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Algorithm for the diagnosis of smear-negative pulmonary tuberculosis in high-incidence resource-constrained settings

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Abstract

OBJECTIVES Diagnosis of smear-negative pulmonary tuberculosis (SNPT) remains a challenge, particularly in resource-constrained settings. We evaluated a diagnostic algorithm that combines affordable laboratory tools and a clinical prediction rule (CPR).

METHODS We derived, based on published evidence, a diagnostic algorithm for SNPT. Sputum concentration constitutes its first step. In suspects with negative results, SNPT probability is classified with a CPR as low (excluded), high (confirmed) or intermediate. For intermediate patients, sputum Middlebrook 7H9 liquid culture is performed, and they are assessed after 2 weeks. If clinically deteriorated, with still negative liquid culture, bronchoscopy is offered. Otherwise, results of Middlebrook 7H9 culture are awaited. We prospectively evaluated this algorithm against a reference standard of solid and liquid cultures in two reference hospitals in Lima, Peru.

RESULTS 670 SNPT suspects were included from September 2005 to March 2008. The prevalence of SNPT was 27% according to the reference standard. The algorithm's overall accuracy was 0.94 (95% CI 0.91–0.95), its sensitivity was 0.88 (95% CI 0.82–0.92) and its specificity, 0.96 (95% CI 0.94–0.98). Sputum concentration, the CPR, Middlebrook 7H9 sputum culture and bronchoscopic samples defined a diagnosis of SNPT according to the algorithm in 57 (37%), 25 (16%), 63 (41%) and 8(5%) of patients, respectively. 65% of patients were diagnosed within 3 weeks.

CONCLUSIONS The algorithm was accurate for SNPT diagnosis. Sputum concentration, CPR and selective Middlebrook 7H9 culture are essential components.

keywords diagnosis, smear-negative, tuberculosis, pulmonary, algorithms, decision-making

Introduction

For decades, the diagnosis of pulmonary tuberculosis (TB) has relied on the search for acid-fast bacilli (AFB) in sputum smears, a procedure that has a sensitivity of only 50–60% (Aber *et al.* 1980; Lipsky *et al.* 1984; Siddiqi *et al.* 2003). A case of smear-negative pulmonary tuberculosis (SNPT) is defined, according to WHO (2010), as a clinical suspect with at least two negative AFB smears and a positive culture, or two negative smears, radiographical abnormalities consistent with active pulmonary TB, no improvement with a course of broad-spectrum antibiotic (if HIV-negative), and the decision to treat for TB.

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The existing guidelines for diagnosing SNPT in resource-constrained settings, published by the International Union Against Tuberculosis and Lung Disease (Ait-Khaled *et al.* 2010), WHO (2010) and the American Thoracic Society (Blumberg *et al.* 2003), are somewhat vague. In general, they hinge on 'strong clinical evidence', 'medical officer's judgment' or 'additional investigations', without detailing the diagnostic work-up. Nowadays, some affordable and accessible options are available for a more refined diagnosis of SNPT. Direct examination of concentrated sputum is more sensitive than conventional microscopy (Steingart *et al.* 2006). Fluorescence microscopy improves the diagnostic yield of sputum samples (WHO 2011a). Cultures in solid media, classically the reference standard, have been superseded by liquid cultures in terms of accuracy and time to diagnosis

(WHO 2007), and liquid cultures have become more widely available. Work performed on clinical prediction rules (CPR) for SNPT also shows encouraging results (Bah *et al.* 2002; Mello *et al.* 2006; Soto *et al.* 2008b).

We aimed to construct and evaluate an efficient diagnostic algorithm for SNPT in resource-constrained settings that combines these laboratory tools with a CPR.

Materials and methods

Setting and study population

The study was conducted in Lima, Peru. Peru has a tuberculosis incidence of 101/100.000, a 2.9% prevalence of HIV infection among TB cases and 22.5% smear negativity among pulmonary cases (WHO 2012). We recruited patients from the outpatient internal medicine and pneumology clinics, the emergency room and the pneumology and internal medicine wards in two-third-level public hospitals, Hipólito Unanue and Cayetano Heredia. They are referral centres for a population of about two million.

