A Review of the Diagnosis and Treatment of
Smear-Negative Pulmonary Tuberculosis

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Summary

Recommendations on the management of smear-negative pulmonary tuberculosis (TB) are still based on the behaviour of this disease in populations unaffected by the human immunodeficiency virus (HIV). Studies prior to the HIV epidemic estimated that there were 1.22 cases of smear-negative and extra-pulmonary TB for each smear-positive case. Patients with smear-negative pulmonary TB were found to be less infectious and to have a lower mortality but a significant proportion (ie. 50-71%) progressed to active disease justifying treatment. Moreover, a wide variety of regimens also proved effective in the treatment of smear-negative disease in HIV-negative patients.

The advent of HIV has changed many of these parameters. Countries affected by both HIV and TB have experienced a disproportionate increase in smear-negative disease. While apparently remaining less infectious than smear-positive cases, HIV-positive patients with smear-negative pulmonary TB are generally more immunocompromised, have more adverse drug reactions, and suffer higher mortality rates on treatment. Clinical decision making has also been complicated because HIV co-infection broadens the differential diagnoses of smear-negative pulmonary TB to include diseases such as Pneumocystis carinii pneumonia (PCP), pulmonary Kaposi’s sarcoma, and Gram-negative bacteraemia.
Our approach to smear-negative pulmonary TB must therefore adapt to these changed parameters. Management algorithms based on several features (eg. clinical symptoms, response to antibiotic trials, smear investigations, and chest radiography) have been developed to improve case detection. These algorithms must be validated in each locale because their performance will vary depending on numerous local factors such as the regional prevalence of PCP. Alternative methods of specimen collection (eg. sputum induction) and processing must be evaluated. National tuberculosis programmes should also consider extending the use of rifampicin-based short-course chemotherapy (SCC) to new patients with smear-negative disease. This latter intervention, and the much-needed establishment of additional microscopy and culture facilities, will depend on increased financial and technical support from the international community.
Introduction

The detection and management of smear-positive pulmonary disease are quite rightly the principal aims of national and international tuberculosis (TB) control programs.\(^1\) However, smear-negative disease (ie. patients with clinical and radiological evidence of pulmonary TB but repeatedly negative sputum investigations) is a common clinical problem,\(^2\) particularly in countries affected by the dual TB/human immunodeficiency virus (HIV) epidemics.\(^3,4\) Despite the high and increasing frequency of smear-negative pulmonary TB, a search of the medical literature has found only two editorials and two reviews on this topic in the last 20 years.\(^4-7\) This article will describe the incidence, natural history and differential diagnoses of smear-negative pulmonary TB in HIV-negative and HIV-positive patients. The various strategies that have attempted to address smear-negative TB will then be reviewed highlighting plausible interventions for developing countries and areas for future research.

Incidence and natural history in HIV-negative patients

Our understanding of smear-negative pulmonary TB is based on experience from the pre-HIV era (Table 1). Murray et al extrapolated from data collected in the United States to estimate that there are 1.22 cases of smear-negative and extra-pulmonary TB for every case of smear-positive TB in developing countries.\(^2\) In HIV-negative populations, smear-negative pulmonary TB is more common among children and the elderly.\(^2,6,8,9\) For example, less than 10% of cases aged 0-14 years in the US (1985-1987) and Norway (1951-1972) were smear-positive.\(^2\) The low rate of smear-positive disease among
children may be explained by the fact that children generally have primary
disease without extensive cavitation. Difficulties in collecting adequate
specimens from children and misdiagnosis of other paediatric illnesses as TB
may also be contributing factors.

About 5,000-10,000 acid-fast bacilli (AFB) per millilitre of sputum must be
present for detection by smear whereas culture requires only 10-100 viable
organisms.¹⁰ Hence, smear-negative culture-positive patients generally have
minimal disease with low bacillary counts rather than far-advanced cavitary
TB with heavy bacillary burdens (Table 1).⁵,⁶,¹¹ For example, a US study of
HIV-negative culture-positive patients estimated that negative smears are
obtained from 60%-80% of patients with minimal disease, from 30%-40% of
cases with more extensive disease, but from only 5%-10% of patients with
extensive cavitary lesions.¹¹

The association of AFB-negative smears with lower bacillary burdens and
minimal pulmonary lesions would imply that the infectivity and the mortality
of smear-negative disease should be lower, and that less-intensive
chemotherapy may adequately treat this condition (Table 1). Rouillon et al
reviewed five studies comparing the bacteriological status of the source case
with the prevalence of infection (as measured by tuberculin reactivity) among
household contacts aged less than 15 years.¹⁰ The prevalence of infection
among children exposed to smear-positive cases varied between 39%-65%
whereas the tuberculin reactivity rate was only 4.7%-26.8% among contacts
of smear-negative patients, whose TB diagnoses were based on positive cultures or radiography. However, in studies in high incidence environments in India and Africa, the infection rate among young contacts of smear-negative cases was similar to that in the general population or in households without documented TB.\textsuperscript{10} Nonetheless, a recent DNA-fingerprinting study from San Francisco attributed 17\% of tuberculosis transmission in this low-prevalence setting to patients with smear-negative culture-positive pulmonary TB.\textsuperscript{12}

