Evaluation of a diagnostic algorithm for smear-negative pulmonary tuberculosis in HIV-infected adults

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Objectives. To evaluate the diagnostic accuracy of and reduction in diagnostic delay attributable to a clinical algorithm used for the diagnosis of smear-negative pulmonary tuberculosis (SNPTB) in HIV-infected adults.

Design. An algorithm was designed to facilitate clinico-radiological diagnosis of pulmonary TB (PTB) in HIV-infected smear-negative adult patients. A folder review was performed on the first 58 cases referred for empirical TB treatment using this algorithm.

Setting. Nolungile HIV Clinic, Site C, Khayelitsha.

Subjects. Subjects included 58 HIV-infected adult patients with suspected PTB consecutively referred to the local TB clinic for outpatient TB treatment using this algorithm between 12 February 2004 and 30 April 2005.

Outcome measures. Outcome measures were response of C-reactive protein, haemoglobin, weight and symptoms to TB treatment, and TB culture result. Diagnostic delay (in days) was calculated.

Results. Thirty-two of the 58 patients (55%) had positive TB cultures (definite TB). Initiation of TB treatment occurred on average 19.5 days before the positive culture report. A further 21 patients (36%) demonstrated clinical improvement on empirical treatment (probable/possible TB). Two patients did not improve and subsequently died without a definitive diagnosis. Three patients defaulted treatment.

Conclusions. SNPTB is more common in HIV-infected patients and leads to diagnostic delay. This algorithm allowed for earlier initiation of TB treatment in HIV-infected patients presenting with symptoms of PTB and negative smears or non-productive cough in a high TB incidence setting.

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Tuberculosis (TB) is the commonest cause of morbidity and mortality in HIV-infected people in sub-Saharan Africa.[1] The mortality associated with TB is considerably higher in HIV-infected than HIV-negative patients.[2] Furthermore, TB is a more rapidly progressive disease in HIV-infected people.[3] This means that any delay in the diagnosis of active TB in HIV co-infected patients is more likely to result in clinical deterioration and greater morbidity and mortality.[4] The diagnostic window of opportunity is therefore narrower. Active TB has been found at autopsy in around 50% of AIDS-related deaths in Africa,[5,6] and in almost half the diagnosis was not made ante-mortem.[5]

This situation is complicated by the fact that TB is more difficult to diagnose in HIV-infected patients. HIV modifies the clinical presentation of TB, particularly in those with more advanced immunosuppression. HIV-infected patients are twice as likely to have sputum smear-negative, culture-positive pulmonary TB (PTB).[7-9] This results from their compromised immune response causing less cavity formation.[10] The sputum smear has traditionally been used as the method for making an early diagnosis of PTB. The reduction in sensitivity of this test in HIV-infected patients leads to diagnostic delay. Sputum culture is a more sensitive method of diagnosing PTB in such cases, but can take up to 8 weeks before a result is available. The patient's condition invariably deteriorates during this interval. Other factors contributing to diagnostic delay are that patients with HIV-associated PTB present more commonly with atypical or normal chest radiographs[11,12] and extrapulmonary tuberculosis (EPTB) is more common in the context of HIV infection.[13]

This diagnostic delay also results in increased hospitalisation and increased costs to the health system. It has also been proposed that delay in the initiation of TB treatment may accelerate HIV infection.[14]

It is imperative that efforts be made to expedite the diagnosis of TB in HIV-infected people. While new diagnostic techniques are being developed and methods such as sputum induction have been shown to increase the yield of the sputum smear,[15] these are unavailable in most primary care settings in South Africa. It is often necessary to make a clinico-radiological diagnosis of smear-negative TB (SNTB) using an algorithm and to initiate empirical TB treatment while awaiting culture results. The sensitivity and specificity of clinical algorithms

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used for the diagnosis of TB will vary depending on where they are implemented and will be influenced by local factors such as HIV prevalence, prevalence of conditions that mimic TB and the acumen of local clinicians. It is therefore necessary to validate an algorithm in a specific locality before widespread use.[16]

This study aimed to validate one such algorithm in a primary care setting at the Nolungile HIV Clinic in Site C, Khayelitsha, which is a township on the outskirts of Cape Town with a population of approximately 400 000 people and a TB incidence rate of 1 612/100 000 in 2005 (Virginia Azevedo, Khayelitsha District Manager, City of Cape Town – personal communication).

