Original Article

Clinical and Laboratory Diagnosis of the Patients with Sputum Smear-negative Pulmonary Tuberculosis

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Abstract

Background: The objective of this study is to describe the proportion of patients with chronic cough and negative smear microscopy appropriately diagnosed as tuberculosis (TB) and to identify clinical features that could be used in developing a diagnostic scoring system for smear-negative patients.

Methods: Records of patients with chronic cough and ≥ 3 negative sputum smears for acid fast bacilli who attended a reference University hospital in south-eastern Iran and screened by culture were retrospectively reviewed. We compared confirmed smear-negative pulmonary TB (PTB; culture-positive) and unconfirmed smear-negative patients (culture-negative) to describe the appropriateness of treatment and their characteristics. Features independently predictive of smear-negative PTB (SNPTB) were entered into a logistic regression to create a diagnostic rule.

Results: This study enrolled 350 patients, of which 52 (14.8%) were culture-positive and 298 (85.2%) culture-negative. Of these, 38 out of 52 (sensitivity 73%) confirmed SNPTB were diagnosed as TB and 283 out of 298 (specificity 95%) unconfirmed sputum-negative patients were diagnosed as non-PTB. Variables associated with confirmed SNPTB were the presence of night sweats, family history of TB, typical chest radiography, erythrocyte sedimentation rate > 45 mm and white blood cell count < 11000/mL. The score constructed with these variables had a sensitivity of 94% and specificity of 74% with an area under the curve of 0.90.

Conclusion: The clinical differences between SNPTB and control patients could be used to develop a clinical scoring system to identify patients with SNPTB.

Keywords: Adults, clinical features, Iran, laboratory diagnosis, pulmonary tuberculosis

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Introduction

Despite efforts to develop new diagnostic methods for tuberculosis (TB), sputum smear microscopy remains the most widely used diagnostic test in low and middle income countries (LMIC). This method however has low sensitivity and, although a culture is more sensitive it lacks the timeliness necessary to provide information when the initial diagnosis is made. In the absence of more sophisticated tests, such as the Nuclear Acid Amplification tests (NAAT), health staff often make the diagnosis of smear-negative pulmonary TB (SNPTB) with information obtained through the clinical history, physical examination and chest X-ray.[1,2] This information is not specific and the diagnosis and management of SNPTB patients is an important clinical problem in LMIC. According to World Health Organization (WHO) guidelines, SNPTB is defined as: i) a patient with at least three sputum that are negative for acid fast bacilli (AFB), radiological abnormalities consistent with pulmonary TB (PTB) and a lack of clinical response to at least two weeks of a broad spectrum antibiotic; ii) a severely ill patient with at least two sputum specimens negative for AFB, radiological abnormalities consistent with extensive PTB and decision by a medical officer to treat with full course of TB chemotherapy; or iii) a patient whose sputum smears are negative but whose sputum culture result is positive.[3]

Patients with SNPTB who do not receive treatment have a high mortality, while patients without SNPTB inappropriately treated with anti-TB drugs frequently experience side effects, especially if co-infected with human immune deficiency virus (HIV).[4–6] Thus it is important to make the correct decision in SNPT patients before starting anti-TB treatment.

This study therefore aims to determine the diagnostic accuracy of patients treated for SNPTB in a reference TB clinic in Iran and to identify the characteristics that could be used to identify individuals with confirmed SNPTB.

Materials and Methods

This was a retrospective case series of patients > 15 years old with cough for ≥ 3 weeks duration who attended the Infectious Diseases Department of Boo-Ali University Hospital. The hospital is a referral center for TB located in Zahedan, Sistan, and Baluchestan (southeast Iran), which is the largest province and has the highest incidence of TB in the country.[7]

Patient selection

The records of all patients who attended between January 2007 and January 2008 with ≥ 3 negative sputum smears for AFB and remained symptomatic after a course of broad spectrum antibiotics were analyzed. We excluded patients previously treated for TB, those with known malignancies or connective tissue disorders and those who received corticosteroids or chemotherapy. Data extracted from the clinical records included demographic factors, the presence of signs and symptoms and family history of TB. All patients

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Table 1. Clinical characteristics of patients with confirmed and unconfirmed SNPTB.