The supposition that mortality is lower among HIV-negative smear-negative cases has also been verified. Longitudinal surveys conducted between 1961 and 1963 among untreated TB patients in Bangalore District, South India, found that the mortality rate during the first 18 months of follow-up was 34.7\% for smear-positive patients, 14.1\% for smear-negative culture-positive cases, and about 5.0\% for smear-negative culture-negative cases diagnosed radiologically.\textsuperscript{5,13}

The natural progression of smear-negative disease was studied in a chemotherapeutic trial in Hong Kong.\textsuperscript{14} Table 2 describes the outcome after 30-months follow-up for a sub-group of 283 smear-negative patients who were randomly assigned to receive chemotherapy only when active disease was confirmed by culture, radiography, or clinical deterioration. At least 71\% of these patients initially had or developed active disease requiring treatment. Importantly, nearly half of the smear-negative cases who required treatment
developed active disease within the first three months. Follow-up of untreated smear-negative patients must therefore concentrate on this early period after initial presentation. Surveys in Bangalore also followed the progression of smear-negative patients to active disease and found similar results to the Hong Kong study. Over 50% of 457 patients with radiographic suspicion of TB but one negative smear progressed to active disease within 12 months (cited in reference 5).

Finally, smear-negative pulmonary TB in HIV-negative patients can be successfully treated with a wide variety of regimens.\textsuperscript{5,15} For example, a study in Hong Kong found that treatment with streptomycin, isoniazid, rifampicin, and pyrazinamide for four months cured all 293 patients with smear-negative pulmonary TB and had a relapse rate of only 2% after five years. Longer, cheaper, less-intensive therapies are also effective. The World Health Organisation (WHO) therefore recommends one regimen (ie. 2 EHRZ/4 HR) for Category I smear-positive patients and a different treatment (eg. 2 HRZ/6 HE) for Category III smear-negative cases who are not severely ill and do not have extensive parenchymal involvement.\textsuperscript{1} African studies from the pre-HIV era have justified the treatment of smear-negative cases with even cheaper less-aggressive 12-month regimens (eg. 2 STH/10 TH, 2 EHZ/10 HT).\textsuperscript{4,16}

**The impact of the HIV epidemic**

Since the advent of HIV, the annual incidence of TB has more than doubled in some African countries,\textsuperscript{3,4,17,18} and there has been a disproportionate increase
in the reported rate of smear-negative disease (Table 1).\textsuperscript{19-21} For example, the number of notified TB cases in Blantyre, Malawi, tripled from just over 400 cases in 1986 to more than 1200 in 1991; this increase was almost entirely attributable to an ‘additional’ 800 smear-negative cases in 1991 compared with 1986.\textsuperscript{20} This apparent predominance of smear-negative disease may be partly due to (i) heavy workloads increasing the likelihood of false-negative laboratory errors, and (ii) misdiagnosis of other HIV-related pulmonary conditions as smear-negative TB, but several studies have found that smear-negative disease is actually more common among HIV-positive patients.\textsuperscript{20,22-26} For example, Elliot et al found that 43\% of 72 HIV-positive patients with culture-proven pulmonary TB in Lusaka, Zambia, were smear-negative compared with 24\% of 37 HIV-negative cases (p=0.003).\textsuperscript{24}

Other researchers have not detected a difference in smear positivity rates between HIV-positive and HIV-negative cases.\textsuperscript{27-30} The apparent discrepancies between these studies may be due to differences in the study populations. Some studies were conducted among patients seen at specialist centres who may be more or less likely to be smear-positive depending on the referral procedure. The level of immunosuppression among the HIV-positive patients in the various studies may also have differed. Less severely immunocompromised HIV-positive patients tend to have classic cavitary TB which is smear-positive.\textsuperscript{3,19,25} As the level of immunocompromise increases with advancing HIV disease, atypical pulmonary features predominate and smear examinations prove less sensitive (Table 1).
Overall, population trends and most clinical-based studies do suggest that HIV-positive patients have a higher rate of smear-negative disease.\textsuperscript{20,22-26} Furthermore, while having no apparent affect on the infectiousness of smear-negative pulmonary TB,\textsuperscript{31,32} HIV infection does seem to alter some other features of this disease (Table 1). For example, adverse drug reactions are more common among HIV-positive patients.\textsuperscript{33-35} These side effects include life-threatening cutaneous reactions to thioacetazone,\textsuperscript{33} which is commonly used in ‘standard’ chemotherapy regimens for smear-negative pulmonary TB.