Assuming an overall accuracy of the tested algorithm of 80%, 95% confidence level and 10% loss to follow-up, the sample size needed was 546 patients. From September 2005 to March 2008, all patients aged 18 years or more with clinical suspicion of pulmonary tuberculosis and at least two negative AFB sputum smears were eligible for inclusion. Clinical suspicion was defined as cough for a minimum of 2 weeks and fever, weight loss of more than 3 kg or dyspnoea.

The algorithm

The algorithm for SNPT was developed by the research team and local experts, based on the literature review and using the Delphi panel method (Collecting group data 2010). Sputum concentrate AFB smear examination constitutes its first step (Figure 1). If positive, patients are referred to the National TB programme for treatment. If negative, a previously developed CPR (Soto *et al.* 2008b) is applied to assess the probability of tuberculosis. It considers as clinical predictors of TB the presence of hemoptysis (2 points), weight loss (1 point), ability to expectorate (−1 point) and age older than 45 years (−1 point) and as radiographical predictors the presence of apical infiltrate (3 points) and miliary pattern (4 points). A score less than 0 points indicates a very low probability of having SNPT (except for HIV positive patients, who are then considered to have intermediate probability). The patient is, according to the algorithm, deemed not to have tuberculosis. A score of more than 4 points corresponds to a high probability of the disease (except in patients with a history of previous tuberculosis, who are then considered to have intermediate probability). The patient is classified as having tuberculosis and referred to the national TB programme for treatment.

Patients with scores from 0 to 4 points or otherwise intermediate probability of SNPT are given a course of antibiotics (doxycycline 100 mg bid for 10 days), and a liquid culture in Middlebrook 7H9 (hereafter referred as liquid culture) taken from their sputum concentrates is performed. They are evaluated after 2 weeks, when preliminary liquid culture results are available. If the culture is negative, the clinical status is assessed through patient's appreciation and clinical examination. Patients who improved are no longer followed up, but they are traced back if their liquid culture result eventually turns out to be positive. Patients with unchanged clinical condition are given an appointment 4 weeks later, when the final results of liquid culture are available. Patients with clinical deterioration are offered fiberoptic bronchoscopy (FBO) with bronchial aspirate for AFB smear and liquid culture.

Study Procedures

For all eligible patients that gave informed consent, we recorded signs and symptoms, demographic information and medical history. HIV counselling and testing was offered, and those testing positive were referred to the national HIV programme for corresponding management.

Microbiological investigation included Ziehl-Neelsen and auramine-O (Van Deun *et al.* 2008) staining of concentrated sputum specimens. Liquid culture in Middlebrook 7H9 was a step in the algorithm, but to construct the reference standard, it was performed in all patients entering the algorithm, along with mycobacteria growth indicator tube (MGIT) and solid (Ogawa) culture. Sputum decontamination and concentration, as well as manual MGIT (Becton-Dickinson™) cultures were performed as previously reported (Soto *et al.* 2008a). Ogawa cultures followed conventional methodology (Kent & Kubica 1985). Due to stock shortage from September 2005 to January 2006 and from July to September 2006, MGIT could not be performed for 130 patients. Middlebrook 7H9 (DIFCO™) vials were prepared according to manufacturer instructions and used in conjunction with OADC (oleic acid, albumin, dextrose and catalase) supplement and the antimicrobial combination PANTA (polymyxin B, amphotericin B, nalidixic Acid, trimethoprim and azlocillin). The appearance of lump-like colonies was considered as a positive result. Positive cultures in MGIT or Middlebrook 7H9 were recultured in Ogawa

