen of the 15 countries with the highest incidence rates of TB per capita lie within this region.[1] Moreover, TB is the leading cause of death among HIV-infected persons.[2] Alarmingly, fewer than half of TB cases in HIV-infected patients are diagnosed before death.[3–6]

Accepted: March 9, 2007
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The factors underlying our failure to rapidly diagnose and effectively treat TB are multiple. Patients co-infected with HIV and *Mycobacterium tuberculosis* (*M. tb*) have a greatly increased risk of developing active TB (annual risk 10% compared with a lifetime risk of only 8–10% in those solely infected with *M. tb*) and progress much more rapidly from infection to active disease.[1] This reduces the time available for diagnosis and instigation of treatment. Issues surrounding stigmatization of persons living with HIV lead to reluctance to seek medical assistance,

British Medical Bulletin 2007; **81 and 82**: 149–165
DOI: 10.1093/bmb/ldm009
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and limited health-care provision in many resource-poor countries undoubtedly compounds the access to treatment.

However, perhaps the major factor hampering our ability to diagnose TB in HIV patients is lack of a sensitive, specific, rapid point-of-care diagnostic test. The World Health Organization (WHO) Directly Observed Therapy Short Course (DOTS) programme relies on the microscopic identification of *M. tb* in sputum (smear positivity) to diagnose TB. However, due to alteration of the normal host immune response to *M. tb* in persons with HIV, cavitation and transfer of bacilli into respiratory secretions is markedly reduced. As over 60% of new cases of pulmonary TB in some developing countries are now co-infected with HIV,[1] the WHO approach to TB diagnosis and treatment is failing in a large number of HIV-infected patients. Indeed, smear-negative TB has been linked to poor treatment outcome, including death.[7,8] In addition, as is seen in children, the increased prevalence of extrapulmonary forms of TB in HIV-infected patients is a further challenge to the management of TB in resource-poor settings, where access to histopathology and advanced imaging tests are limited or absent. The lack of a reliable, rapid test for smear-negative TB in adults and children not only means that we often miss the correct diagnosis, but also that patients are often started on antituberculous therapy erroneously on the basis of clinical presentation alone. As many other opportunistic infections found in HIV patients mimic the clinical presentation of TB, this leads to drugs being administered unnecessarily, risking adverse drug reactions, drug interactions and drug resistance.

This paper reviews the current diagnostic tests available for the management of TB in HIV-infected patients and examines the alternative methods that show promise.

Sputum microscopy

Two methods are available for the direct examination of sputum: conventional staining with carbol-fuschin [Ziehl–Neelsen (ZN)] or Kinyoun stain using light microscopy and auramine-based stains (auramine O or auramine–rhodamine) based on fluorescent microscopy. Both methods rely on the retention of stain following the application of acid, resulting from tight binding of the stain to mycolic lipids in the cell wall of the bacillus. The sensitivity of conventional microscopy is highly variable depending on the setting, ranging from 20 to 80%.[9–11] A heterogenous group of studies have compared conventional with fluorescence-based methods for diagnosing pulmonary TB, (excellently reviewed by Steingart *et al.*[12]). Overall,

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fluorescent microscopy was approximately 10% more sensitive than conventional staining methods in studies that used culture as the gold standard diagnostic test. In those that did not use culture, the mean incremental yield (number positive by fluorescence−number positive by conventional stain) was 9%. The advantage of fluorescence over conventional microscopy was maintained in studies from countries with high or low burden of TB. Patients with paucibacillary disease were more likely to be diagnosed by the fluorescence-based method. Specificity was equivalent for both conventional and fluorescent microscopy.

Comparative studies of microscopy method in HIV patients are limited, although two have reported increased sensitivity of fluorescence-based methods.[13,14] In a cross-sectional study of 1398 TB suspects in Nairobi using Löwenstein–Jensen culture as gold standard, the sensitivity of fluorescence microscopy was unaffected by HIV status and was twice that of ZN staining.[13] The specificity of fluorescence microscopy was similarly unaffected. A second study from India, of 200 patients diagnosed with TB by clinical and radiographic means in an area of high HIV prevalence, showed an incremental yield of 26% for fluorescence microscopy over ZN staining for those co-infected with HIV.[14] Hence, although the number of studies is limited, fluorescence-based staining techniques show promise in increasing diagnostic yield over conventional staining in HIV-infected individuals. The added advantage of this technique is the reduced time taken by laboratory technicians to view the sample (1 min compared with 4 min for conventional methods).[15] However, the requirement of electricity for fluorescence microscopy and concerns regarding the stability of reagents for this technique under field conditions bring the practicality of its use into question, in more rural settings.