In developing countries, treatment outcomes in the presence of HIV infection have been studied almost exclusively in patients with smear-positive TB. These studies have found that HIV-positive TB patients who survive and complete treatment have a similar response to therapy as HIV-negative populations.\textsuperscript{36-38} However, mortality while on treatment is consistently higher in the HIV-positive group. This excess mortality is largely attributed to diseases other than TB (eg. gram-negative bacteraemia).\textsuperscript{3,4,36,37} Furthermore, a recent study from Malawi has confirmed the clinical impression that HIV-positive patients with smear-negative pulmonary disease (which is an indicator of advanced immunosuppression) do even worse than HIV-positive patients with smear-positive disease.\textsuperscript{39} The mortality rate was 3.9 times greater among smear-negative patients (who received ‘standard’ chemotherapy with 1 STH/11TH) than in smear-positive patients (who received more intensive treatment with 2 SHRZ/6 HT); the prevalence of HIV
in this TB patient population was 77%. ‘Standard’ chemotherapy regimens containing streptomycin, isoniazid and thioacetazone have also been associated with higher relapse rates among HIV-positive patients.38

In summary, the HIV epidemic has been associated with an increased incidence of smear-negative pulmonary TB, more adverse drug reactions, higher mortality rates on treatment, and perhaps higher relapse rates on ‘standard’ chemotherapy. The clinical management of patients suspected of having smear-negative pulmonary TB has also been complicated because the differential diagnoses of this condition are broadened by HIV co-infection.

**Differential diagnoses of smear-negative disease**

Microscopic examination of sputum smears for AFB forms the basis of TB diagnosis in developing countries.1 AFB microscopy is rapid, simple and cheap.2,40-43 Murray et al estimated that a single smear examination in Tanzania costs less than US$ 0.25.2 Unfortunately, AFB microscopy lacks sensitivity compared with culture. In patients with culture-confirmed pulmonary TB, the sensitivity of AFB microscopy ranges from 22 to 80%.5,11

Table 3 outlines the factors that may produce false-negative smear results in patients with pulmonary tuberculosis. In under-resourced over-worked TB control programs, laboratories cannot cope with the influx of diagnostic and follow-up smear examinations. Smears may not be done at all! For example,
in Botswana in 1992, 48% of patients reported with pulmonary tuberculosis had not had any smears performed.\textsuperscript{44}

Alternatively, the sputum specimens collected may be inadequate in quality or number. Similar incremental yields from serial smear examinations have been reported from industrialised and developing countries. Ipuge et al found that 83.4\% of smear-positive cases were detected on the first specimen, 12.2\% on the second, and 4.4\% on the third, by Ziehl-Neelsen (Z-N) staining under routine program conditions in Tanzania.\textsuperscript{53} Using an auramine-rhodamine stain in a diagnostic laboratory in the US, Nelson et al reported that 73\% of smear-positive cases were found on the first specimen, 14\% on the second, 7\% on the third, and 6\% on the fourth or later.\textsuperscript{54} At least two good-quality specimens must therefore be examined to reliably detect smear-positive pulmonary TB.

Finally, the performance of the smears may be technically inadequate. Declining quality of smear examination is a particular problem in overburdened laboratories in HIV-endemic countries. For example, as part of an epidemiological study of TB and HIV in Tanzania, Chum et al compared the sputum microscopy results obtained in local and reference laboratories.\textsuperscript{55} Twenty-nine per cent of new smear-negative cases (on the basis of local microscopy) were found to be smear-positive by the reference laboratory. False-negative results can be due to inadequate staining, under- or over-decolourisation, or inspection of too few fields (ie. a minimum of 100 fields of
a Z-N smear must be examined before reporting a negative result and this examination takes about 5-10 minutes).\textsuperscript{56,57} Finally, overly thick smears can obscure the presence of AFB or may fall off the slide.\textsuperscript{41-43}

Table 3 also describes the other medical conditions that may be misdiagnosed as smear-negative pulmonary TB, and highlights the differential diagnoses of increased importance in HIV-positive patients. Bacterial pneumonia is the main differential diagnosis in HIV-positive and HIV-negative individuals while \textit{Pneumocystis carinii} pneumonia (PCP), cryptococcosis, and nocardiosis are of increased importance in HIV-positive subjects.\textsuperscript{30,45-52} The reported rates of PCP in African HIV-positive patients with respiratory symptoms vary between 0-33\%.\textsuperscript{30,45-48,50} This variation has not been fully explained but has been attributed to differences in patient selection, the level of immunodeficiency of HIV-positive patients in Africa, the limited availability of specialised laboratory diagnostics, the failure to diagnose PCP in the presence of multiple other infections, and geographic differences in the prevalence of PCP.\textsuperscript{48,50}

HIV-associated nocardiosis may also be under-diagnosed. Lucas et al conducted an autopsy study of 247 HIV-positive cadavers in Abidjan, Ivory Coast, and found one case of nocardiosis for each nine TB cases.\textsuperscript{49} While nocardiosis is less common than TB, a simple Gram stain of AFB-negative sputa may prove a worthwhile investigation. Surprisingly, a recent study of HIV-positive patients in a respiratory medicine unit in Abidjan reported that 9\% had a Gram-negative bacteraemia (usually due to non-typhoid
salmonellae). Kamanfu et al found a similar percentage (ie. 10.4%) of HIV-positive patients hospitalised with acute respiratory disease in Burundi had Salmonella bacteraemia, usually with S. typhimurium. Blood culture facilities are required to make this diagnosis but are rarely available. These two studies highlight that Gram-negative bacteraemia must be considered in patients presenting with pulmonary disease but negative sputum smears, particularly if they are HIV-positive.

