Smear-Negative Pulmonary Tuberculosis

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Abstract

Tuberculosis (TB) is the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus. There are almost 2.5 million new smear-positive pulmonary TB cases and 1.9 million new smear-negative pulmonary TB cases in the world in 2012 according to the World Health Organization (WHO) Global Tuberculosis Report 2013. Smear negativity in pulmonary TB is a common clinical problem. Clinicians have difficulty in diagnosing smear-negative pulmonary TB without bacteriological confirmation. It is very important to decide whether or not to treat a patient with smear negative pulmonary TB when the culture results are pending or negative. New diagnostic methods are required for the diagnosis of smear-negative pulmonary TB. In addition to the development of new microbiological and serological diagnostic tests, clinical prediction scoring systems and algorithms including clinical and radiological findings of smear-negative pulmonary TB patients in our country, should be established to facilitate the diagnosis of smear-negative pulmonary TB.

Keywords: Pulmonary, smear negative tuberculosis, tuberculosis

INTRODUCTION

Pulmonary tuberculosis is still one of the most common infectious diseases in the world. According to the Tuberculosis Control Report published by World Health Organization (WHO) in 2013, approximately 2.5 million new smear-positive pulmonary tuberculosis and 1.9 million new smear-negative pulmonary tuberculosis (SNPT) patients were reported (1). It is seen that among new cases, the ratio of smear-positive pulmonary tuberculosis is 57% and most patients are SNPT.

When the data from Turkey were examined, 4595 new cases of smear-positive pulmonary tuberculosis and 3829 new cases of SNPT or smear-unknown pulmonary tuberculosis were detected in 2012 (2). Among new cases of pulmonary tuberculosis, the rate of smear-positive pulmonary tuberculosis is 54%. In Turkey, according to the 2012 Tuberculosis Control Report, 60.1% of the total pulmonary tuberculosis patients have smear-positive pulmonary tuberculosis, 28.5% have SNPT and 11.4% have smear-unknown pulmonary tuberculosis. The rate of smear-unknown pulmonary tuberculosis cases, which were 20.8% in 2005, has decreased with time (2). However, a relative increase has been observed in the following years in SNPT cases, whose rate was 22.4% in 2005.

Definition

There are serious unsolved problems regarding the definition and, accordingly, the diagnosis of SNPT. There are different recommendations in various guidelines. According to the WHO definition, SNPT is defined as follows:

a. Pulmonary tuberculosis cases with negative sputum smears but growth positive in cultures;
b. The patients who meet all of the four criteria stated below:
   1. At least two negative sputum smears,
   2. Radiological findings consistent with radiologically active pulmonary tuberculosis,
   3. No response to a course of broad-spectrum antibiotics (except patients with clinically validated HIV infection or with strong clinical findings suggesting a HIV infection),
   4. A decision made by a clinician for the treatment with antituberculosis medication (1).
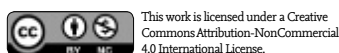
According to the Tuberculosis Diagnosis and Treatment Guideline published in Turkey 2011, SNPT is present in patients who have the following:

a. Negative sputum smears but growth positive in cultures,
b. Clinical and radiological findings consistent with tuberculosis, three negative sputum smears, and a decision of tuberculosis treatment in a hospital with opportunities for a differential diagnosis after no response has been obtained despite the use of broad-spectrum antibiotics (without quinolone) for at least a week (3).

There are differences as well as similarities in the two definitions. First, although WHO includes at least two smear-negativities in the definition, the Turkey guideline states that at least three smear-negativities are required. SNPT, which is defined as patients with smear-negative and with findings radiologically consistent with pulmonary tuberculosis in WHO, guideline is defined as patients with findings clinically and radiologically consistent with pulmonary tuberculosis in the guidelines of Turkey. For HIV-negative patients with a pre-diagnosis of SNPT, antituberculosis treatment should be initiated following the elimination of other pulmonary infections according to the response on the clinical and control chest X-ray after taking at least two sputum samples on different days and the use of quinolone-free antibiotics for a week on average (5–30 days).

In patients with a pre-diagnosis of SNPT, the selection and period of the antibiotic treatment is controversial. In antibiotic selection, the use of fluoroquinolones should be avoided because of the antituberculosis effectiveness. Samples for the causes of non-tuberculosis pulmonary infection should be taken from patients and further investigations should be performed. Trial treatments with antituberculosis medication are not recommended for patients with a pre-diagnosis of SNPT because the factors of the non-tuberculosis infection can respond to this treatment. The algorithm suggested by WHO for SNPT diagnosis in HIV-negative patients is shown in Figure 1 (4).