Various methods of concentrating sputum based on centrifugation have been shown to increase diagnostic yield when used prior to microscopy (see review by Steingart *et al.*[16]). Whether the increase in sensitivity holds for HIV patients remains to be defined, particularly in those who are smear negative.

Does induction of sputum with nebulized saline increase diagnostic yield?

A number of studies have investigated whether sputum induction by inhalation of nebulized hypertonic saline prior to expectoration improves diagnostic yield. In infants and children, examination of gastric lavage specimens has long been the preferred method of sampling sputum, as

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this age group tends to swallow expectorated sputum. Two studies from sub-Saharan Africa have challenged gastric lavage as the test of choice.[17,18] Zar *et al.*[17] compared sputum induction with gastric lavage in 254 TB suspects admitted to a tertiary paediatric referral centre. Sputum induction could be used successfully in children as young as 1 month of age and a single specimen of induced sputum gave an equivalent diagnostic yield to three consecutive gastric lavage samples.[17] HIV status of the child did not influence the rate of smear or culture positivity. Similarly, a cross-sectional study of children aged 2 months to 5 years from a population with high prevalence of HIV in Uganda yielded a 12% smear-positive and 30% culture-positive rate using induced sputum from TB suspects.[18] There was no difference found in the results of induced sputum cultures between HIV-infected and uninfected children.

Adult studies confirm the usefulness of sputum induction in the diagnosis of TB. In high-income countries, a single specimen of induced sputum is comparable in diagnostic yield with bronchoalveolar lavage specimen from fiberoptic bronchoscopy.[19,20] The yield is further increased with repeat specimens. Moreover, in resource-poor settings, sputum induction has also been shown to be of use in the diagnosis of extrapulmonary forms of TB.[21] In a study of 84 patients with pleural TB, culture of induced sputum compared favourably (52% sensitivity) with that of pleural biopsy culture (62%). Furthermore, sputum induction provided bacteriological confirmation of diagnosis in 55% of patients with normal lung parenchyma on chest X-ray (CXR). Yield from sputum induction was again independent of HIV status. Most interestingly, in this study, 12% of all patients with pleural TB were smear positive following sputum induction, challenging the perceived wisdom that patients with pleural TB are non-infectious. In a study of smear-negative TB in HIV patients in South Africa, 72% of cultures from sputum induction in 110 patients were positive for *M. tb*, including 56% of cases that did not have pulmonary infiltrates on CXR.[22]

Importantly, sputum induction is well tolerated by patients. Bronchospasm is limited by the routine use of nebulized bronchodilator prior to saline inhalation and sputum induction can be performed as an outpatient procedure. However, due to the high risk of nosocomial transmission of *M. tb* to health-care workers and other patients, induced sputum should only be performed under strict infection control settings, in a room with adequate local exhaust ventilation. A proper protective respiratory mask must be worn by health-care workers to reduce nosocomial transmission. Summarizing the available evidence, sputum induction is a useful tool for increasing the diagnostic yield in patients with smear-negative TB.

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Rapid mycobacterial culture

Traditional solid-phase culture techniques such as Löwenstein–Jensen culture remain the gold standard diagnostic test for TB in most resource-poor countries. The development of early, manual broth-based culture systems such as BACTEC 460 (BBL; Becton Dickenson Microbiology Systems) which measured growth radiometrically have now been largely superseded by newer, fully automated non-radiometric systems. They include systems based on fluorescence such as BACTEC 9000 (Becton Dickinson) and MGIT (Mycobacterial Growth Indicator Tube, Becton Dickinson), those that use a colorimetric CO_2 sensor such as MB/BacT (BioMérieux) and the ESPII system (TREK Diagnostic Systems, Inc) that measures the pressure changes in vial head-space. The BACTEC 9000 system has the added advantage of using a compatible medium for mycobacterial blood culture (MYCO/F lytic medium), which is of particular use in detecting dissemination of mycobacteria in blood of HIV patients, who have an increased rate of bacteraemia.[23] A comprehensive review of the numerous studies comparing various culture techniques, has been published.[24] For most systems, culture becomes positive within 21 days, although those from smear-positive sputum are detectable earlier, within approximately 14 days. Although time to diagnosis may be shortened using automated liquid systems, most studies highlight the incremental benefit of using a combination of liquid and solid-phase culture to increase the overall culture positivity,[25] as is recommended by the Centers for Disease Control.[26]