These medical conditions account for significant morbidity and mortality in patients presenting with ‘smear-negative pulmonary disease’ in HIV- and TB-endemic developing countries (Table 3). However, the pre-eminent position of TB as the major pathogen in these circumstances must be emphasised. The lowest rate of TB reported in the studies cited in table 3 was still 22.5% (in a study that selected smear-negative patients for bronchoscopy) and the highest rate was 64%.

**Addressing the problem of smear-negative pulmonary TB**

**Improving selection of patients with TB**

Various management algorithms have been proposed to optimise the number of patients correctly treated for smear-negative tuberculosis while minimising over-treatment of patients who do not have the disease. Samb et al investigated which symptoms could be effectively included in these diagnostic algorithms. They studied 182 smear-negative patients with respiratory disease (41 with culture-confirmed TB and 141 with non-TB disease) in
Burundi and Tanzania; 71% of the patients were HIV-positive. Four symptoms were associated with TB: cough >21 days, chest pain >15 days, absence of expectoration, and absence of dyspnoea. Presence of any two of these symptoms diagnosed TB with 85% sensitivity but only 67% specificity. If at least three of these symptoms were required for a diagnosis of TB, the specificity improved to 86% but the sensitivity fell to 49%. Presence of lymphadenopathy and haematocrit <30% aided discrimination.

An additional indicator of smear-negative pulmonary TB could be failure to respond to a trial of antibiotics. Wilkinson et al studied 237 South African patients with suspected TB, including 56 smear-negative culture-positive cases. Smear-negative patients were given a course of ampicillin (500mg qid for 7-10 days). A final diagnosis of TB was correctly made in 28 (50%) of the smear-negative culture-positive cases (ie. the sensitivity of Z-N smear alone was 61% and rose to 80% when combined with a failed clinical response to an antibiotic trial). However, the remaining 28 culture-positive patients appeared to respond to antibiotic therapy, either because of unrelated fluctuations in disease severity or successful treatment of a superimposed bacterial infection, and were incorrectly discharged. Furthermore, 32 culture-negative patients failed to respond to antibiotics and were misdiagnosed with TB (ie. specificity of Z-N alone was 94% but fell to 78% when combined with the outcome of an antibiotic trial).
Failure of non-TB patients to respond to a trial of antibiotics may be due to infection with a resistant organism. Unfortunately, little data is available on the prevalence of antibiotic resistance in developing countries. For example, the serotypes and resistance profiles of 5,000 invasive isolates of *Streptococcus pneumoniae* from the United States were published in 1996 but similar information was available for <500 strains from the entire African continent.\(^6\)\(^1\) Reported levels of antibiotic resistance in developing countries have also varied widely due to study design and the *in vitro* susceptibility tests employed.\(^6\)\(^2\) Nonetheless, the few published African studies have failed to detect penicillin-resistant *S. pneumoniae* (ie. minimum inhibitory concentration $\geq 2.0 \, \mu g/mL$) that would adversely affect successful treatment of pneumococcal pneumonia with penicillin but have found pneumococci with reduced susceptibility (ie. MIC 0.1-2.0 $\mu g/mL$).\(^6\)\(^1\)-\(^6\)\(^3\)

**Improving selection of patients with other conditions**

Failure of non-TB patients to respond to a trial of antibiotics could also be due to the presence of a disease other than bacterial pneumonia. In some countries, PCP, nocardiosis, *Salmonella* bacteraemia, and pulmonary Kaposi’s sarcoma are important differential diagnoses in HIV-positive patients presenting with respiratory disease (Table 3). Various clinical predictors of PCP, bacterial pneumonia, and TB have been proposed in HIV-positive patients.\(^4\),\(^5\)\(^0\),\(^6\)\(^4\) Selwyn et al studied 229 cases of pulmonary disease in HIV-positive patients in the US.\(^6\)\(^4\) The independent predictors were exertional dyspnoea, an interstitial infiltrate, and the presence of oral thrush for PCP; a
‘toxic’ appearance, a lobar infiltrate, and a fever ≤ 7 days for bacterial pneumonia; and cavitary infiltrate, fever > 7 days, and weight loss for TB. In Zimbabwe, Malin et al found similar clinical indicators for PCP: respiratory rate > 40/min, fine reticulonodular shadowing on the chest radiograph, and severe hypoxia.50