Smear-negative pulmonary tuberculosis occurs less frequently than smear-positive pulmonary tuberculosis, but it is an infectious communicable form of tuberculosis. In a cohort study conducted in Holland, it was revealed that smear-negative culture-positive pulmonary tuberculosis was responsible for 13% of the contagion of tuberculosis (5). In another study conducted in San Francisco, it was detected that at least 17% of the contagion of tuberculosis was due to SNPT (6). SNPT is an infectious and a communicable contagious disease. SNPT and extrapulmonary tuberculosis are responsible for 13-41% of the tuberculosis contagion (7).

Smear-negative pulmonary tuberculosis is differentiated from smear-positive pulmonary tuberculosis in some aspects. There are 1.22 SNPT and extrapulmonary tuberculosis cases per one smear-positive pulmonary tuberculosis patient in the society (8). In SNPT, minimal disease is more frequent because of small amount of bacillary load,
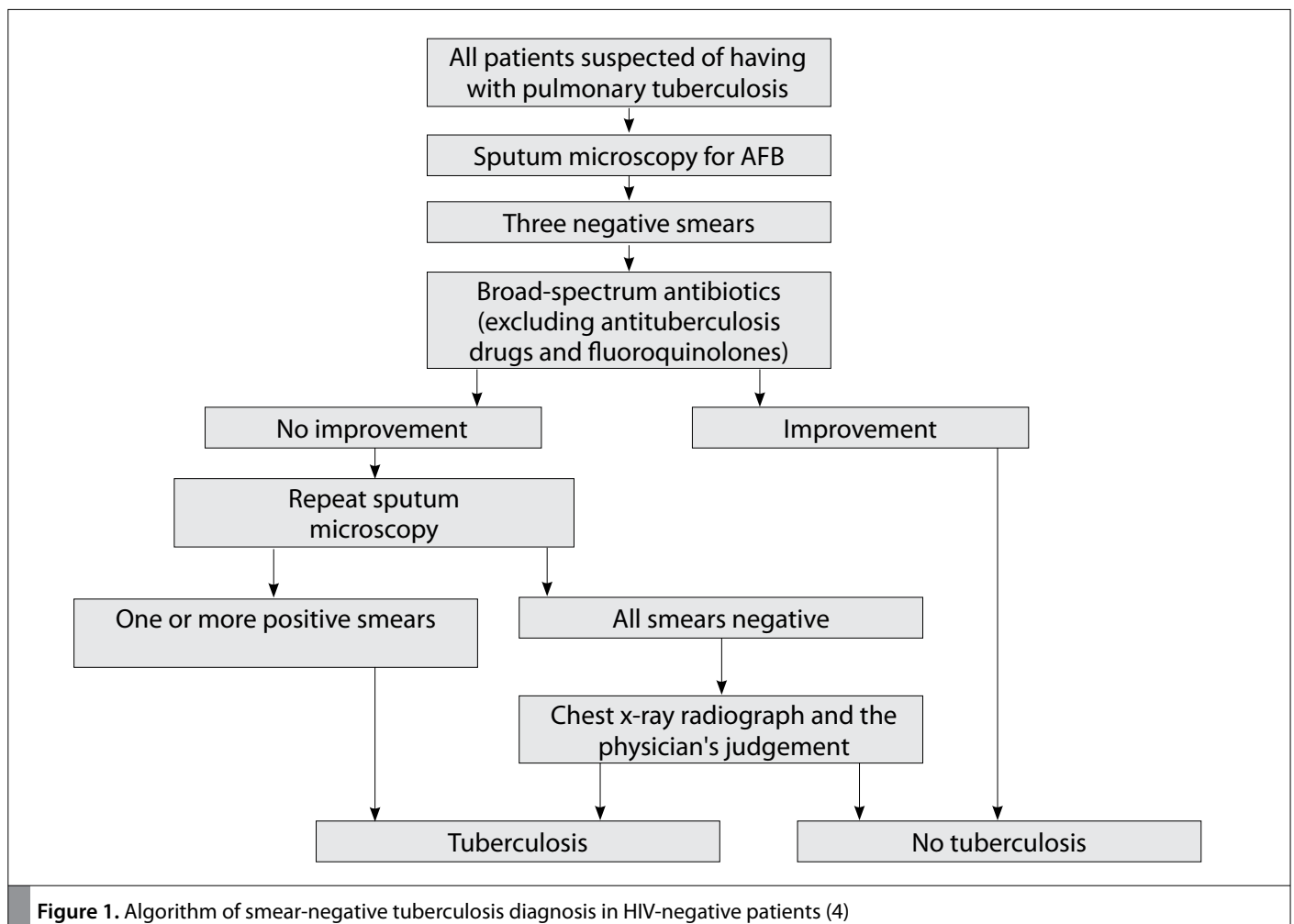