Characteristics	SNPTB		*P*-value
	Confirmed N = 52	Unconfirmed N = 298	
Age (mean ± SD)[CI: 95%]	55.2 (14.5) [51.2–59.2]	56.6 (15.7) [54.7–58.2]	0.6
Male (%)	30 (58%)	162 (54%)	0.6
Origin			0.01
Iranian	41 (79%)	270 (91%)	
Afghani	11 (21%)	28 (9%)	
Fever	36 (69%)	241 (81%)	0.06
Sputum	47 (90%)	263 (88%)	0.7
Hemoptysis	9 (17%)	52 (17%)	0.9
Chest pain	15 (29%)	64 (22%)	0.3
Dyspnea	21 (40%)	138 (46%)	0.4
Weight loss	19 (37%)	73 (25%)	0.08
Anorexia	31 (60%)	162 (54%)	0.5
Night sweats	17 (33%)	35 (12%)	< 0.0001
Abnormal breath sounds	43 (83%)	239 (80%)	0.7
Lymphadenopathy	6 (12%)	19 (6%)	0.2
HIV serology	2/35 (6%)	4/108 (4%)	0.6
Smoker	30/52 (58%)	194/298 (65%)	0.3
Family history of TB	28/52 (54%)	55/298 (18%)	< 0.0001

underwent chest X-rays and other tests such as white blood cell count (WBC); hemoglobin (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and tuberculin skin test (TST) at the time of their first consultation. HIV infections are not prevalent in this area and tests for HIV were not requested for all patients. The chest X-ray was classified either having typical (presence of parenchymal infiltrates, localized cavities in the upper/apical zone) or atypical patterns (presence of findings other than typical images, such as lobar or diffuse infiltrations, mediastinal lymphadenopathy, miliary patterns, pleural effusions, etc.), or normal. Patients with pleural effusions but without pulmonary infiltrations were excluded, as they were considered to have extrapulmonary TB. Five tuberculin units of protein purified derivative (PPD-S, Razi Company, Tehran) were used to apply the TST. TST indurations ≥ 10 mm were considered positive, 5 to 10 mm intermediate and < 5 mm negative. Three or more morning sputum specimens were collected from each patient. Patients who could not produce sputum underwent induced sputum and the specimens obtained were digested and decontaminated by N-acetyl-L-cysteine, NAOH, and citrate solution. Smears were stained using the Ziehl-Neelsen (ZN) method and one specimen per patient was cultured in Löwestein-Jensen medium. Patients confirmed by culture to have SNPTB were located to initiate treatment, but patients with negative culture who had initiated treatment were not asked to discontinue treatment.

Statistical analysis

Data were analyzed using SPSS (version 15.0). The characteristics of the patients were described using summary statistics stratified by culture results. The culture was used to classify patients with negative smear microscopy as having confirmed (culture-positive) or unconfirmed (culture-negative) PTB. Chi-squared, Fisher's exact tests and student t-tests were used to compare the

Table 2. Chest X-ray and laboratory characteristics of patients with confirmed and unconfirmed SNPTB.