A novel, simplified colorimetric culture method has recently been developed based on TK Medium™ (Salubris, Inc., MA, USA). Metabolic activity of dividing mycobacteria is detected by a change in the colour of the medium from red to yellow, whereas growth of contaminating fungi or bacteria is indicated by a change from red to green (Figure 1). Early studies indicate that time to culture positivity is around 14 days,[27,28] with sensitivity equivalent to Löwenstein–Jensen medium. Larger studies are now required to validate these results and answer the critical question of how this test will perform in smear-negative HIV patients which will be a key indicator to its usefulness in high HIV-prevalent populations.

Recent studies from Peru have brought the implementation of a cheap, rapid liquid culture-based detection system for *M. tb* a step closer.[29] The microscopic-observation drug-susceptibility (MODS) assay relies on light microscopy to visualize the characteristic cording morphology of *M. tb* in culture (Figure 2). Decontaminated sputum specimens are incubated with Middlebrook 7H9 broth, nutrients and

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Fig. 1 TK medium rapid culture system for tuberculosis. Mycobacterial growth changes the colour of the medium from red to yellow. Common contaminants, such as fungi or Gram-negative bacteria, change the colour from red to green. Image courtesy of T. Kocagoz, Salubris, Inc. (MA, USA) and reproduced with permission.

an antibiotic cocktail to prevent bacterial and fungal overgrowth, in a standard 24-well culture plate.[30] Cultures are sealed in a ziplock bag to prevent contamination and studied daily for cording using an inverted light microscope in a standard laboratory setting. In one study of three cohorts in Lima, Peru: unselected TB suspects, TB suspects at high risk for TB or multi-drug resistant TB (MDR-TB) and unselected hospitalized HIV patients, MODS was more sensitive and as specific as automated (MB/BacT system) and solid-phase (Löwenstein–Jensen) culture.[29] In addition, the time to culture positivity for MODS [median 7 days (IQR 6–8)] was less than automated [13 days (IQR 10–16)] or solid-phase culture [26 days (IQR 21–33)]. Rapid determination of drug susceptibility of *M. tb* cultures can also be assayed by addition of antimycobacterial drugs to other wells of the culture plate. Median times from initial sample processing to results of drug-susceptibility testing were markedly reduced for MODS (7 days) compared with automated (22 days) and Löwenstein–Jensen (68 days) culture.[29]

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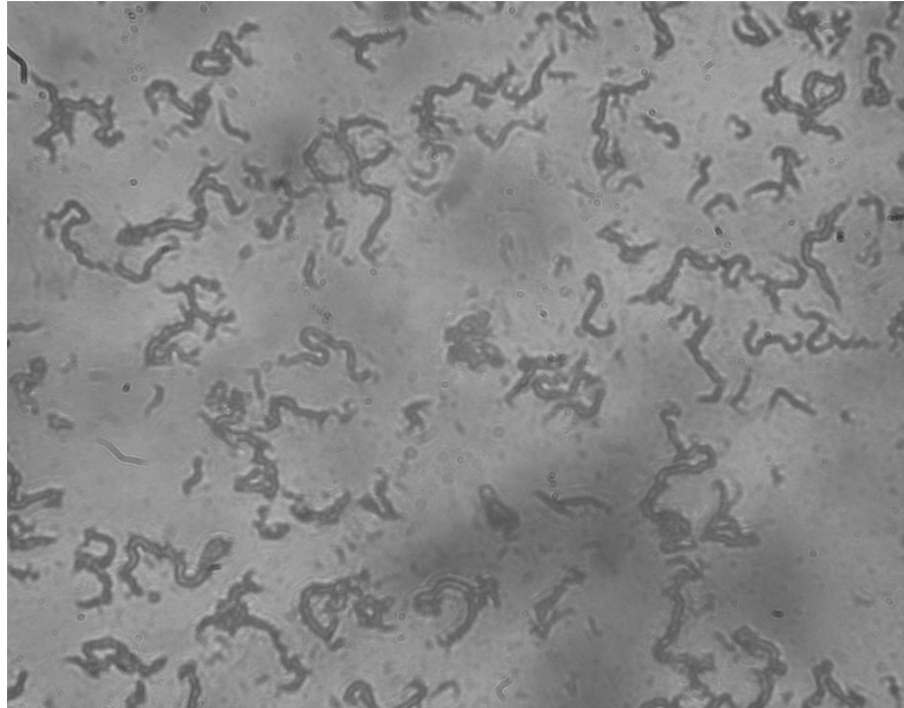