Methods

A smear-negative algorithm (Fig. 1) that could be used to initiate TB treatment empirically in HIV-infected adults with symptoms of PTB but negative sputum smears or non-productive cough, was designed by doctors working in Khayelitsha in 2004. This algorithm was based on the World Health Organization (WHO) criteria[17] and a study undertaken previously in Cape Town[18] to validate case definitions for SNTB. The WHO diagnostic criteria for SNTB at the time were: at least 3 negative sputum smears, radiographic abnormalities consistent with active PTB, no response to a broad-spectrum antibiotic, and the decision by a clinician to treat with a full course of antituberculosis treatment.[17] Patients were referred by an HIV clinician to the local TB clinic using this algorithmic approach from February 2004.

All referred patients were clinically unwell and deteriorating but well enough to be treated (at least initially) as outpatients. Those with suspected TB who were too sick to be treated as outpatients were referred to a secondary hospital for admission and were not included in this analysis.

In order to increase specificity for the diagnosis of active TB and not overload the TB service, the following criteria had to be met before referral for empirical TB treatment: (*i*) cough with night sweats and/or weight loss for more than 2 weeks;

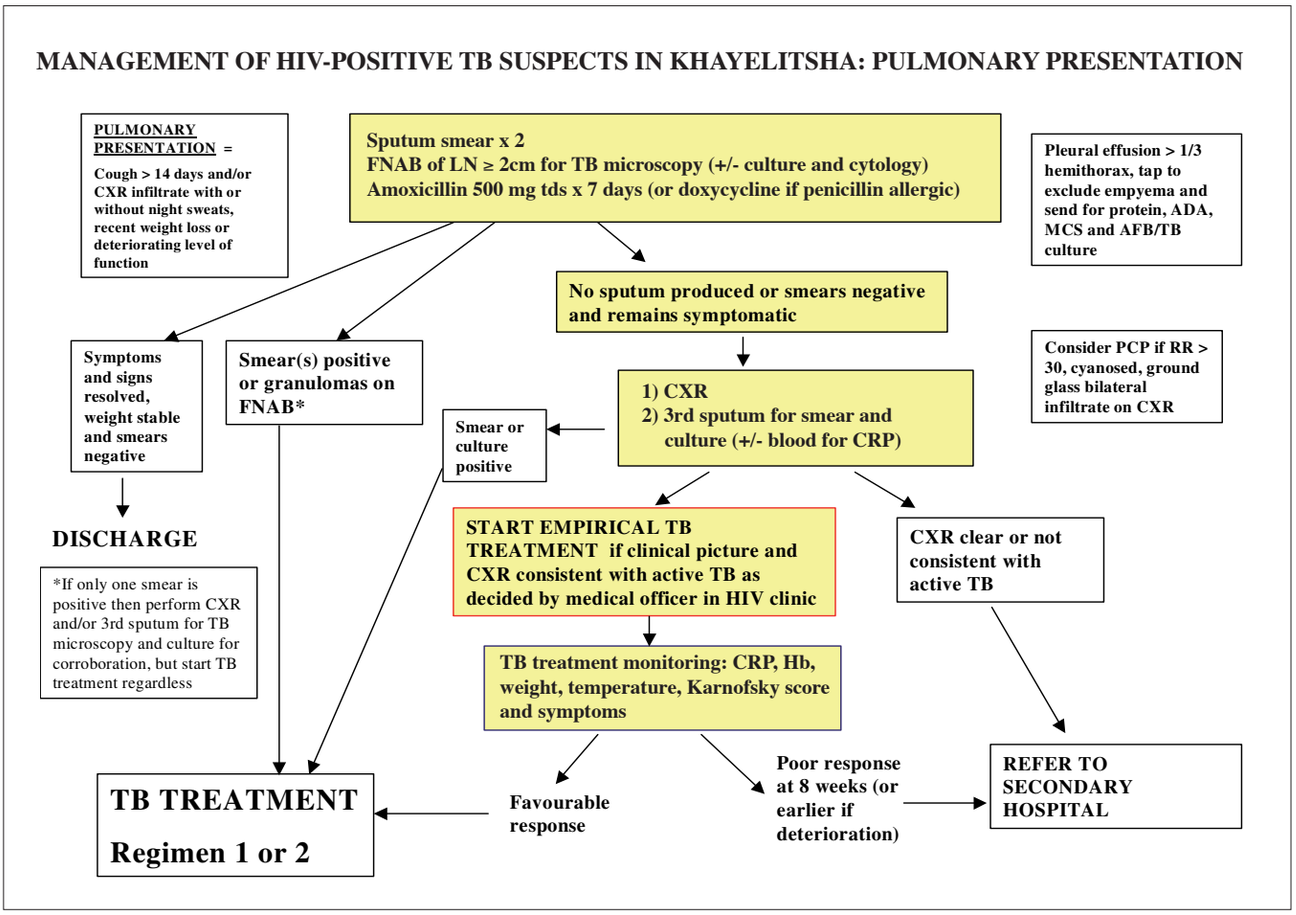

Fig. 1. The smear-negative algorithm. (CXR = chest radiograph; FNAB = fine-needle aspiration biopsy; LN = lymph node; ADA = adenosine deaminase; MCS = microscopy, culture and sensitivity; AFB = acid-fast bacilli; RR = respiratory rate; PCP = pneumocystis pneumonia; CRP = C-reactive protein; Hb = haemoglobin.)

(*ii*) inadequate clinical response to broad-spectrum antibiotics; (*iii*) deteriorating level of function; (*iv*) chest radiograph consistent with active PTB or miliary TB; (*v*) pleural fluid sent for adenosine deaminase (ADA) testing if effusion present; (*vi*) fine-needle aspiration biopsy (FNAB) of any peripheral lymph nodes > 2 cm in diameter if lymphadenopathy present.

Patients who were diagnosed with TB and referred for treatment on the basis of a positive smear from a FNAB were not included in this cohort. Once initiated on empirical TB treatment, patients were monitored every 2 - 3 weeks at the HIV clinic. Outcomes were assessed after 4 - 8 weeks of empirical TB treatment and were determined according to the following criteria: (*i*) symptom response; (*ii*) weight gain (or loss); (*iii*) serial C-reactive protein (CRP); (*iv*) serial haemoglobin; and (*v*) TB culture result from sputum, pleural fluid, or FNAB.