Figure 1. Algorithm of smear-negative tuberculosis diagnosis in HIV-negative patients (4)

and cavitation is observed less frequently compared to smear-positive pulmonary tuberculosis patients (9). Thus, contagiousness of SNPT cases is lower. Although approximately 70% of smear-positive pulmonary tuberculosis cases die in 10 years when there is no treatment, this rate is 20% for SNPT cases (1). According to these results, the mortality rate of SNPT is lower than of smear-positive pulmonary tuberculosis; however, patients with SNPT have high mortality rates and must be diagnosed and treated immediately.

Smear-negative pulmonary tuberculosis can be confused with many diseases because of diagnostic problems and therefore, its differential diagnosis is very important (9). Especially, it is very important to make a differential diagnosis of pneumonia and other pulmonary pathologies. In the differential diagnosis of SNPT, bacterial pneumonia, interstitial pneumonias, pulmonary nocardiosis, pneumocystis jiroveci pneumonia, cryptococcal pneumonia, and histoplasmosis are included.

Diagnosis

A. Microbiology
Isolation of *Mycobacterium tuberculosis* is the "golden standard" in tuberculosis diagnosis. Sputum, gastric juice, bronchial lavage, pleural fluid, tissue biopsy samples, synovial fluid, cerebrospinal fluid (CSF), and urine taken for this purpose are investigated for bacillus. Microscopic investigation of sputum is still the most commonly used method for tuberculosis diagnosis. Typically, the Ziehl–Neelsen procedure (Carbolfuchsin method) is used in the microscopic investigation of sputum smears. The sensitivity of the microscopic investigation of sputum is 20-60% and ≥80% in some cases (10). Instead of direct microscope, sensitivity is increased by methods such as processing of the phlegm with centrifuge and chemicals such as NaOH before smear investigations (10). Although microscopic investigation is the fastest diagnostic method present, its sensitivity is relatively low. For a positive result, samples must contain more than 10.000 bacilli per milliliter. The diagnostic success of the microscopic investigation depends on the prevalence of the disease, presence of cavity, and the quality of the sample. Sputum smears give a negative result for approximately half of the pulmonary tuberculosis patients. Causes of negative results of the sputum smear include insufficiency or mistakes during taking, collection, processing, or investigation of sputum (11). Accurate results can be obtained by improvements and caution. For the patients who cannot take out sputum, sputum induction, bronchoscopy, or gastric juice collection must be considered. The sensitivity of standard microscopy can be increased by 10% with fluorescent microscopy (12). Another advantage of fluorescent microscopy is that samples can be evaluated faster. Light emitting diode (LED) fluorescent microscopy is a valid alternative to traditional methods (short arc mercury lamp) and its low cost creates an important advantage (13).

Culture is still the reference standard method for pulmonary tuberculosis diagnosis. In the presence of 10–100 bacilli in a milliliter of the sample and culture, a positive result can be obtained. The sensitivity of sputum culture is around 81.5% and the specificity is around 98.4% (14). Solid mediums such as Löwenstein–Jensen or liquid mediums such as Middlebrook H12 are used for culture. In recent years, methods such as BACTEC 460TB, BACTEC MB9600, and MGIT, which provide rapid results, are also used. WHO suggests the use of both solid and liquid mediums for the culture of samples (1). An advantage of the culture apart from sensitivity and specificity is that it enables the identification of species, the test for drug resistance, and gene typing for epidemiological studies. Microbiological culture results are obtained in approximately 2–6 weeks. Because the culture results are obtained after a longer time for patients with a pre-diagnosis of SNPT, clinicians remain undecided on whether to initiate tuberculosis treatment. Thirty-eight percent of pulmonary tuberculosis patients are smear- and culture-positive, 48% are smear-negative and culture-positive, and 14% are smear-negative and culture-negative (11). All advanced investigations, including high-resolution CT (HRCT) of the thorax and bronchoscopy, should be performed for patients with SNPT who are culture-negative.