	SNPTB		*P*-value
	Confirmed N = 52	Unconfirmed N = 298	
X-ray appearance			< 0.0001
Typical of TB	9 (17%)	4 (1%)	
Atypical	42 (81%)	276 (93%)	
Normal	1 (2%)	18 (6%)	
Cavities	1 (2%)	2 (1%)	0.4
Mediastinal Lymph nodes	6 (12%)	6 (2%)	0.004
Pleural effusion with infiltrations	5 (10%)	31 (10%)	0.9
Miliary TB	1 (2%)	—	—
Thorax side affected			0.07
Right	23 (44%)	124 (42%)	
Left	12 (23%)	98 (33%)	
Bilateral	16 (31%)	58 (20%)	
Normal	1 (2%)	18 (6%)	
WBC count/dL (mean±SD) [CI: 95%]	6887 (1635)[6426–7355]	10507 (4237) [999111017]	< 0.0001
Hb gr/dL (mean ± SD) [CI: 95%]	12 (1.6) [11.5–12.4]	13 (1.4) [12.8–13.1]	< 0.0001
ESR mm/1st hr (mean ± SD) [CI: 95%]	55 (19) [49.8–60.4]	38 (21) [35.7–40.6]	< 0.0001
CRP	41 (79%)	192 (64%)	0.1
PPD			0.05
< 5	23 (44%)	166 (56%)	
5–10	4 (8%)	40 (13%)	
> 10	25 (48%)	92 (31%)	

SD = standard deviation; WBC = white blood cells; Hb = hemoglobin; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; PPD = purified protein derivative.

Table 3. Multivariate analyses of patients with confirmed and unconfirmed SNPTB.

	AOR	CI: 95% C	SE	*P*-value
WBC count < 11000/mL	38.2	4.7–309.6	1.067	0.001
Typical chest X-ray	24.4	1.9–308.6	1.295	0.014
Night sweats	7.19	2.7–18.8	0.492	0.000
ESR > 45 mm/hr	6.3	2.8–14.3	0.414	0.000
TB family history	3.7	1.8–7.9	0.384	0.001

AOR = adjusted odd ratio; SE = standard error; WBC = white blood cells; CXR = chest X-ray; ESR = erythrocyte sedimentation rate.

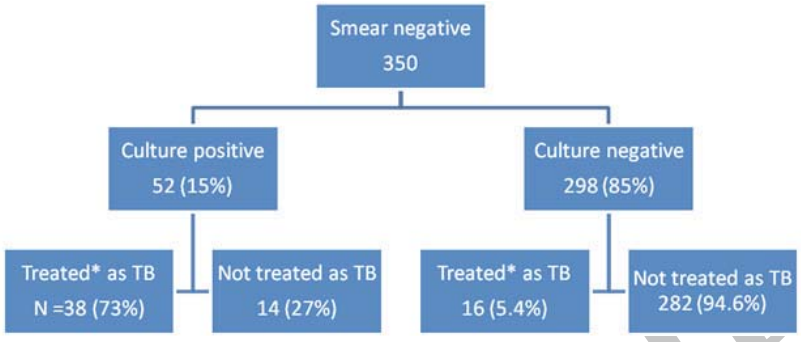

Figure 1. Study profile.*Treatment before culture results.

characteristics with confirmed and unconfirmed SNPTB, as appropriate. Variables with $P < 0.05$ were selected for logistic regression to identify those associated with SNPTB. Characteristics independently predictive of SNTB were modeled to create a diagnostic rule. A score was assigned to each patient according to the presence or absence of the predictive variable. The presence of the predictive variable was allocated the adjusted odd ratio (AOR) value and the absence of the predictive value was given a score of zero. The total score was calculated for each patient and a receiver operating characteristic (ROC) curve was drawn to identify patients with SNPTB based on the best-fit model.

All patients signed an informed consent at the time of diagnosis. The Ethics Committees of the Zahedan University of Medical Sciences in Iran and the Liverpool School of Tropical Medicine, UK approved the study protocol.

Results

A total of 350 patients with negative smear microscopy were registered over the study period. Of these, 52 (14.8%) had confirmed (culture-positive) and 298 (85.2%) unconfirmed (culture-negative) SNPTB. There were 54 (15%) patients who had been clinically diagnosed by the treating physician as having SNPTB (before culture results were available) and 296 (85%) as having other conditions. Of the 52 patients with confirmed SNPTB, 38 (73%) were correctly identified by the physician and 282 (94.6%) of 298 patients with unconfirmed SNPTB were considered to have other conditions; (Figure 1). Therefore, 320 (91%) patients were correctly and 30 incorrectly classified. All 52 culture-positive and 16 culture-negative patients were treated for TB after culture results.