If there was a favourable clinical response to empirical TB treatment, then the patient received a full course of TB treatment regardless of the culture result. If there was a poor clinical response to empirical TB treatment, then the patient was referred to a secondary hospital for further management.

A folder review was performed to assess the outcomes of patients referred using the algorithm between 12 February 2004 and 30 April 2005. Approval for the study was granted by the University of Cape Town Research Ethics Committee.

Results

Fifty-eight patients were referred to the adjacent TB service using the algorithm. Four patients had 1 sputum sample that was 'scanty positive' for AFB, but they were included in the cohort because they would not have been eligible to start TB treatment according to the National TB Programme Protocol. The other 54 patients either could not produce sputum or had 1 or more negative smears. Baseline data for the cohort are shown in Table I.

The outcomes of the 58 cases are shown in Fig. 2. Thirty-two cases (55.2%, 95% confidence interval (CI): 42 - 68%) were diagnosed as having 'definite' TB on the basis of positive cultures for *Mycobacterium tuberculosis* (MTB). Twenty-four of these positive cultures were sputum specimens, 4 were pleural fluid specimens, 3 were lymph node aspirates, and 1 was from a chest abscess aspirate.

Four of the 32 positive cultures were not from initial specimens; these cultures were positive on subsequent specimens done for various reasons. Two of these 4 patients defaulted TB treatment and had culture-positive sputum specimens when they returned after treatment interruption. The third case had a dry cough and initially improved after 8 weeks of empirical TB treatment, only to deteriorate subsequently; subsequent sputum culture and sensitivity showed multidrug-resistant (MDR) MTB. Initially the fourth case did not improve clinically on empirical TB medication, and subsequently developed a chest wall abscess. Needle aspiration of this abscess grew drug-sensitive MTB.

Sensitivity results from the 28 cases with initial cultures that were positive demonstrated an additional 2 cases of MDR TB. All 3 MDR cases had initial clinical improvement at 8 weeks of empirical TB medication, but deteriorated subsequently.

Five cases without positive cultures were classified as having 'probable' TB, defined as clinical improvement after 8 weeks of TB treatment and a miliary pattern on chest radiograph or pleural effusion with an ADA level > 30 U/l. Four of these cases were unable to produce sputum specimens (Table II).