Routine usage of bronchoscopy for the diagnosis of SNPT is controversial. In most of the guidelines, there are no direct suggestions regarding this. Bronchoscopy is suggested as a support procedure for the diagnosis of SNPT (15, 16). Especially, when it is used with thoracic HRCT, its sensitivity is increased in SNPT patients (17). Postbronchoscopic sputum sampling both helps the diagnosis and increases the culture positivity by 7% (18). However, in the same study, increased contamination was observed in one-third of the patients from whom phlegm was taken after bronchoscopy. On the samples taken with bronchoscopy, nucleic acid amplification tests (NAATs) and investigations with Xpert MTB/RIF can be performed (19, 20).

B. Clinical Guess Score
A large number of patients who are given antituberculosis treatment because of pulmonary tuberculosis cannot be diagnosed bacteriologically with smear and culture despite advanced diagnostic opportunities. Clinicians experience difficulty in deciding whether to initiate the treatment for patients with a pre-diagnosis of pulmonary tuberculosis because it takes a long time to obtain culture results if the smear is negative. Antituberculosis treatment treats the disease, prevents its progress, and decreases the contamination. On the other hand, because of the possibility of a misdiagnosis and potential side effects of the medication, clinicians hesitate to start treatment.

To ease the diagnosis of SNPT, the clinical score and/or guess systems and a variety of management algorithms were developed, but very few of them were validated. Most of the studies were conducted in populations with a high HIV prevalence. Moreover, local differences between countries are present. Samb et al. (21) revealed that for patients with a pre-diagnosis of SNPT who were hospitalized, the symptoms associated with tuberculosis were (1) cough (>21 days), (2) chest pain (>15 days), (3) absence of sputum, and (4) absence of dyspnea. The presence of any two of these four symptoms was found to have 85% sensitivity and 67% specificity. In this study, the prevalence of HIV was high in patient and control groups (71%) and only hospitalized patients were included. Kanaya et al. (22) developed a tuberculosis prediction score (TPS) by identifying clinical, demographic, and radiological characteristics of patients. TGS is used for patients whose cultures are going to result positive among SNPT patients. Tuberculin skin test (TST) positivity is independently together with increased culture-positivity and in contrast, radiological non-typical findings for tuberculosis and cough with sputum are indicators of decreased culture positivity (Table 1). In places where the tuberculosis prevalence is high, a conservative threshold value of TGS≥0 should be used for tuberculosis treatment. In places where tuberculosis prevalence is low, waiting is required, instead of starting the empirical treatment, until the culture results are obtained. This study that was conducted

Table 1. Prediction system of tuberculosis in smear-negative lung tuberculosis (22)

Predictors	Multivariant OR (95% CI)	p value	Score	LR
			-2	0.2
Expectoration	0.3 (0.1-0.6)	0.002	-1	0.5
			0	1.8
TST positivity	4.8 (2.0-11.9)	0.001	+1	7.1
Atypical infiltration for TB	0.3 (0.1-0.7)	0.006	-1	
HIV positivity and mediastinal lymphadenopathy	7.2 (1.4-36.0)	0.02	+2	

HIV: Human immunodeficiency virus; LR: likelihood ratio; OR odds ratio; TB: tuberculosis; TST: tuberculin skin test

in a society with a low HIV and tuberculosis prevalence can also be suitable for Turkey. Various algorithms based on local epidemiological data and clinical score systems were developed for the diagnosis of SNPT (23). Most of them were validated only locally and not used commonly. However, in countries where SNPT is a serious public health issue, these algorithms can set a starting point for the development of diagnostic rules specific to that society and contribute to national tuberculosis programs (23).