The clinical and laboratory characteristics of patients with confirmed and unconfirmed SNPTB are shown in Tables 1 and 2. Patients with confirmed SNPTB were more likely to have night sweats ($P < 0.0001$), a family history of TB ($P < 0.0001$) and to be Afghani ($P = 0.01$). Their X-ray patterns were more likely to be considered typical of PTB by the radiologists ($P < 0.0001$) and to have mediastinal lymphadenopathy ($P = 0.004$). In addition, confirmed SNPTB patients were more likely to have lower WBC counts and Hb, higher ESR and positive TST (> 10 mm) than patients with unconfirmed SNPTB.

The logistic regression identified that night sweats (AOR = 7.19), a family history of TB (AOR = 3.7), the presence of typical X-ray findings (AOR = 24.4), a WBC count < 11000 cells/ml (AOR = 38.2), and an ESR > 45 mm/hour (AOR = 6.3) were independently associated with confirmed SNPTB, as shown in Table 3. After calculating the total score for all patients, a cut off of 45 points had a sensitivity of 94% and specificity of 74% with an area under the curve of 0.90 (95% CI: 0.88 – 0.92 (Figure 2) **.** Therefore, the score would correctly identify 48 out of 52 (94%) confirmed SNPTB and 219 out of 298 (73.5%) unconfirmed SNPTB patients, with 79 patients being incorrectly classified.

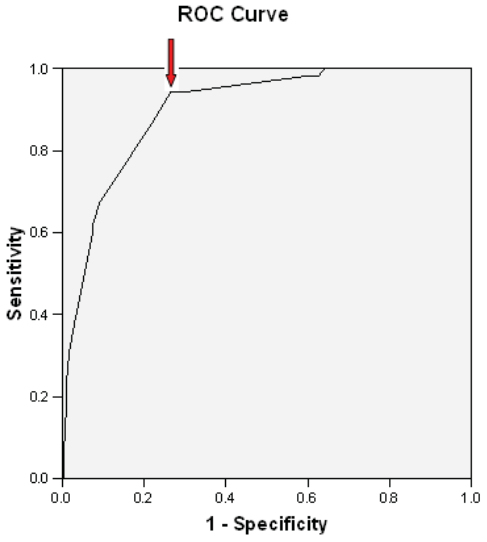

Figure 2. Receiver operating characteristic curve derived from the logistic regression model shows the cut-off point (arrow) with sensitivity and specificity of 94% and 74%, respectively.

Discussion

The diagnosis of PTB in patients with negative smear by microscopy remains an important clinical problem in LMIC and often supplementary tests are used to improve the diagnostic accuracy for these patients.[8] There is, however, no point of care diagnostics available with the sensitivity, specificity and timeliness needed for initial clinical management and most tests have limited benefit to the information obtained through a careful clinical history and examination. In the setting of this study, the clinical judgement of the clinician resulted in 73% of patients with SNPTB being correctly diagnosed with 95% specificity.

Several studies have tried to develop algorithms to identify patients with PTB based on smear microscopy and response to antibiotic therapy. In South Africa, a setting with high HIV prevalence, an algorithm based on light smear microscopy and response to a trial of antibiotics with amoxicillin and erythromycin in patients with negative smear microscopy had a sensitivity of 89% and specificity of 84% to identify patients with culture-positive TB. This setting had a high positive predictive value (95%) but relatively low negative predictive value (70%).[9] A further study in Malawi reported that the careful clinical and radiological selection of patients plus ZN smear microscopy and the response to a trial of antibiotics resulted in an improved diagnosis of TB. Seven out of 15 culture-positive patients (47%) in that setting who were followed for three months developed a chest radiogram compatible with PTB and were treated for TB.[10] In practice many patients with a diagnosis of SNPTB are not confirmed by culture, even if these are taken. For example, only 60% of cases notified in the UK are confirmed by culture[11] and the proportion of cases with active TB with negative culture is poorly defined. Our diagnostic score is similar to the South African score.[9] By using this score, clinicians would be able to identify patients with confirmed SNPTB. However, its relatively low specificity would result in an overall higher number of patients with unconfirmed sputum-negative patients being treated for TB.