Of the remaining 21 TB suspects, 16 had improved clinically at 4 - 8 weeks on treatment without proof of TB by positive culture and were regarded as 'possible' TB cases. Nine of these 16 cases were unable to produce a sputum specimen and therefore cultures were not done. The other 7 cases had negative sputum cultures but significant clinical improvement (weight gain and/or improvement in TB symptoms). Six of these 16 cases had follow-up CRP measurements, 4 of which showed lowering of CRP. In addition, 3 of the 16 cases had a documented rise in haemoglobin on empirical TB medication. The median weight gain in these 16 cases was 3.5 kg, which occurred over a median duration of 9 weeks of empirical TB treatment. Clinical details of these 16 'possible' TB cases are summarised in Table III.

Two cases did not improve significantly after 8 weeks of TB treatment and required referral to a secondary hospital for further investigations. Both died without the cause of their illness or death being determined. The remaining 3 patients defaulted TB treatment. Attempts were made to trace these patients. One patient, who had initially improved symptomatically with weight gain after 2 weeks of TB treatment and then defaulted, had died at a secondary hospital 4 weeks later. Another patient defaulted TB medication and died 5 weeks later. The outcome of the third case could not be determined.

Analysis of the 28 cases confirmed to have active TB by positive culture on initial specimens showed that they were started on TB treatment a median 19.5 days before cultures became positive. The date used for the positive culture result was the date that the laboratory reported growth of acid-fast bacilli (AFB). In 1 of these 28 patients TB was also confirmed by a positive smear from a lymph node FNAB that became available after empirical TB treatment was commenced, but before the culture result. Subsequent identification showed *M. tuberculosis* in all patients with a positive culture result.

The chest radiographs of 50 cases were reviewed after the study period by an experienced chest radiologist. Thirty-nine (78%) were assessed as being compatible with active PTB, whereas 11 (22%) were assessed as having no features of active TB: 7 were reported as normal or 'virtually' normal, 2 as

Table I. Baseline data for the 58 smear-negative patients

Characteristic	Value
Study participants (*N*)	58
Age (years)	
Median	32.5
Interquartile range	28.0 - 36.8
Female sex (*N* (%))	37 (64)
WHO clinical stages before TB diagnosis (*N* (%))	
Stage 2	6 (10)
Stage 3	33 (57)
Stage 4	19 (33)
CD4 cell count (cells/µl)	
Median	119
Interquartile range	71 - 196
Antiretroviral treatment exposure (*N* (%))	
Before starting empirical TB	13 (22)
Started during first 8 weeks of TB treatment	2 (3)
Prior TB episodes (*N* (%))	
None	28 (48)
One	18 (31)
Two	7 (12)
Three	3 (5)
Unknown	2 (3)
Symptoms and signs at time of initial designation as a TB suspect	
Cough duration (days)	
Median	14
Interquartile range	7 - 28
Weight loss (kg)	
Median	4.2
Interquartile range	2.7 - 6.5
Weight loss duration (weeks)	
Median	7
Interquartile range	4 - 17
Interval from time of designation as TB suspect to initiation of empirical TB medication	
Weight loss during this period (kg)	
Median	0.9
Interquartile range	0.0 - 2.0
Duration (days)	
Median	7
Interquartile range	4 - 15
Antibiotics before starting empirical TB medication (*N* (%))	
One course	23 (40)
Two or more courses	35 (60)
Amoxicillin	42 (72)
Doxycycline	20 (34)
Erythromycin	16 (28)
Ciprofloxacin	8 (14)
Cotrimoxazole	4 (7)
Co-amoxyclavulanic acid	2 (3)
Metronidazole	1 (2)
Flucloxacillin	2 (3)
Smear results (*N* (%))	
No sputum due to dry cough	19 (33)
One scanty positive sputum smear	4 (7)
One negative sputum smear	12 (21)
Two or more negative smears	23 (40)
Chest X-ray interpretations by HIV clinician (*N*)	
Miliary pattern	7 (12)
Other infiltrates	16 (28)
Pleural effusion/thickening	16 (28)
Adenopathy	11 (19)
Infiltrate and adenopathy	5 (9)
Pleural effusion/thickening and infiltrates	2 (3)
Pleural effusion and adenopathy	1 (2)
Haematological and chemistry results (*N*)	
Initial haemoglobin (g/dl, *N* = 54)	
Mean	9.9
Standard deviation	2.3
Follow-up haemoglobin (g/dl, *N* = 20)	
Mean	10.8
Standard deviation	1.8
Initial C-reactive protein (mg/dl, *N* = 49)	
Mean	110
Standard deviation	65
Follow-up C-reactive protein (mg/dl, *N* = 10)	
Mean	47
Standard deviation	32

past PTB without evidence of active TB, 1 showed features suggestive of Kaposi's sarcoma, and 1 had smoking-related changes. Of the 11 assessed to have no features of active TB, 5 had positive TB cultures and improved on TB treatment (including the 1 with features suggestive of Kaposi's), 3 had 'possible TB', 2 defaulted, and 1 deteriorated and died.