C. Auxiliary Tests

Most SNPT patients are diagnosed on the basis of clinical and radiological findings. Laboratory tests for the diagnosis or for aiding diagnosis are also used. TST shows the infection, not the disease. TST can give positive result because of the sensitivity to atypical mycobacteria in societies using the BCG vaccine. Interferon gamma emission releasing assays (IGRAs) that have become common lately and do not get affected by BCG are also not suggested for the diagnosis of the active disease. IGRA is suggested in Turkey for patients who are TST negative (with booster), who are highly suspected of a tuberculosis infection, who have suppressed immunity, or who are candidates for suppressive treatment (3). Nucleic acid amplification test has low sensitivity (40.4–44.2%) especially in smear-negative samples (24). Gene Xpert is a new molecular test recently used in tuberculosis treatment frequently. This test is suggested by WHO for HIV-positive patients or for patients suspected of tuberculosis resistant to drugs (1). In SNPT patients, the sensitivity of Gene Xpert for one sample is 50-70% and it increases with the sample number (25). Positivity of the Gene Xpert test has been found to be related to the basil load in the sample (26). Sensitivity of Anda-TB IgG, which is a serological diagnostic test developed in recent years, in smear-positive patients is 63-85% and its specificity is 73-100% and no sufficient information about the use of this test in smear-negative patients is available (27). WHO suggests not using serological tests in pulmonary and extrapulmonary tuberculosis diagnosis because of the inconsistency and impreciseness of their sensitivity and specificity values (28).

D. Radiology

Radiology is an extremely valuable diagnostic tool for SNPT. The radiological findings of SNPT patients are different from those of smear-positive patients. Radiological changes in SNPT patients are

less significant and cavitation is less frequently seen (23). The specificity of chest X-ray in pulmonary tuberculosis diagnosis is high (78%) and its sensitivity is low (51%) (29). The low sensitivity of the chest X-ray limits its usage. Radiological findings differ between primary tuberculosis and secondary (reactivation, postprimary) pulmonary tuberculosis. In primary tuberculosis, parenchymal disease, lymphadenopathy (43-96%), miliary disease (1-7%), and pleural effusion (25%) are seen most commonly (30). In postprimary tuberculosis, parenchymal disease (consolidation, cavity), airway involvement (10-40%), and pleural involvement (18%) are observed more commonly (30). Pulmonary tuberculosis typically involves the apical and posterior segments of the upper lobes in 85% of patients. In a lower rate, tuberculosis is detected in the anterior segment of the upper lobe and anterior segment of the lower lobe. Infiltration and cavity screenings are seen typically on chest X-rays. Cavities seen in pulmonary tuberculosis are generally thin walled. Acinar infiltration and pneumonia can arise in the lower lobes in association with endobronchial spread. HRCT enables the lesions that cannot be seen on chest X-ray to be monitored (31). Based on the HRCT findings, guessing systems that predict sputum smear positivity have been previously tried to be developed (32). In SNPT patients, many, lesions have been identified on HRCT. By evaluating radiological findings and localizations in these patients, it has been suggested that HRCT can be used for guessing patients with high risk of SNPT (33). The most common radiological findings detected on HRCT in SNPT patients are centrilobular nodules (73%), nodules (71%), and consolidation (54%) (34).

Treatment

In Tuberculosis Diagnosis and Treatment Guideline published in 2011, the quadruple therapy of H (isoniazid), R (rifampicin), Z (pyrazinamide), and E (ethambutol) is recommended for 2 months at the beginning of SNPT treatment. Subsequently, during the maintenance period, the dual therapy of HR is recommended for 4 months and standard antituberculosis treatment is recommended for 6 months (3). In the tuberculosis treatment guideline published by WHO in 2010, the suggested treatment regime, including triple therapy of HRZ for 2 months and dual therapy of HR for 4 months, was switched to quadruple therapy of HRZE for 2 months, dual therapy of HR for 4 months, and standard treatment for 6 months (35).

CONCLUSION

Smear-negative pulmonary tuberculosis is still one of the most problematic issues of tuberculosis. In preliminary diagnosed SNPT patients, microbiological samples (sputum, induced sputum, gastric juice, bronchoscopy, and bronchoalveolar lavage, when necessary) should be taken, radiological investigations (chest X-ray, HRCT included, where necessary) should be performed, elimination of the diseases included in the differential diagnosis should be made, and antituberculosis treatment should be initiated following the diagnosis of SNPT established by experienced centers after completion of all these procedures. In recent years, important developments in the diagnosis of SNPT have been reached with advanced radiological examinations such as HRCT and new microbiological diagnostic methods. In our country, new studies that can create clinical guessing score systems and algorithms, including clinical, laboratory, and radiology, and the development of diagnostic tests with high sensitivity are warranted.

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