Unfortunately, many features of these pulmonary syndromes overlap hence syndromic diagnoses lack both sensitivity and specificity. For example, Selwyn et al found that 81% of PCP patients complained of exertional dyspnoea but so did 33% of TB and 43% of pneumonia patients.64 The specificity of these syndromic diagnoses was improved by combining indicators but with an invariable reduction in sensitivity (eg. a combination of exertional dyspnoea and an interstitial infiltrate diagnosed PCP with 92% specificity but the sensitivity fell to 58%).64 Multiple pathologies can also co-exist in HIV-positive patients further complicating the clinical diagnosis of pulmonary syndromes. For example, Malin et al found that 6 of 21 HIV-positive Zimbabwean patients with PCP also had TB.50

In an attempt to further differentiate patients with TB from those with other pulmonary diseases, Harries et al have recently recommended that smear-negative TB suspects receive a second trial of antibiotics.58 Ideally, this second drug should be unrelated to the β-lactams (eg. ampicillin) which are generally used in the initial antibiotic trial. Cotrimoxazole is a cheap, widely-available antibiotic that could be reasonably used in this second treatment
trial. Cotrimoxazole is a recognised treatment for pneumonia,\textsuperscript{62,63} and has activity against \textit{Salmonellae, Pneumocystis carinii}, and nocardia.\textsuperscript{65} Unfortunately, high-dose cotrimoxazole (ie. trimethoprim 15-20 mg/kg/day-sulfamethoxazole 75-100 mg/kg/day) is required for effective treatment of PCP and nocardiosis, and side effects, such as rash and neutropenia, are not uncommon.\textsuperscript{65} High-dose cotrimoxazole would therefore be problematic as the ‘second antibiotic trial’ in a diagnostic algorithm and could only be justified by documenting significant rates of PCP and nocardiosis in a locale.

Chest radiography has also been included in algorithms for selecting patients with smear-negative pulmonary tuberculosis. However, CXR changes may be atypical or may be due to other infections, particularly in HIV-positive patients.\textsuperscript{5} Harries et al have also shown that using CXR, rather than AFB microscopy, as the initial screen in a diagnostic algorithm is less sensitive and more expensive.\textsuperscript{66} Hence, collection of 2-3 sputum specimens for microscopy should precede CXR in any diagnostic algorithm. In developing countries, tuberculin skin testing (TST) is confounded by the high coverage of BCG vaccination, asymptomatic TB infection, the presence of nontuberculous mycobacteria, and anergy due to HIV or malnutrition.\textsuperscript{67} TST therefore has no place in diagnostic algorithms in these settings.

Figure 1 outlines an algorithm for managing TB suspects which is based on several studies.\textsuperscript{1,6,30,58-60,66,68} The sensitivity and specificity of this algorithm will vary depending on the acumen of the local clinicians, and the prevalence
of HIV, of antibiotic-resistant *S. pneumoniae* and *H. influenzae* that may cause the treatment trials to fail, and of the diseases that can mimic smear-negative pulmonary tuberculosis (Table 3). Such algorithms must therefore be validated and optimised in each country.

While most steps in this algorithm are broadly accepted, controversy surrounds the correct management of smear-negative patients who have failed two courses of antibiotics. The physician has several choices: (i) to make a definitive diagnosis of smear-negative TB and to treat accordingly, (ii) to consider other diagnoses, or (iii) to observe and re-investigate for TB over the next three months. Some authors have also suggested instituting a two-month ‘therapeutic trial’ of antituberculosis treatment. However, inappropriate implementation of ‘therapeutic trials’ for TB could lead to treatment failures, acquired drug resistance, and subsequent difficulties in correctly categorising patients into treatment and re-treatment programs. These ‘therapeutic trials’ may also lack specificity because patients with other infections may respond to the broad antibacterial effect of rifampicin-containing regimens. Definitive antituberculosis treatment is therefore recommended for patients who complete the management algorithm (Figure 1) and are still suspected of having TB.

**Improving specimen collection**

Two or three high-quality sputum specimens are required to reliably detect smear-positive pulmonary TB. Occasionally patients cannot produce
adequate specimens. Simple chest physiotherapy may be worthwhile. Pithie and Chicksen have shown that Z-N staining of a fine-needle aspirate from a palpable extra-thoracic lymph node is another worthwhile investigation for HIV-positive patients with suspected smear-negative TB in developing countries.

In industrialised countries, bronchoscopy would be the preferred investigation for such patients. Rao has demonstrated the usefulness of bronchoscopy among smear-negative patients in India. Of 55 sputum smear-negative patients, 15 (27.3%) had AFB-positive bronchial washings (cultures were not performed); bronchial carcinoma was diagnosed in another 5 patients. However, bronchoscopes are expensive and require on-going disinfection and maintenance. Bronchoscopy is also an invasive procedure with recognised complications. In fact, Daley et al evaluated the role of bronchoscopy among 237 patients in Tanzania, and found that, if an algorithm such as figure 1 was used, only 17 (7%) patients required bronchoscopy and a treatable disease was found in only three. They concluded that bronchoscopy had little place in the investigation of smear-negative TB patients in resource-poor areas.