Discussion

Traditionally, the TB programme in Khayelitsha and other parts of South Africa has required patients to have 2 positive smears, 1 positive culture, or a referral from a secondary or tertiary hospital before TB treatment would be initiated. Clinicians working in Khayelitsha found that difficulty making the diagnosis of TB in HIV-infected people when following these protocols led to delays in the initiation of TB treatment, with consequent clinical deterioration, hospitalisation and mortality. The algorithmic approach was developed to address this.

Of the 58 patients commenced on TB treatment using this algorithm, 55% had 'definite' TB and 36% had 'probable' or 'possible' TB. It is possible that some in the 'possible' category may have had bacterial chest infections that responded to rifampicin, but the approach of giving an antibiotic before empirical TB treatment was intended to minimise this. A total of 91% of patients (53/58) commenced on empirical TB treatment based on a clinico-radiological diagnosis therefore showed a response to therapy. Diagnostic delay was also reduced. In those patients who eventually had positive TB cultures, the TB treatment was initiated almost 3 weeks before the culture result was available. If logistical factors are taken into account such as the time it takes to receive the laboratory report and recall the patient, the delay would have been even longer. A further 21 patients who responded to TB treatment were culture-negative or

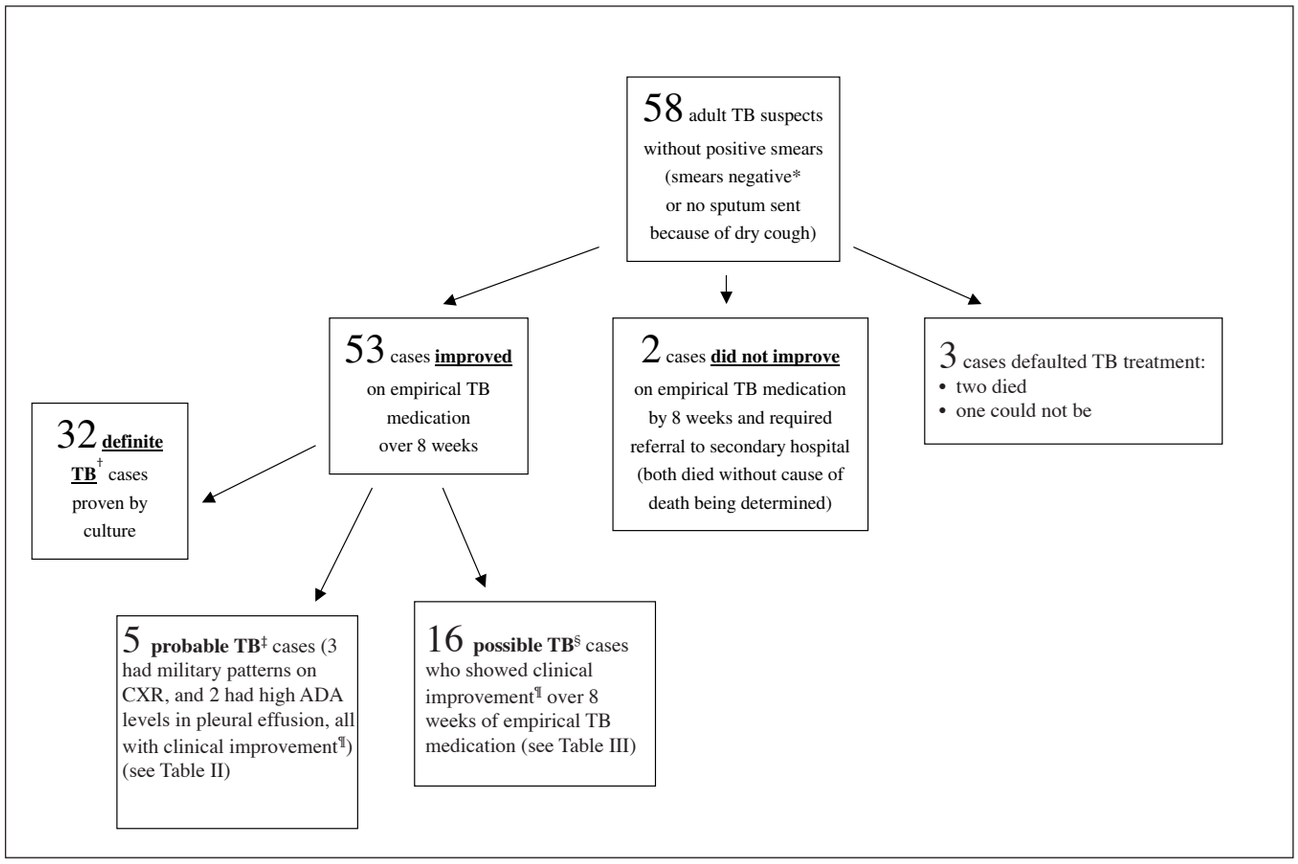

*Fig. 2. Outcomes of 58 consecutive adult cases referred for TB treatment using the smear-negative algorithm by an HIV clinician in a high-incidence TB setting. (*Four of these smear-negative cases had 'scanty positive' sputum results; †A positive culture was considered to be 'definite TB'; ‡Case definition of 'probable