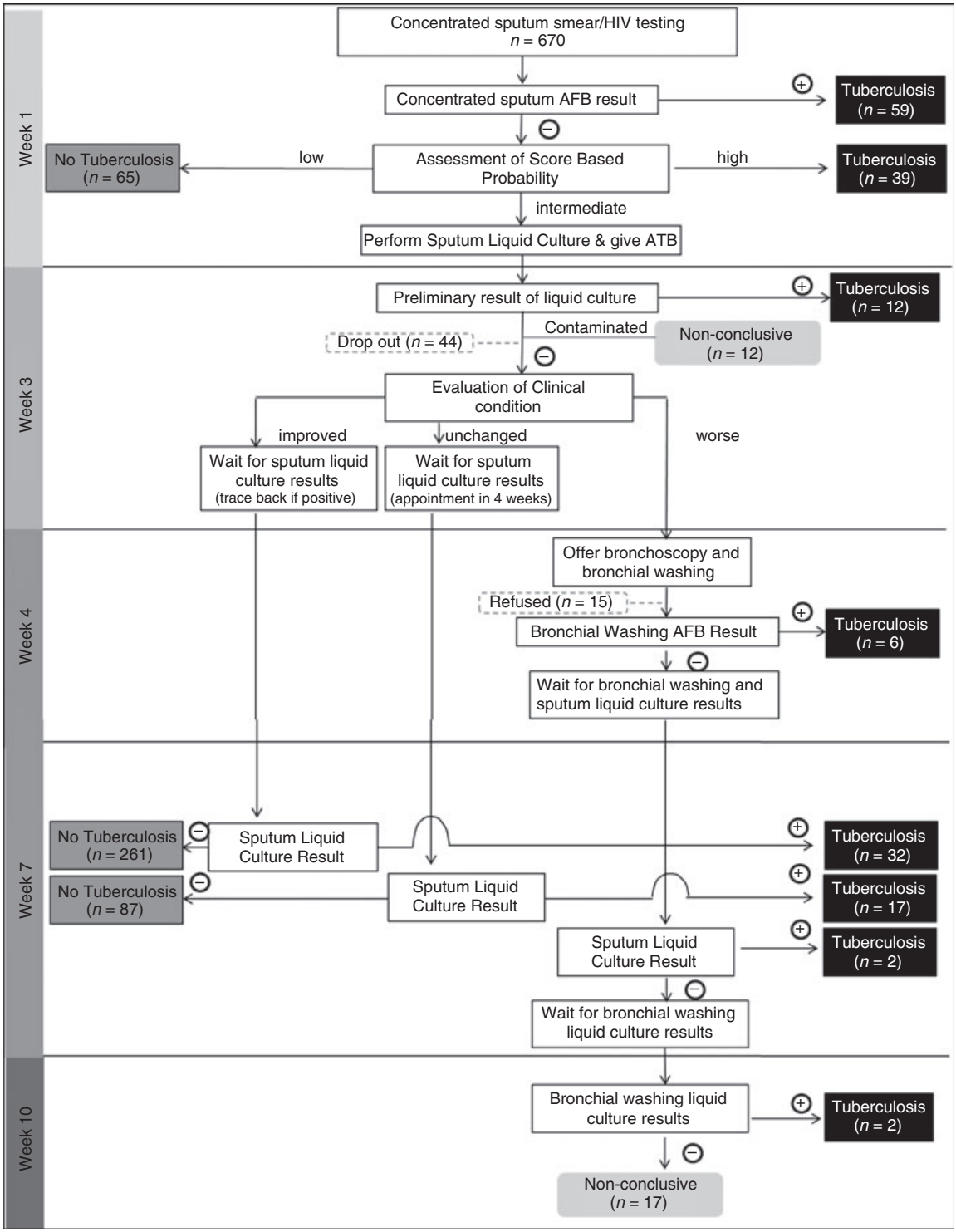

Figure 1 Flow of smear-negative pulmonary tuberculosis (SNPT) suspects through and diagnosis with the SNPT algorithm. Lima, Peru 2005–2008. Right (black) boxes represent patients diagnosed with SNPT and left (grey) boxes patients with SNPT excluded according to the algorithm.

for *M. tuberculosis* typification. Cultures were evaluated twice weekly for up to 6 weeks for liquid cultures and 8 weeks for Ogawa.

Analysis

For evaluating the performance of the SNPT algorithm, the reference standard for the diagnosis of SNPT was defined as a positive *M. tuberculosis* culture in Ogawa or a positive culture in MGIT or Middlebrook 7H9 confirmed by reculture in Ogawa.