Sputum induction with nebulised hypertonic saline is a realistic alternative investigation in such settings. The technique has been used widely in industrialised countries for diagnosing PCP in patients with AIDS. However, it was originally used in the 1960s to obtain adequate sputum samples for cytological examination and TB investigations. Anderson et al found that
sputum induction performed as well as bronchoscopy in the diagnosis of 101 smear-negative Canadian patients.\textsuperscript{71} Smear microscopy on bronchoscopic and induced sputum specimens had sensitivities of only 12% and 19%, respectively, but culture of the bronchoscopic and induced sputum specimens had similar improved sensitivities (ie. 73% and 77%, respectively).

Culture is generally not available in resource-poor countries. Nonetheless, Parry et al have demonstrated the usefulness of sputum induction in Malawi.\textsuperscript{20} Among 82 patients who could not produce sputum or were smear-negative, induced sputum was smear-positive in 18 (22%); positive cultures were obtained from these 18 induced sputum specimens and from an additional 12 smear-negative samples. A nurse or physiotherapist could perform the procedure on 7-8 patients in one morning. To prevent the spread of TB to other patients or staff, the procedure was done in a well-ventilated room and contaminated equipment was washed then soaked in glutaraldehyde. In fact, infection control measures are the problematic factor in using sputum induction in developing countries. For example, Parry et al could not establish this technique as a routine procedure after their study because of an unreliable supply of glutaraldehyde for decontamination. Nonetheless, sputum induction appears the best procedure for obtaining better specimens from smear-negative patients in resource-poor countries.
Improving laboratory diagnosis

Before the HIV epidemic, two well-performed sputum examinations were considered as sensitive as a single culture.\(^5\) With the increasing rate of smear-negative disease and the rising prevalence of drug resistance, culture facilities may need to be more widely available in developing countries and new convenient TB diagnostics are urgently required.\(^73,74\)

In the meantime, can sputum microscopy be improved? The carbol fuchsin staining procedures (ie. Z-N and Kinyoun stains) generally used in developing countries are less sensitive than fluorochrome-stained smears, which take only 1-2 minutes to read.\(^41-43,73\) The cost-effectiveness of fluorescence microscopy largely depends on local labour costs but may become worthwhile when a laboratory processes more than 30 smears per day.\(^43\) The major disadvantages of fluorescence microscopy are the need for a reliable electricity supply, the extra capital outlay (ie. a fluorescence microscope is 4-5 times as expensive as a light microscope), and the additional maintenance (eg. halogen lamps must be replaced after 200 hours of use).\(^41-43,73\)

Standard light microscopy may be improved by homogenisation and concentration of the sputum specimen. Various methods have been described (eg. N-acetyl-L-cysteine plus 2% NaOH, 4% NaOH, dithiothreitol plus 2% NaOH) and are generally followed by concentration by centrifugation.\(^40-43\). The efficiency of these techniques depends on the toxicity of the digestant-decontaminant solution, heat build-up in the centrifuge, and the centrifugal
force applied. This final factor is of most importance. The mycobacterial cell wall has a high lipid content and is therefore naturally buoyant. Hence, the specific gravity of the suspending fluid must be minimised and the relative centrifugal force (RCF, measured in g) maximised to optimise the recovery of AFB. Adequate sedimentation efficiency (95%) can be achieved by centrifugation at 3,000 x g for 15 minutes. Unfortunately, centrifuges in many laboratories, particularly in developing countries, cannot attain this RCF. Acceptable recovery rates (ie. >70%) are only attainable at lower RFC if the centrifugation time is prolonged (eg. 2,000 x g for 20 minutes).

Gebre et al have reported using household bleach (sodium hypochlorite, NaOCl) to liquefy sputa and then centrifugation to concentrate the mycobacteria. Initial homogenisation with NaOCl produced better recovery of AFB than did digestion with either NaOH or dithiothreitol, and this improved efficiency was seen following centrifugation at different RCFs. In practical terms, the sensitivity of AFB microscopy increased from 30.8% for a direct smear to 69.2% using the NaOCl technique. Miörner et al simplified the technique by showing that AFB could be concentrated as effectively by overnight sedimentation as by centrifugation. Household bleach is readily available and is mycobactericidal thereby reducing the risk of laboratory-acquired infections. Microscopy examinations are easier because liquefaction with NaOCl removes background debris and a higher density of AFB per microscope field is obtained by concentration. The main disadvantage of these techniques is the additional processing time.
Unfortunately, these techniques have not been widely applied in the field.\textsuperscript{73} In the only published evaluation, Wilkinson and Sturm found that NaOCl liquefaction and subsequent centrifugation did not increase the overall diagnostic sensitivity of smear microscopy.\textsuperscript{78} While investigating 166 consecutive TB suspects in Hlabisa Hospital, South Africa, an extra 12 smear-positive specimens were detected after processing. However, 13 specimens that were positive by direct smear were negative after processing, presumably because AFB were not effectively pelleted during centrifugation.