TB': clinical improvement after 8 weeks of TB treatment, PLUS miliary pattern on chest radiograph OR pleural effusion with high ADA level; §Case definition of 'possible TB': clinical improvement after 8 weeks of TB treatment, with or without significant lowering of CRP after 2 weeks; ¶'Clinical improvement' is defined as weight gain and/or improvement in TB symptoms.)*

Table II. Outcomes of five cases with probable tuberculosis

Patient	CXR findings	Clinical improvement on empirical TB treatment	Other findings
1	Pleural effusion	Yes, with 2.4 kg weight gain at 10 weeks	High ADA* (63 U/l)
2	Pleural effusion	Cough improved, but 6.2 kg weight loss at 8 weeks (had vomiting with TB medication)	High ADA (70 U/l)
3	Miliary pattern	Yes, with 10.7 kg weight gain at 4 months	CRP 125 → 37 mg/dl
4	Miliary pattern	Symptoms improved at 2 weeks, then patient moved to Eastern Cape	
5	Miliary pattern	Symptoms improved; 0.1 kg weight gain at 7 weeks	Hb 6.7 → 10.3 g/dl

*Values > 30 U/l are suggestive of TB, but ADA is also raised in parapneumonic and malignant effusions.
CXR = chest radiograph; ADA = adenosine deaminase; CRP = C-reactive protein; Hb = haemoglobin.

unable to produce sputum and may potentially have had even longer diagnostic delay.

In our cohort TB was confirmed by culture in 55% of cases. This is higher than the rates reported in other African cohorts of smear-negative TB suspects. Hargreaves *et al.*[19] reported that TB was confirmed in 39% of patients referred for TB treatment with a diagnosis of SNTB in Malawi. The study done by Wilson *et al.*[18] in Cape Town reported higher rates of confirmed TB than in our study. They used a set of case definitions to diagnose smear-negative TB at a referral hospital. Of the patients in that study who fulfilled the pulmonary infiltrate case definition, 77% had confirmed TB by culture

