REVIEW

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Diagnosis of Pulmonary Tuberculosis: Recent Advances and Diagnostic Algorithms

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Pulmonary tuberculosis (TB) persists as a great public health problem in Korea. Increases in the overall age of the population and the rise of drug-resistant TB have reinforced the need for rapid diagnostic improvements and new modalities to detect TB and drug-resistant TB, as well as to improve TB control. Standard guidelines and recent advances for diagnosing pulmonary TB are summarized in this article. An early and accurate diagnosis of pulmonary TB should be established using chest X-ray, sputum microscopy, culture in both liquid and solid media, and nucleic acid amplification. Chest computed tomography, histopathological examination of biopsy samples, and new molecular diagnostic tests can be used for earlier and improved diagnoses, especially in patients with smear-negative pulmonary TB or clinically-diagnosed TB and drug-resistant TB.

Keywords: Lung; Tuberculosis; Diagnosis

Introduction

Tuberculosis (TB) is a global health concern for both developing and developed countries and has recently become more complex due to persistence in aging populations and the rise of drug-resistant strains, even in Korea[1,2]. In clinical practice, rapid TB diagnosis can be difficult, and early pulmonary TB detection continues to be challenging for clinicians. Prompt diagnosis of active pulmonary TB is a priority for TB control, both for treating the individual and for public health intervention to reduce further spread in the community[3]. Chest X-ray is useful but is not specific for diagnosing pulmonary TB. Moreover, TB can present with symptoms and atypical radiologic findings that are indistinguishable from those of community-acquired pneumonia[4,5]. As a result, it is not unusual for clinicians to prescribe a number of courses of antibiotics for pneumonia before the pulmonary TB is correctly diagnosed[6,7]. Therefore, an acid-fast bacilli (AFB) smear and bacteriological culture tests should be performed for patients with symptoms that are compatible with or suggestive of TB. However, mycobacterial culture, which has the highest sensitivity for diagnosing and confirming active TB, requires 2 to 6 weeks for interpretation[3]. Although sputum smear microscopy is a rapid, simple, and inexpensive tool for diagnosing pulmonary TB, it has low and variable sensitivity[3]. Recently, non-molecular and molecular assays have been developed for early detection of active TB with or without drug resistance detection. The diagnosis of TB is suspected from a combination of context, symptoms, clinical signs and investigations. Pulmonary TB refers to any bacteriologically-confirmed or clinically-diagnosed case of TB that involves the lung parenchyma or the tracheobronchial tree based on the revised previous standard case definitions for TB by the World Health Organization (WHO) in 2013[8].

In this article, recent advances that allow better and earlier

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diagnosis of active pulmonary TB are summarized and diagnostic algorithms in clinical practice are recommended, focusing on the intermediate burden setting in Korea, based on updated literature reviews and standard guidelines[3,9-13].

Diagnostic Methods

1. Radiologic study

Anyone with a cough that lasts for two weeks or more or with unexplained chronic fever and/or weight loss should be evaluated for TB[11]. Chest X-ray is the primary radiologic evaluation of suspected or proven pulmonary TB. Radiological presentation of TB may be variable but in many cases is quite characteristic. Radiology also provides essential information for management and follow-up of these patients and is extremely valuable for monitoring complications. Chest X-ray is useful but is not specific for diagnosing pulmonary TB, and can be normal even when the disease is present[4,5]. Therefore, it cannot provide a conclusive independent diagnosis and needs to be followed by sputum testing. Many of the so-called unusual manifestations of adult TB are actually usual manifestations of primary disease. Post-primary TB in adults typically manifests as a heterogeneous, often cavitary opacity in the apical and posterior segments of the upper lobes and the superior segments of the lower lobes. Lymphadenopathy is rare. Cavitation is the hallmark of post-primary TB and appears in about half of all patients. Patchy, poorly defined consolidation in the apical and posterior segments of the upper lobes and in the superior segment of the lower lobe is also commonly observed[4,5,14,15]. However, post-primary disease activity cannot be accurately assessed by chest radiography. Radiographic stability for 6 months and negative sputum cultures is the best indicator of inactive disease[16]. The descriptive terms 'inactive' or 'old' TB should be discarded in favor of radiographically stable TB, as viable bacilli may persist despite adequate therapy. Post-primary TB heals with parenchymal scarring and nodules. An important task for radiology is to determine whether these residual findings are indicative of active disease. For this, chest X-ray has limited value, since it can only establish that a lesion is stable, and stable lesions can contain active bacilli[4,5].

Although chest X-ray is the primary diagnostic tool for evaluating pulmonary TB, chest computed tomography (CT) is generally required to detect fine lesions that can be overlooked on chest X-ray, to define equivocal lesions, or to evaluate complications[4,14]. Chest CT is an effective diagnostic method when plain films are normal or inconclusive, and it provides valuable information for managing the illness. Chest CT can add valuable information for detecting bacterial activity. Branching opacities, cavitation, or consolidation are clear signs of active TB, but active disease must be confirmed by analyzing sputum for the presence of bacilli. A significant radiologic finding in chest CT is the "tree-in-bud" pattern, consisting of multiple branching linear structures that represent bronchogenic dissemination of disease with caseating necrosis in the respiratory and terminal bronchioles[14]. These branching opacities have a lobar or segmental distribution and are considered reliable markers of activity. Tree-in-bud opacities are also seen in other infections, but when visualized in combination with cavitation or nodular opacities in the upper and posterior lung segments, and in the appropriate clinical setting, a specific diagnosis of pulmonary TB can be established[17]. While chest CT is useful for clarifying confusing findings, it has not been conclusively shown to have a significant impact on patient management[14], therefore microbiological identification of TB by culture should follow this test[11].