Overall accuracy was defined as the percentage of correct results obtained by the algorithm. Sensitivity, specificity, predictive values and likelihood ratios for the algorithm were calculated and reported according to international recommendations (Bossuyt *et al.* 2003). We conducted a stratified analysis for patients with or without previous TB history and with or without HIV infection. We also evaluated the diagnostic performance of sputum concentration and compared the diagnostic yield and growing times of Middlebrook 7H9, manual MGIT and Ogawa media. Proportions and means were compared with chi-squared and *t*-test, respectively, using paired approaches when necessary. *P*-values < 0.05 were considered significant. Data analysis was performed with STATA statistical software (Release 10.0; Stata Corporation, College Station, TX, USA).

Ethics

The research protocol was approved by the ethics committees of the participating hospitals, Cayetano Heredia University and the Institute of Tropical Medicine, Antwerp. Written informed consent was obtained from all patients included in the study.

Results

A total of 1010 patients were eligible for the study. Of these, 670 entered the algorithm. The reasons for exclusion are detailed in Figure 2. Seven patients had contaminated results in all three culture media and were excluded from the evaluation of the algorithm. Of the remaining 663 patients, 182 (27.5%) had, according to the reference standard, a final diagnosis of SNPT (Figure 2). Clinical, sociodemographic, laboratory and radiological findings are summarised in Table 1. Patients with SNPT were younger and more likely to have hemoptysis, fever, weight loss, an abnormal Chest X-ray and apical infiltrates than patients without SNPT. Previous tuberculosis, being able to expectorate and clubbing were negatively associated with a final diagnosis of tuberculosis. The presence of an abnormal physical lung examination and a positive HIV serology were not associated with SNPT.

The patient flow through the algorithm is shown in Figure 1. By the second visit, 59 of the 670 patients (8.8%) had a positive concentrated sputum smear. 57 of these had SNPT according to the reference standard and constituted 31% of all SNPT patients. The remaining 611 patients were evaluated with the clinical prediction rule (CPR) during the same visit, which allowed taking a decision in 104 (15.5%) further patients. 39 had a high probability of SNPT and were classified, following the algorithm, as having tuberculosis. 25 of these (64.1%) had TB according to the reference standard. On the other hand, 65 patients had a low probability and were classified as not having SNPT. Two of these (3.1%) had TB according to the reference standard.

The 507 patients that had an intermediate CPR probability received a course of antibiotics and were scheduled for a follow-up visit 2 weeks later (week 3). By then 12 of these had a positive liquid culture; for another 12 the culture was contaminated. The latter were classified as non-conclusive by the algorithm, but three of them eventually had a reference standard diagnosis of TB. 44 patients did not show up and were classified as non-evaluable by the algorithm (5 of them eventually were diagnosed with TB according to the reference standard).

439 patients with intermediate CPR probability and negative cultures by week 3 were available for clinical re-evaluation. 293 had improved. Liquid culture became positive in 32 (10.9%) by week 7. The remaining 261 were classified as not SNPT by the algorithm, but nine (3.4%) had tuberculosis according to the reference standard. 104 patients had unchanged clinical status, of whom 17 (16.3%) eventually had a positive and 87 (83.7%) had a negative liquid culture. Of the latter, 10 (11.5%) had tuberculosis according to the reference standard.

Forty-two patients clinically deteriorated and were offered bronchoscopy. Fifteen did not accept the procedure and were classified as non-evaluable by the algorithm. None of them had TB according to the reference standard. Of the 27 patients who consented, six had a positive AFB from bronchial aspirate, two subsequently had a positive liquid culture from the initial sputum sample and two had a positive culture from bronchial aspirate only. Liquid cultures from sputum and bronchial aspirate samples of the remaining 17 patients were negative and they had non-conclusive results according to the algorithm. Their cultures were also negative in MGIT and Ogawa media, and they had no TB according to the reference standard.