Fig. 2 MODS. Characteristic cording morphology of *Mycobacterium tuberculosis* seen at ×20 magnification by inverted light microscopy. Image courtesy of David AJ Moore and Luz Caviedes, and reproduced with permission.

The cost effectiveness of MODS (∼$2 per sample) compared favourably with Löwenstein–Jensen culture ($6 per sample) and MB/BacT culture ($52 per sample).[29] Hence, MODS assay represents a major potential advance in rapid culture-based diagnosis of TB, especially in resource-poor countries with basic laboratory facilities. However, further studies are needed to address the performance of MODS in smear-negative TB cases, as the Peruvian study was performed in predominantly smear-positive disease (only 21% of treated cases in Peru are smear negative).

Mycobacteriophage-based diagnostic assay

Phage-based systems for detection of *M. tb* such as FASTPlaqueTB (Biotech, Ipswich, Suffolk, UK) utilize a mycobacterial phage to infect *M. tb* in sputum samples that have first been decontaminated and concentrated.[31] A potent virucide is then added to eliminate any remaining free phage and the mixture is plated with a suspension of *M. smegmatis* in molten agar. The phage replicates within *M. tb* inducing lysis of the

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bacilli. Free phage then infects the surrounding *M. smegmatis*. Subsequent lysis of *M. smegmatis* by replicating phage results in plaques in the agar, visible as small clear circles to the naked eye. Prospective trials have investigated the use of FASTPlaqueTB comparing it with microscopy and solid-phase culture in developing countries.[32–34] Although when combined with microscopy FASTPlaqueTB is able to correctly diagnose >80% of specimens that are culture-positive, high false-positive rates have been reported. The phage is not *M. tb*-specific; hence it can infect any mycobacteria present in sputum yielding a false-positive result. This would have a particular effect on specificity of phage-based tests in areas with low TB prevalence, where the contribution of non-tuberculous mycobacteria in sputum will be greater. High false-positive rates in smear-negative samples have been reported (8% from Karachi[33] and 19% from Cape Town[32]). False-negative results have also been identified as a problem in bacteriophage assays. For the test to be positive, viable *M. tb* must be present. Hence, any factor that reduces *M. tb* viability such as antituberculous treatment and delay in processing samples will increase false-negative rates and reduce sensitivity. False-negative rates of ∼13% were found in two studies in culture-confirmed samples,[32,33] and in 21% from a further study in Pakistan.[34] The use of FASTPlaqueTB in conjunction with initial liquid culture to screen for rifampicin resistance (as a marker for MDR-TB) has also been proposed.[35]

In summary, although the specificity of phage-based tests in non-HIV infected patients shows promise, the sensitivity of the test varies markedly and concerns regarding performance in paucibacillary disease remain. Studies to date have not adequately defined the numbers of HIV-infected individuals involved, making sub-analysis of the data unfeasible. Further studies of how phage-based assays perform in smear-negative TB and HIV populations are therefore required.

Nucleic acid amplification tests

Nucleic acid amplification tests detect targeted regions of the *M. tb* genome by amplifying specific regions of mycobacterial DNA. A number of meta-analyses have recently been published studying the performance of commercially available and in-house tests for diagnosis of smear-positive and smear-negative TB (see review by Greco *et al.*[36]). It is evident from these studies that the main use of nucleic acid amplification tests is restricted to confirming the presence of *M. tb* in smear-positive sputum samples where pre-test probability of TB is high. This would be predominantly in developed countries with low TB burden.