Of the diseases that mimic smear-negative pulmonary TB (Table 3), only nocardiosis could be detected by a simple investigation. In direct sputum smears, nocardia appear as gram-positive beaded branching filaments and are acid-fast using a modified Z-N stain.\textsuperscript{79} Gram and modified Z-N stains of sputum may therefore be worthwhile examinations if nocardiosis is prevalent in a particular locale, such as in Abidjan, Ivory Coast.\textsuperscript{49}

\textbf{Improving treatment}

The recommended management of smear-negative pulmonary TB is still based on the characteristics of this disease in HIV-negative populations (Table 1).\textsuperscript{1,15,16} Developing countries are encouraged to concentrate their limited resources on the effective treatment of smear-positive cases, who are more infectious and who were considered to have a higher mortality. There is also a fear that inclusion of 'smear-negative' cases in TB programs may lead to
over-treatment of patients who do not have TB. Treatment of smear-negative disease is therefore restricted in many developing countries, such as Rwanda and the DRC,\textsuperscript{80,81} or prolonged inferior regimens (eg. 2 STH/10 TH, 2 EHZ/10 HT) are used.\textsuperscript{82,83}

Can inferior regimens continue to be recommended for smear-negative TB in the face of the HIV/TB dual epidemic? Firstly, local diagnostic facilities have been so over-burdened that smear microscopy may be either unavailable or unreliable.\textsuperscript{44,55} Remember our earlier example that nearly one-third of patients in Tanzania defined as smear-negative based on local microscopy were found to be smear-positive by a reference laboratory.\textsuperscript{55} Classification of patients as smear-positive and –negative (and, more importantly, prescription of treatment based on this differentiation) becomes almost arbitrary in these circumstances.

Secondly, the HIV epidemic has changed some basic characteristics of smear-negative pulmonary TB (Table 1). HIV-positive patients with smear-negative TB may actually have a higher mortality rate than smear-positive cases.\textsuperscript{39} ‘Standard’ 12-month regimens containing thioacetazone have also been associated with higher rates of adverse reactions and with higher relapse rates in HIV-positive patients.\textsuperscript{33-35}

In countries affected by the dual HIV/TB epidemic, De Cock and Wilkinson have therefore suggested that short-course chemotherapy (SCC) based on
rifampicin (with DOT supervision) be recommended for all new cases of TB irrespective of smear status. Harries et al have made a similar suggestion. Short-course regimens have been associated with improved survival in HIV-positive TB patients. For example, in a Ugandan study of 191 HIV-positive patients with smear-positive TB receiving standard (2 STH/10 TH) or short-course (2 HRZ/7 HR) chemotherapy, the relative risk of death for standard compared with short-course chemotherapy was 1.57 (95% CI 1.0-2.48). This improved survival has been partly attributed to the prevention/treatment of other intercurrent infections by the broad antibacterial activity of rifampicin. The survival advantage of SCC has not been studied specifically in smear-negative HIV-positive patients but they should benefit equally from the broad activity of rifampicin.

A single SCC regimen for all new TB cases would also avoid confusion, simplify guidelines, increase completion and cure rates, and reduce non-adherence and side effects. Fears of over-treating non-TB patients should be assuaged if validated management algorithms (eg. figure 1) are followed. In the end, cost is the only prohibitive factor to universal SCC for new TB cases. In 1995, Chaulet calculated that the WHO Category I regimen for smear-positive cases (eg. 2 EHRZ/4 HR) cost US$ 32.34 while the Category III regimen (ie. 2 HRZ/6 HE) for smear-negative patients cost US$ 21.40. A similar US$ 10-15 price differential existed between various Category I and III regimens that contained streptomycin, ethambutol or thiocetazone. This additional cost is significant but some savings could come from the
streamlined approach of a universal regimen, from bulk purchasing of a single regimen, and from the reduced number of patients requiring re-treatment or developing resistance. The additional cost of using SCC for all new cases must be weighed against the definite benefits, and the international community must facilitate the introduction of a universal SCC regimen for new TB cases if it proves cost-effective.