Table 1 Sociodemographic characteristics, signs, symptoms and results of examinations in smear-negative pulmonary tuberculosis suspects. Lima, 2005–2008

Variable	All (n = 663)	SNPT(n = 182)	No TB (n = 481)	P value
Sociodemographic characteristics				
Mean Age (SD)	41.4 (17.2)	36.3 (15.9)	43.3 (17.3)	<0.001
TB contact -no. (%)	314 (47.4)	91 (50.0)	223 (46.4)	0.40
Male gender (%)	370 (55.8)	108 (59.3)	262 (54.5)	0.26
History of previous TB (%)	239 (36.1)	43 (23.6)	196 (40.8)	<0.001
Comorbidities				
Alcoholism (%)	41 (6.2)	11 (6.0)	30 (6.2)	0.93
Diabetes (%)	16 (2.4)	6 (3.3)	10 (2.1)	0.36
Drugs (%)	29 (4.4)	10 (5.5)	19 (4.0)	0.39
Renal failure (%)	10 (1.5)	1 (0.6)	9 (1.9)	0.21
Symptoms and physical examination				
Weight loss (%)	417 (62.9)	130 (71.4)	287 (59.7)	0.01
Expectoration (%)	443 (66.8)	108 (59.3)	335 (69.7)	0.01
Hemoptysis (%)	205 (30.9)	77 (42.3)	128 (26.6)	<0.001
Abnormal lung examination (%)	435 (65.6)	122 (67.0)	313 (65.1)	0.64
Lymphadenopathy (%)	35 (5.3)	14 (7.7)	21 (4.4)	0.09
Hepatomegaly (%)	45 (6.8)	18 (9.9)	27 (5.6)	0.05
Mean blood pressure (SD)	84.0 (9.22)	84.1 (9.6)	84.0 (9.1)	0.91
Mean Temperature (SD)	36.7 (0.5)	36.9 (0.6)	36.7 (0.4)	<0.001
Mean BMI (SD)	22.4 (4.14)	22.0 (4.0)	22.5 (4.2)	0.30
Clubbing (%)	54 (8.1)	4 (2.2)	50 (10.4)	<0.01
Ancillary examinations				
Mean WBC count $\times 10^3$	8.38 (3.6)	8.83 (3.4)	8.21 (3.7)	0.06
Mean Lymphocyte count $\times 10^3$	2.01 (1.1)	1.98 (1.2)	2.02 (1.0)	0.66
Chest X-ray findings(%)				
Abnormal CXR (%)	538 (81.2)	165 (90.7)	373 (77.6)	<0.001
Apical infiltrate (%)	379 (57.2)	132 (72.5)	247 (51.4)	<0.001
Miliary infiltrate (%)	5 (0.7)	3 (1.7)	2 (0.4)	0.10
ELISA HIV serology				
Positive (%)	98 (14.8)	23 (12.6)	75 (15.6)	0.08
Negative (%)	310 (46.8)	98 (53.9)	212 (44.1)	
Refused (%)	255 (38.5)	61 (33.5)	194 (40.3)	

After excluding non-evaluable patients (n = 59) and those with non-conclusive results (n = 29) according to the algorithm, 576 patients with non-contaminated cultures were available for evaluation of the algorithm performance (Table 2). The overall accuracy, sensitivity, specificity, positive and negative likelihood ratios of the algorithm as a whole were 0.94, 0.88, 0.96, 22.1 and 0.13, respectively. The area under the ROC curve was 0.92 (95% CI 0.89–0.94) and the diagnostic Odds Ratio 175.8 (95% CI 89.3–345.8). Algorithm accuracy was better in patients with history of TB (0.98 *vs.* 0.91; P = 0.001) but was not affected by HIV status. The cumulative percentage of the 169 patients diagnosed with SNPT by the algorithm attained 58.1% and 65.1% by the end of weeks 1 and 3, respectively. Losses to follow-up cannot have a significant impact on the estimated accuracy of the algorithm. Even under the extreme assumption that all losses were incorrectly classified, the overall accuracy only drops from 0.94 to 0.87.