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However, current nucleic acid amplification tests exhibit poor sensitivity in those with smear-negative TB.[37] Furthermore, wide variation in reported performance of nucleic acid amplification tests suggests that they may also be highly operator dependent. Such tests are also unable to distinguish live from dead mycobacteria. The development of newer technologies such as loop-mediated isothermal amplification (LAMP, Eiken Chemical Co., Ltd, Tokyo, Japan) with higher efficiency and fast assay times are advancing methodology of such assays.[38] The use of isothermal amplification negates the need for expensive thermocyclers which are less affordable in resource-poor settings. Although, LAMP remains untested in the field in terms of sensitivity and specificity, one study has found the performance of LAMP to be comparable with that of the Amplicor assay (Roche Molecular Diagnostics, CA, USA) in the laboratory.[39]

The role of tuberculin skin testing and interferon-gamma release assays in rapid diagnosis of active TB

At over 100 years old, the tuberculin skin test (TST) represents the second longest standing test in use for TB after sputum microscopy. The TST measures the delayed type hypersensitivity response to a purified mix of mycobacterial antigens, purified protein derivative (PPD). PPD comprises antigens that are found not only in *M. tb*, but also in *M. bovis*-BCG and other mycobacteria. As such, the response to TST lacks specificity in defining both latent TB infection (LTBI) and active disease (see review by Pai *et al.*[40]). In addition, the TST is compromised in HIV infection, where immunosuppression commonly leads to anergy, thereby reducing sensitivity. However, the TST remains a useful determinant of which patients should receive isoniazid prophylaxis to reduce progression of LTBI to active disease (60% reduction in progression to active TB in people who are TST positive).[41]

A specific region of difference in DNA sequence (RD1) exists between *M. tb* and *M. bovis*-BCG, resulting in production of early secretory antigen target 6 (ESAT-6) and culture filtrate protein-10 (CFP-10) by *M. tb*, but not *M. bovis*-BCG. This difference forms the basis of a number of commercially available immunodiagnostic tests for LTBI, measuring interferon-gamma (IFN-gamma) release from T cells stimulated with ESAT-6 and/or CFP-10. Because ESAT-6 and CFP-10 are absent from *M. bovis*-BCG, immunodiagnostic tests perform with greater specificity than the TST in defining LTBI.[40]

Fewer studies have addressed the use of immunodiagnostic tests to accurately diagnose active TB. In a recent analysis of published

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studies, the overall sensitivity of one commercially available test, QuantiFERON-TB gold (Cellestis Ltd, Carnegie, Victoria, Australia) which tests for IFN-gamma release by T cells in whole blood, ranged from 70 to 90%, equivalent to TST.[42] Specificity was higher than that for the TST. The vast majority of studies cited were either in patients with unknown HIV status or in cohorts that were predominantly HIV negative. Two studies have investigated IFN-gamma release in HIV patients.[43,44] Chapman *et al.*[43] studied 50 Zambian patients with clinical and radiological evidence of active TB with at least one positive sputum smear, 39 of whom were HIV positive. T cells in blood of 35/39 HIV positive patients responded to one or more RD1 peptides in an *ex vivo* ELISPOT assay, which involves *in vitro* separation of peripheral blood mononuclear cells from whole blood, prior to stimulation with peptides. Total lymphocyte counts (a surrogate marker for CD4 count) showed no significant difference between the 35 HIV patients who responded and the four HIV patients who did not. This suggests that lack of a positive result was not due to more advanced immunosuppression, although the number of patients in these studies was small. A second study based in Uganda investigated blood cytokine responses to stimulation with PPD or *M. tb* culture filtrate.[44] Reduced IFN-gamma responses were found in HIV-infected healthy recruits compared with those who were HIV uninfected. Differences in test methodology including the use of non-species-specific antigens in the Ugandan study and the type of subjects evaluated are likely to underlie the contrasting results of these two studies.

The advantages of immunodiagnostic tests for TB are that they can be performed on a single visit compared with TST which requires two and that specificity is increased. The main disadvantage of immunodiagnostics in populations with high rates of TB is their inability to distinguish between LTBI and active infection. Additionally, many questions remain concerning the performance of such tests in HIV patients with immunosuppression. However, a recent study by Rangaka *et al.*[45] of 160 adults attending for voluntary counselling and testing in Khayelitsha township in Cape Town suggests that IFN-gamma release assays may be less affected by immunosuppression associated with HIV than is the TST. The study found that that, although the proportion of TST was lower in HIV-infected people than those uninfected, there was no significant difference in the proportion of positive IFN-gamma release assays using either Quantiferon-TB Gold or the T-SPOT.TB assay (based on stimulation of PBMC, rather than whole blood). Larger prospective studies are now required to further define the use of these tests in HIV-infected people with varying degrees of immunosuppression.