Table III. Outcomes of 16 cases with possible tuberculosis

Patient	Specimen sent for culture?	Weight response	Improvements in				Laboratory results after starting TB medication
			Cough	Night sweats	Appetite	Chest pain	
1	Yes	3.4 kg increase over 2 months	√	√	√		CRP 90 → 27 mg/dl after 2 weeks
2	Yes	3.5 kg increase over 10 weeks	√	√	√		CRP 50 → 24 mg/dl after 3 weeks
3	No (dry cough)	5 kg increase over 2 months	√	√	√		–
4	No (dry cough)	3.2 kg increase over 2 months	√			√	–
5	No	0.4 kg increase over 7 weeks	√	√			CRP 104 → 98 mg/dl after 2 weeks
6	Yes (2 x negative)	2.3 kg increase over 10 weeks	√				–
7	Yes (2 x negative)	3.6 kg increase after 11 weeks	√	√			CRP 42 → 35 mg/dl after 2 weeks
8	Yes (1 x negative)	Not weighed due to peripheral neuropathy	√				–
9	No (dry cough)	4.2 kg increase after 3.5 months	√			√	–
10	No (dry cough)	3.0 kg increase after 2 months	√	√	√		–
11	No (no cough), FNAB not diagnostic	6.3 kg increase after 10 weeks		√	√		–
12	No	7.3 kg increase over 3.5 months	√	√	√		–
13	No (dry cough)	7.2 kg increase over 2 months	√	√			Hb 8.7 → 11.7 g/dl after 3 months
14	Yes	4.9 kg increase over 2 months	√	√			CRP 91 → 15 mg/dl, Hb 6.9 → 8.4 g/dl after 10 weeks
15	No	0.7 kg increase over 2 months	√	√			CRP 175 → 12 mg/dl after 6 weeks, Hb 5.5 → 10.6 g/dl after 3 months
16	Yes (1 x negative)	3.1 kg increase over 10 weeks	√	√			–

CRP = C-reactive protein, Hb = haemoglobin, FNAB = fine-needle aspiration biopsy.

or histological analysis and 14% had possible TB, having had a response to empirical TB treatment. This gave a positive predictive value of 92% for this case definition. Although the proportion of patients responding to TB treatment was very similar to that in our study, the culture-positive rate was higher than in our study, probably because multiple cultures were done from different sites and sputum induction was used. Our study site reflects the clinical situation in a busy primary health care clinic where the quality of samples could not be assured; in some patients sputum could not be obtained and sputum induction was unavailable.

The algorithm was designed to encourage the consideration of alternative diagnoses at initial presentation. This process included the consideration of pneumocystis pneumonia in distressed patients, aspiration of pleural effusions to inspect for purulent effusions (empyema) and bloody effusions (suggestive of Kaposi's sarcoma) and a course of a broad-spectrum antibiotic to treat bacterial infections. Three patients in this cohort were found to have MDR TB when they deteriorated after an initial improvement on TB treatment, making it an important consideration in patients not improving on empirical TB treatment. The 2 patients who deteriorated despite being on TB treatment with negative cultures died without a definitive diagnosis being made. In general, HIV-infected patients not improving on empirical TB treatment should be investigated for MDR TB, non-tuberculous mycobacterial infection, systemic fungal infections, lymphoma, Kaposi's sarcoma and nocardiosis.[16] Ciprofloxacin was used in 8 of the TB suspects in this study. However this is not an advisable antibiotic to use in TB suspects as the antituberculous activity of the fluoroquinolones may cause a temporary improvement in TB symptoms, thereby lengthening diagnostic delay, and predispose to MTB fluoroquinolone resistance.[20,21]

A chest radiograph 'consistent with active TB' was one of the criteria required in this algorithm before empirical TB medication could be initiated. Features on the chest radiograph that could suggest active TB include miliary pattern, lymphadenopathy, pleural effusion or nodular infiltrates. However, the HIV clinician's interpretations of the radiographs did not always correlate with the subsequent radiologist's interpretations. The HIV clinician regarded all the radiographs as compatible with active TB, but the radiologist reported that 22% of radiographs were not consistent with active TB. Seventy-three per cent of this latter group were subsequently found to have 'definite' or 'possible' TB. Other studies[12] have also found that up to 19% of patients with sputum culture-positive TB may have normal chest radiographs, and thus it may be feasible to remove the requirement for the chest radiograph to be compatible with active TB from this algorithm.

Many areas in southern Africa have high TB-HIV co-infection rates, but limited access to radiographs, cultures and doctors.

If this smear-negative algorithm was modified to exclude chest radiographs, it could be used in such rural settings. A nurse could use clinical symptoms, Karnofsky performance score, lack of response to broad-spectrum antibiotics and CRP (if available) to initiate and monitor empirical TB therapy. Any such algorithm would first have to be validated.

Conclusions

The smear-negative algorithm allowed for earlier initiation of TB treatment in HIV-infected adults presenting with symptoms of PTB in a high TB incidence setting. This algorithm should be validated and could be modified for use by nurses in rural areas where doctors and chest radiographs are not readily available. Such algorithms could be used in the National TB Programme to reduce diagnostic delay associated with SNTB in HIV-infected patients in South Africa.

The authors wish to acknowledge Professor H Goodman, who reviewed the chest radiographs.

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