Regarding microbiological procedures, 49 patients had positive results for both Ziehl-Neelsen and auramine-O staining of sputum concentrates, two for Ziehl-Neelsen and eight for auramine-O staining only. The incremental diagnostic yield of fluorescence microscopy over Ziehl-Neelsen staining was 16% (08/51) (P = 0.10). Non-contaminated samples for comparison between the three culture media were available for 520 patients. No species other than *M.tuberculosis* were isolated from cultures. The sensitivity of culture in Middlebrook 7H9 (0.77; 95% CI 0.69–0.84) was lower (P = 0.03) than that in manual MGIT (0.86; 95% CI 0.79–0.91) but significantly higher (P = 0.01) than in Ogawa (0.63; 95% CI 0.55–0.71, P < 0.01). The mean growing time (19.4 days; 95% CI 18.1–20.8) was comparable to that for MGIT (20.7 days 95% CI 19.1–22.2) and significantly (P < 0.001) shorter than for Ogawa (31.3 days; 95% CI 28.6–34.0). The combination of Middlebrook 7H9 and

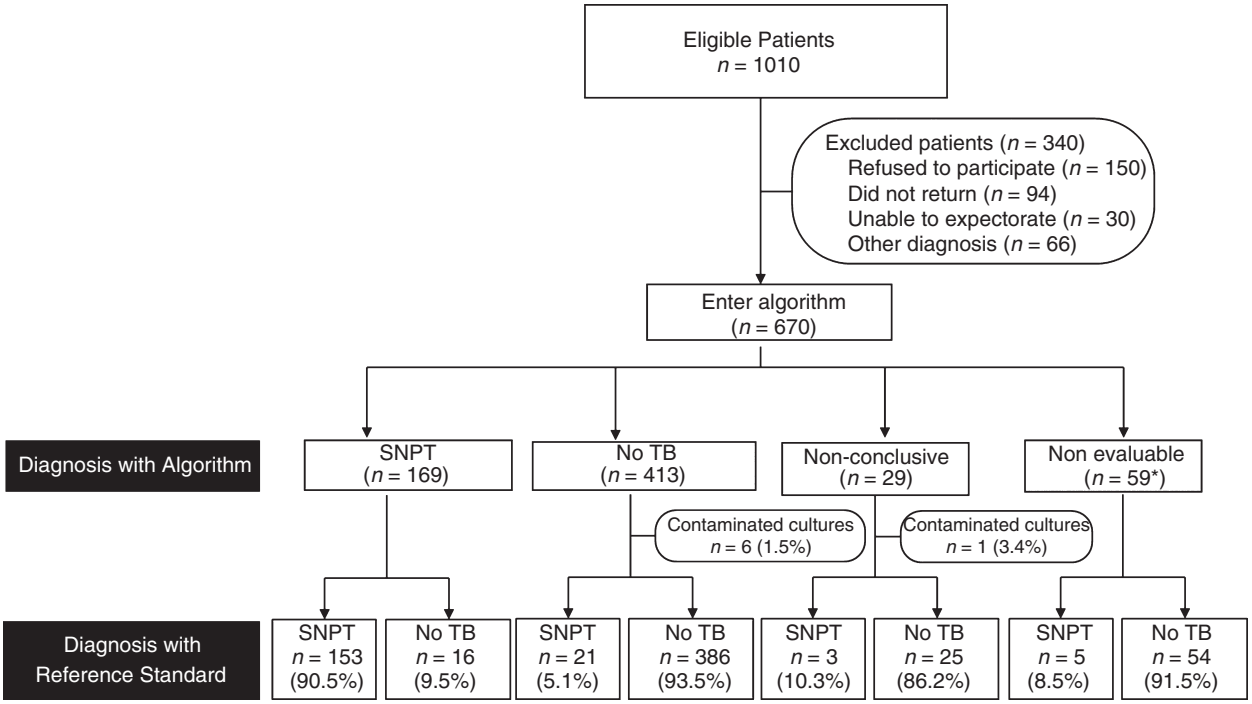

Figure 2 Comparison of the results of the algorithm with smear-negative pulmonary tuberculosis (SNPT) diagnosis according to reference standard. Lima, Peru 2005–2008. *44 lost to follow-up and 15 refusals of bronchoscopy.