Conclusion

Smear-negative pulmonary TB is an increasing clinical problem in developing countries affected by the dual HIV/TB epidemic. Management algorithms that have been validated by local studies should improve case detection. Wider use of sputum induction and evaluation of novel sputum processing techniques may also improve the investigation of these patients. Some authors have argued for the wider availability of TB culture facilities in developing countries, and for universal access to SCC for all TB patients irrespective of smear status. However, these Utopian interventions will require increased financial and technical support from the international community.
Acknowledgements

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References


07/11/08


07/11/08 34


Table 1  Characteristics of smear-negative pulmonary tuberculosis in HIV-negative and HIV-positive populations

**Classic features of smear-negative pulmonary tuberculosis**

- 1.22 cases of SNP and EP TB for each SPP TB case
- Low bacillary burden with minimal disease and no cavitation
- Less infectious than smear-positive cases
- Lower mortality than smear-positive cases
- Effectively treated with a variety of regimens

**Effect of HIV on smear-negative pulmonary tuberculosis**

- Disproportionate increase in incidence of SNP TB
- SNP TB becomes a marker of advanced immunosuppression
- Infectivity of smear-negative cases probably unchanged
- HIV-positive patients with SNP TB have higher mortality rates
- ‘Standard’ chemotherapy associated with more side effects, increased mortality rates, and higher relapse rates

SNP TB, smear-negative pulmonary tuberculosis; EP, extra-pulmonary; SPP, smear-positive pulmonary; ‘standard’ chemotherapy denotes 12-month regimens employing streptomycin, isoniazid, and thiacetazone
Table 2  Outcome at 30 months for 283 Hong Kong patients initially diagnosed with pulmonary tuberculosis despite five negative smears*

<table>
<thead>
<tr>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required treatment</td>
</tr>
<tr>
<td>Initial specimens proved culture positive</td>
</tr>
<tr>
<td>Subsequent progress demanded treatment †</td>
</tr>
<tr>
<td>Evidence of active disease not treated‡</td>
</tr>
<tr>
<td>No evidence of active disease</td>
</tr>
</tbody>
</table>

* Adapted from reference 14
† Developed active disease requiring treatment based on bacteriological, radiological or clinical findings
‡ Had one or more isolated positive cultures and/or radiological evidence of active disease
### Table 3  Important differential diagnoses of smear-negative pulmonary tuberculosis in developing countries

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Frequency*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>False-negative smear results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No smear examination performed</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Technical problems</td>
<td></td>
<td>41-43</td>
</tr>
<tr>
<td>Inadequate specimen quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate number of specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor staining technique</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overly thick smears</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate number of specimens</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other medical conditions**

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Frequency*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial pneumonia</td>
<td>14.0%-41.2%</td>
<td>30, 45-47</td>
</tr>
<tr>
<td>Empyema</td>
<td>2%</td>
<td>30</td>
</tr>
<tr>
<td>Pulmonary nocardiosis‡</td>
<td>0%-4%</td>
<td>46-51</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> pneumonia‡</td>
<td>1%-33%</td>
<td>30, 45-48, 50</td>
</tr>
<tr>
<td>Cryptococcal pneumonia‡</td>
<td>0%-13%</td>
<td>30, 45-48, 50</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>3%</td>
<td>47</td>
</tr>
<tr>
<td>Pulmonary Kaposi’s sarcoma‡</td>
<td>1.3%-9.3%</td>
<td>30, 45-48, 50</td>
</tr>
<tr>
<td>Interstitial pneumonitis</td>
<td>38%</td>
<td>48, 51</td>
</tr>
<tr>
<td>Cytomegalovirus pneumonitis</td>
<td>1.5%</td>
<td>50</td>
</tr>
<tr>
<td>Gram-negative bacteraemia‡</td>
<td>9%-10.4%</td>
<td>46, 52</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1%</td>
<td>52</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
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<tr>
<td>Congestive cardiac failure</td>
<td></td>
<td></td>
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<tr>
<td>Asthma, chronic obstructive lung disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic bronchopulmonary aspergillosis</td>
<td></td>
<td></td>
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<tr>
<td>Occupational lung diseases (e.g. silicosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrinsic allergic alveolitis</td>
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<td></td>
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<tr>
<td>Psittacosis</td>
<td></td>
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</tr>
</tbody>
</table>

07/11/08 41
Legend to Table 3

* Bronchoalveolar lavage and autopsy studies have been performed in developing countries to determine the cause of respiratory disease in HIV-positive and HIV-negative patients. The prevalences of non-tuberculous disease shown are from a compilation of these studies and may be affected by geographic differences in the rates of some diseases, such as cryptococcosis,\textsuperscript{45,48} by patient selection, by the range of laboratory investigations performed, and by the local prevalence of HIV.

\textsuperscript{‡} Conditions of increased importance in HIV-positive patients
Initial screen based on symptoms:
eg. cough > 3 weeks; loss of weight;
no response to ampicillin 500 mg qid x 7-10 days

↓

Microscopy of 2-3 sputum specimens

AFB + ↓

Registration and Treatment for Pulmonary TB

↓ AFB -

CXR and Clinical Consultation

*additional investigations

Second Antibiotic Trial (eg. cotrimoxazole) and Physician Review

↓ if no response

Antituberculosis Treatment to be considered by Physician†

Figure 1

Algorithm for managing TB suspects in developing countries. Adapted from references 1, 6, 30, 58-60, 66, and 68. * Additional investigations may include lymph-node aspiration and sputum induction. 24,69,71 † See text for discussion of the management of smear-negative patients who fail to respond to the second course of antibiotics. AFB, acid-fast bacilli.