As expected, none of the patients with negative culture who initiated treatment were asked to discontinue the drugs. Cultures performed in solid media require two months to be declared negative and at that time, the patients are likely to have undergone clinical improvement. Thus it would be difficult for the clinician to decide whether the patient had TB and responded to therapy or the diagnosis was erroneous and the patient had improved with time. It is thus difficult to discontinue an anti-TB treatment once initiated, as the patient would have reached the end of the intensive phase after two months,[8] when solid media cultures become available. There is thus a worldwide impetus to increase the availability of rapid liquid culture methods (such as BACTEC), which require around seven days to provide positive results and a few weeks to declare a culture as negative.[12] However it remains to be shown whether this shorter time would modify the clinical management of the patients in LMIC, as most patients are given a diagnosis within the first 2 – 3 days of examination.

Currently, the most widely available test for the diagnosis of TB in LMIC is direct sputum smear microscopy. The sensitivity of this test varies between 34% and 80% and has high specificity.[13] Training and strengthening the capacity of laboratory personnel can improve its sensitivity,[14] although its performance is still well below the ideal for a screening test. New diagnostics and diagnostic approaches suitable for the proof of concept of patients in LMIC are thus still urgently needed.

The incidence of all types of TB and of PTB in Zahedan, the center of Sistan and Balouchestan region were 53.8 and 38.1 per 100000 population during the study period. The incidence of sputum smear-positive was 27.1 per 100000 and negative PTB was 11 per 100000 population, during that time.[15]

The study location is a low prevalence HIV area and only two SNPT and four of the control patients out of 143 tested cases for HIV had positive results on ELISA, which were all confirmed by western blot. There was not a statistically significant difference between TB and control groups according to this variable. One of the limitations to be considered was the fact that only 41% of the patients underwent HIV testing. The rate of TB/HIV co-infection in the present sample was approximately 4%, which represents co-infection in a low prevalence area. In high prevalence area, HIV seropositive cases are more likely to have negative sputum smear for AFB than seronegative patients,[16] but we could not find any significant difference because of small number of patients tested for HIV and low prevalence of HIV in this region.

Several characteristics were independently associated with an increased likelihood of a patient being smear-positive. The clinicians awareness of the incidence of TB in different ethnic groups and origin are important to inform the diagnosis[8] and in our setting, patients who were born in Afghanistan were more likely to be culture-positive. In the UK, the rate of TB in African and Indian-born migrants is higher than in whites born in the UK.[11] These differences may be due to genetic predisposition, but are more likely to reflect environmental and social circumstances.

The logistic regression revealed that night sweats, positive family history for TB, typical radiological findings, a WBC < 11000 cells/mL and ESR > 45 mm were more likely to be associated with SNPTB. A score constructed with these variables attained a sensitivity of 94% and specificity of 74%. These values have higher sensitivity (73%) but a lower specificity (95%) than the clinical diagnosis and in a similar number of patients as in this study (350) would result in a higher proportion of patients being incorrectly diagnosed (26%).

Although the diagnostic accuracy of the scoring system based on clinical and radiologic findings had reasonable results, its performance was poorer than the clinical judgement of experienced physicians in a reference center. Such scoring systems however may be useful in locations where the clinical management of the patient is decided by less experienced and trained staff in the community and need to be prospectively tested. Performing one sputum culture instead of three cultures and the small sample size of SNPT patients are the weaknesses of the study. The latter could explain the wide range of 95% confidence interval in some of the predictive variables. The diagnosis of SNPTB remains a major challenge in LMIC.

Conflicts of interest: The authors have no conflicts of interest to declare.

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