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Antigen capture

The immunodiagnostic tests outlined above assay the cell-mediated immune response to *M. tb*. In contrast, numerous tests that assay antibodies to *M. tb*, have been developed over many years and are available on the market. These assays lack sensitivity with highly variable results. Operator and batch variability of tests are important limitations to their use. Moreover, specificity is generally poor, often $<80\%$, and in those assays with higher specificity, sensitivity is particularly poor, with deteriorating performance in HIV coinfection (see review by Laal and Skeiky).[46]

Antigen, rather than antibody-based detection assays are now being developed and show some promise as a future test. One ELISA-based assay detecting lipoarabinomannan (LAM) in unprocessed urine has been developed (Chemogen, USA) and trialled in 231 Tanzanian TB suspects.[47] LAM is a cell wall lipopolyscaccharide specific for the *Mycobacterium* genus, but not to *M. tb* itself. Hence, concerns have been expressed regarding specificity of such an assay. In the Tanzanian study, sensitivity of 80.3% was superior to AFB-stain in culture-positive TB suspects and was unaffected by HIV co-infection. Interestingly, of the smear-negative, culture-positive patients, 76.5% were positive for urinary LAM, suggesting a role for the test in smear-negative disease. Specificity was 99% as judged by 1/103 healthy controls being positive for urinary LAM. Larger studies are now required to validate urinary LAM as a diagnostic test. A dipstick version of the assay is under development.

Expanded clinical case definitions as a tool for diagnosing smear-negative TB

One method of reducing the time to treatment for smear-negative TB cases is to circumvent the use of laboratory-based tests and to use clinical case definitions instead. WHO have recently updated the guidelines for management of smear-negative and extrapulmonary TB.[48] Previous guidelines published in 2003 were largely impractical in resource-poor settings with high HIV burden.[49] The criteria relied heavily on CXR findings that had to be compatible with active TB, although it is well appreciated that such CXRs may appear normal or non-specifically abnormal. Furthermore, guidelines for instigation of therapy in suspected extrapulmonary TB were vague, relying heavily on the individual clinician's expertise. The current guidelines provide algorithms for management of smear-negative pulmonary and extrapulmonary TB in

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HIV-prevalent communities. They emphasize the need for HIV testing of all TB suspects rather than only confirmed TB cases, reduce the number of sputum specimens for collection from three to two and define smear-positive disease as a single sputum smear-identifying acid fast bacilli on microscopy.[48] Furthermore, the diagnostic algorithms used for management of extrapulmonary TB are much clearer, providing more detailed advice on which tests to employ and improving the definitions of common extrapulmonary forms of the disease to aid the clinician.

Wilson *et al.*[22] have studied the use of expanded case definitions based on the 2003 WHO and national guidelines, as a tool for diagnosis of smear-negative TB in HIV patients. The study was set in a busy secondary-level hospital clinic in Cape Town, South Africa, with high HIV prevalence and TB incidence. One hundred and forty-seven HIV-positive patients with smear-negative TB were assessed by means of symptom review, CXR and cultures, which were performed on induced sputum, blood and urine. Where appropriate, cultures were also sent from other sites. Patients with a normal CXR underwent abdominal and pericardial ultrasonography. Overall, expanded case definitions performed well, with high positive predictive values for correctly diagnosing smear negative TB (Table 1). The exception was patients diagnosed in the category of constitutional syndrome, who have previously been identified as having a poor prognosis.[4] Criteria that defined a clinically relevant response to treatment were also characterized: weight gain $\geq$5%, haemoglobin increase $\geq$1 g/dl, reduction in CRP, increase in Karnofsky performance score $\geq$20 ($\geq$10 if baseline score was 80 or 90) and symptom count ratio (number of symptoms better or resolved at 8 weeks follow-up/total number of symptoms at baseline $\geq$0.5). Of the patients presenting at week 8 of follow-up with more than one of these response criteria, 97.5% had confirmed TB. Stopping therapy in those patients who do not have a response to treatment at 8 weeks could reduce the programme costs and limit treatment with potentially harmful drugs, in those who are highly unlikely to have TB.