Table 2 Performance of the SNPT algorithm according to history of tuberculosis and HIV status. Lima, 2005–2008

		History of Tuberculosis		HIV status	
	Overall (*n* = 576)	Yes (*n* = 206)	No (*n* = 370)	Positive (*n* = 89)	Negative/Unknown (*n* = 487)
Accuracy	0.94 (0.91–0.95)	0.98 (0.95–0.99)	0.91 (0.88–0.94)	0.94 (0.87–0.98)	0.93 (0.91–0.95)
Sensitivity	0.88 (0.82–0.92)	0.95 (0.84–0.99)	0.86 (0.79–0.91)	0.86 (0.65–0.97)	0.88 (0.82–0.93)
Specificity	0.96 (0.94–0.98)	0.99 (0.96–1.00)	0.94 (0.90–0.96)	0.94 (0.90–0.99)	0.96 (0.93–0.98)
LR positive	22.1 (13.6–35.8)	78.1 (19.7–310.1)	14.6 (8.7–24.3)	28.9 (7.3–114.4)	21.09 (12.6–35.3)
LR negative	0.13 (0.08–0.19)	0.05 (0.01–0.19)	0.15 (0.10–0.23)	0.14 (0.05–0.40)	0.12 (0.08–0.19)
Prevalence	0.30 (0.26–0.34)	0.20 (0.15–0.27)	0.36 (0.31–0.41)	0.25 (0.16–0.35)	0.31 (0.27–0.36)
PPV	0.91 (0.85–0.94)	0.95 (0.84–0.99)	0.89 (0.82–0.94)	0.90 (0.70–0.99)	0.91 (0.85–0.95)
NPV	0.95 (0.92–0.97)	0.90 (0.96–1.00)	0.92 (0.88–0.95)	0.96 (0.88–0.99)	0.95 (0.92–0.97)

SNPT, Smear-negative pulmonary tuberculosis; PPV, Positive predictive value; NPV, Negative predictive value; LR, Likelihood ratio; 95% Confidence intervals between parentheses.

MGIT attained a sensitivity of 0.93 (95% CI 0.88–0.97) against the reference standard, while the combination of Middlebrook and Ogawa attained a sensitivity of 0.91 (95% CI 0.85–0.95).

Discussion

The algorithm provides a structured and feasible approach towards the diagnosis of SNPT and permits an efficient use of laboratory resources, which is of particular importance in low and middle income countries. Its diagnostic performance is good. Sputum concentration, the CPR and selective liquid culture are its essential components. The overall accuracy is 0.94 regardless of HIV status. The attained positive (22.1) and negative (0.13) likelihood ratios provide, according to proposed standards (Dujardin *et al.* 1994; Jaeschke *et al.* 1994), convincing diagnostic evidence in favour and against disease,

respectively. Moreover, in a study population with a prevalence of 27% of SNPT, the positive predictive value was 0.91 and the negative, 0.95. This underlines that the algorithm can be used for clinical decision-making. It even performs better than culture of all sputum samples in solid medium alone (0.88 *vs.* 0.63 of sensitivity) and allows more timely decision-making.

AFB smears of concentrated sputum, a simple and cheap technique, detect 31% of cases of SNPT and substantially reduce the diagnostic delay. A systematic review (Steingart *et al.* 2006) showed a 17% increase of sensitivity over direct sputum examination, and similar yields for the diagnosis of SNPT have been reported previously (Apers *et al.* 2004). The false positive rate was very low (3%) and the corresponding patients had wasting and radiographic abnormalities consistent with tuberculosis. Most probably they were culture-negative SNPT cases. Bleach sedimentation has been proposed as an alternative to centrifugation of sputum specimens (Bonnet *et al.* 2008), but a recent meta-analysis found no convincing evidence for its utility (Cattamanchi *et al.* 2010).