Further studies are now needed to extend the analysis of sensitivity and specificity of expanded definitions in a broader group of TB suspects and in a setting of lower HIV-TB prevalence. At this stage, the use of expanded case definitions marks a major potential advance in the management of smear-negative TB in HIV patients, especially in rural settings and resource-poor communities. However, if clinical case definitions are ever to be used effectively alongside the current WHO programme in the developing world, a major shift in consciousness of staff employed in the clinics and managers of national programmes needs to occur, away from the purely smear-positive-based algorithms

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Table 1 Smear-negative tuberculosis case definitions

	Positive predictive value of case definition (%)*
Pulmonary	92
Cough for >21 days +Pulmonary opacification or nodular infiltrate on CXR +*Pneumocystis jirovecil* pneumonia excluded (using CDC clinical case definition)[†] +No resolution after treatment with broad spectrum antibiotics (except in the case of patients with diffuse micronodular [miliary] infiltrate on CXR, who are started on antituberculosis treatment after cultures are sent.	
Lymphadenopathy	94
Peripheral	
Significant asymmetrical peripheral nodes (long axix ≥ 3 cm) +fever > 38°C on two occasions OR drenching sweats for ≥ 2 weeks	
Visceral	94–96
Visceral nodes (mediastinal/hilar or abdorminal nodes on imaging) + fever ≥38°C on two occasions OR drenching sweats for ≥ 2 weeks	
Serositis	
Pleural effusion	85
Lymphocytic infiltrates	
Pericardial effusion	90
Effusion on ultrasound + fever ≥ 38°C on two occasions OR drenching sweats for ≥2 weeks (aspirate reserved for patients with haemodynamic compromise)	
Ascites	100
Lymphocytic exudate + fever ≥ 38°C on two occasions OR drenching sweats for ≥2 weeks	
Constitutional syndrome	36
Wasting (BMI < 18.5) OR documented weight loss >5% body weight within a month + fever > 38°C on two occasions OR drenching sweats for ≥2 weeks	

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*For confirmed (culture positive or histological features) and possible (objective response to treatment) tuberculosis.
[†]Bilateral interstitial infiltrate, exertional dyspnoea (onset <3 months), hypoxia or desaturation 5% or more on effort.
CDC, Centers for Disease Control and Prevention; BMI, body mass index.

for diagnosis of TB currently in effect, allowing for a wider definition of what constitutes a positive diagnosis of TB.

The use of high throughput assays to define biomarkers for future diagnostic tests

New approaches to the development of TB diagnostics remains a priority if our goals of developing a more sensitive, specific and affordable test that can be applied at the point of source is to be met. One such approach that we are investigating is to employ high throughput

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screening of gene and protein signatures in blood or body fluids, using DNA microarray and proteomics to define host or microbe biomarkers of active TB. Such an approach has recently led to a novel diagnostic test for African trypanosomiasis.[50] The concept that various pathogens can induce differential regulation of host immune response genes is supported by *in vitro* studies of gene expression following infection of blood cells.[51,52] Although common patterns of gene expression were induced, pathogen-specific gene regulation was also a feature of the response.[51,52] On this background, we are currently undertaking a study of gene and protein signatures in blood of carefully characterized cohorts of adults and children with TB, in the setting of high HIV and tuberculosis prevalence to define host biomarkers of active TB in HIV patients.

Conclusion

In summary, current diagnostic tests for TB are failing to help stem the tide of deaths from smear-negative disease in patients with HIV. The use of expanded clinical case definitions for smear negative TB, although requiring further evaluation in the field, show great promise in resource-poor settings. Likewise, the MODS assay represents a major potential advance in reducing delay to diagnosis and determination of drug resistance in developing countries. The limited number of studies of many new diagnostic modalities in smear-negative TB patients needs to be rapidly and rigorously addressed, as does the search for novel diagnostic targets on which to base a rapid, sensitive, point-of-care test.

Acknowledgements

The author would like to thank Dr Mark Nicol and Dr Robert Wilkinson for providing critical comments.

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