The clinical prediction rule, based on symptoms and chest X-ray findings, which we use in the algorithm has been evaluated previously as stand-alone tool (Soto *et al.* 2011a). It identified well patients with high and low probabilities of SNPT and should permit to rationalise the use of liquid culture by reserving it for suspects with intermediate probability. After the CPR step, the algorithm has detected 45% of SNPT cases, but three-quarters of all suspects who entered in the present study still proceeded to culture and antibiotic trial, a higher proportion than we expected. Possibly, many suspects with low SNPT probability never reach our referral hospitals because their symptoms subside. This is in line with the high prevalence of SNPT (30%) in our suspect patients. On the other hand, patients with high probability might have been recognised as such at lower levels of care and put on treatment without further investigations. We hypothesise that the yield of the CPR and the number of cultures foregone would be higher at lower levels of care, but this remains to be verified. At any rate, while the place of the CPR needs further assessment through implementation studies under operational conditions, it hardly produces false negatives (3%), even in the challenging conditions of a referral setting like ours. Furthermore, part of the false positive CPR cases could well be culture-negative SNPT cases, in line with historical studies where 50% of culture-negative SNPT cases diagnosed on clinical and radiographic grounds, became bacteriologically positive during follow-up (Hong Kong Chest Service 1981).

It is of note that the frequency of SNPT differs little between patients with improved (11%) and unchanged (16%) clinical condition after being given an antibiotic course and that a differential approach to their follow-up seems hardly warranted. A high proportion of suspects with intermediate CPR probability responded favourably to antibiotics. Among them, over 10% had a final diagnosis of SNPT. Improvement must be viewed with caution and does not permit to exclude the disease, as also demonstrated in an evaluation of the current WHO algorithm for HIV-negative smear-negative SNPT suspects (Soto *et al.* 2011b). In the present study, all SNPT patients could eventually be put on antituberculous treatment, but if culture is not performed in suspects who improve under broad-spectrum antibiotics, they are lost or their diagnosis will be seriously delayed.

Deteriorating under an antibiotic trial, however, is associated with an increased probability of TB, even in the event of a negative liquid culture result. For this smaller group of patients, more sensitive but costly diagnostic techniques could be considered. Our results suggest that bronchoscopy can be useful, but it is limited by the small number of eligible patients and the high refusal rate. We feel that the decision to perform bronchoscopy should be individualised based on expert evaluation of the patient's clinical condition until its value for SNPT is more firmly established.

A complementary finding of our study is that the yield of the liquid cultures is much higher in SNPT suspects than the yield of conventional solid cultures. This is in line with results of other studies (Muyoyeta *et al.* 2009; Shinu *et al.* 2011) and recent WHO (2007) recommendations that encourage the use of liquid media. Middlebrook 7H9 is cheap and more sensitive than conventional solid cultures. Furthermore, the combination of Middlebrook 7H9 with classic solid media gives better yields than each method alone, based on our results. Surprisingly, in spite of being the basis of methods like MGIT or MODS (Moore *et al.* 2006), the literature on the diagnostic performance of Middlebrook 7H9 in itself is scarce.

Alternative diagnostic techniques, such as polymerase chain reaction, are prohibitive in terms of costs in most settings, and their diagnostic performance in smear-negative tuberculosis is controversial (Sarmiento *et al.* 2003). The recent Xpert MTB/RIF assay (Boehme *et al.* 2010) may become more affordable for less developed countries after its endorsement by WHO (2011b). However, technical requirements make it unsuitable as a tool for lower levels of care and, above all, its sensitivity for the diagnosis of SNPT is just around 0.75 (Chang *et al.* 2012). Our algorithm was not designed for primary health care

centres either, but we demonstrated that it can validly guide the management of SNPT suspects at levels where SNPT suspects are generally referred to: intermediate or reference hospitals with perhaps limited resources but radiographic and culture capacity.

The algorithm needs further adaptation before being applied at programmatic level or in a population with lower TB incidence, but our results are already of value for refining current SNPT diagnostic guidelines. The sequential use of sputum concentration and liquid culture, particularly Middlebrook 7H9, should be encouraged, and an antibiotic course should be disregarded as diagnostic tool. The role and place of CPRs definitely needs more operational research. Finally, research on the diagnosis of SNPT has focused on single tests. However, diagnostic test results should not be considered in isolation but contribute to the overall evidence on which a diagnosis is made (National Institute for Health & Clinical Excellence 2011). The thoughtful combination of diagnostic tools, the conceptual basis for algorithm design, should be the focus of future research rather than the quest for a single test.

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