2. AFB smear microscopy and culture

For pulmonary TB, sputum is the most critical sample for laboratory testing. Direct sputum smear microscopy is the most widely used method for diagnosing pulmonary TB and is available in most primary health-care laboratories at the health-center level[3,12]. Smear microscopy may, however, be costly and inconvenient for patients, who must make multiple visits to health facilities and submit multiple sputum specimens over several days. Fortunately, good-quality microscopy of two consecutive sputum specimens has been shown to identify the vast majority (95%–98%) of smear-positive TB patients[18,19]. The WHO policy on case detection by microscopy was, therefore, revised to recommend a reduction in the number of specimens examined, from three to two in settings with appropriate external quality assurance and documented high-quality microscopy[18]. In addition, the WHO recommends that countries that have successfully implemented current WHO policies for a two-specimen case-finding strategy consider switching to same-day diagnosis, especially in settings where patients are likely to default from the diagnostic process[18]. The Korean guideline for TB recommends that presumptive TB patients should have multiple sputum samples, at least two, possibly three, and allows first sputum sample for front-loaded or same-day microscopy[11].

Conventional light microscopy of Ziehl-Neelsen–stained smears prepared directly from sputum specimens is the most widely available test for diagnosing TB in resource-limited settings. Ziehl-Neelsen microscopy is highly specific, but its sensitivity is variable (20%–80%). Conventional fluorescence microscopy is more sensitive (10%) than the Ziehl-Neelsen and takes less time, but it is limited by the high cost of mercury vapor light sources, the need for regular maintenance, and the dark room requirement[20]. Light-emitting diodes (LED) have been developed to offer fluorescence microscopy without the associated costs. The efficacy of LED microscopy was assessed by the WHO and evaluated by standards appropriate for the accuracy and the effect of new TB diagnostics on both

patients and public health[21]. The results showed that the accuracy of LED microscopy was equivalent to that of international reference standards; it was more sensitive than conventional Ziehl-Neelsen microscopy, and it had qualitative, operational, and cost advantages over both conventional fluorescence and Ziehl-Neelsen microscopy. Based on these findings, the WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy[21].

Laboratory diagnosis of TB relies on direct microscopic examination of sputum specimens. However, the technique, although specific, has low and variable sensitivity and cannot identify drug-resistant strains. Clinicians have been advised to obtain culture confirmation of TB whenever possible. This not only confirms the diagnosis, but also obtains material for crucial drug susceptibility testing (DST)[3,9,12]. Mycobacterial culture is more sensitive, but growth of TB bacilli on traditional solid medium requires 4–8 weeks, which delays appropriate treatment in the absence of a confirmed diagnosis. Culturing mycobacteria is mainly done on solid media, the Lowenstein-Jensen slope, or in broth media. These methods are slow, with cultures from microscopy-positive material taking from 2–4 weeks and for microscopy-negative material from 4–8 weeks. Therefore, liquid media remains the mycobacteriology gold standard for initial isolation, because it is significantly faster (between 10 and 14 days) and is better for isolation, compared to solid media. For DST, the delay may be reduced to as little as 10 days compared to 4–6 weeks with conventional solid media. Liquid systems are more sensitive for detecting mycobacteria and may increase the case yield by 10% over solid media. With increased sensitivity and reduced delays, liquid systems may contribute significantly to improved patient management. Liquid systems are, however, more prone to contamination by other microorganisms. In experienced laboratories, approximately 5%–10% of specimens fail to yield results because of contamination. Procedures to prevent cross-contamination (due to carryover of bacilli from positive to negative specimens) should also be strictly followed, especially where increased numbers of positive specimens are processed in high-incidence countries[22]. Solid media are made of agar, egg, and malachite green to limit the growth of remaining contaminants (Lowenstein, Stonebrink, or Ogawa medium). Liquid culture and DST systems are more complex and sensitive than solid culture and DST media. Increased bacterial contamination and an increased frequency of nontuberculous mycobacterial (NTM) isolation must be addressed. A rapid method to differentiate the *Mycobacterium tuberculosis* complex from other mycobacterial species is essential. Several manufacturers have recently marketed tools that can automatically detect *M. tuberculosis* growth in the laboratory, such as the Bactec "Mycobacterial Growth Indicator Tube 960" (MGIT 960; Becton-Dickinson, Sparks, MD, USA) and the MB/Bact Alert 10 3D (Biomérieux, Durham, NC, USA). Unfortunately, these automatized incubators are expensive, they do not give rapid mycobacterial species' identification, and they do not identify contaminated or mixed cultures. Conversely, cultures on solid media provide all of this information with a simple observation of colonies. The current guideline recommends that all specimens cultured on liquid media also be inoculated on solid media to ensure purity and sufficient strengthen for the diagnosis[9,11].

3. Molecular methods

1) Nucleic acid amplification testing: Nucleic acid amplification (NAA) tests are a reliable way to increase the specificity of diagnosis, but the sensitivity is too poor to rule out disease, especially in smear-negative (paucibacillary) disease where clinical diagnosis is equivocal and where the clinical need is greatest[23,24]. Compared with AFB smear microscopy, the added value of NAA testing lies in (1) its greater positive predictive value (PPV) (>95%) with AFB smear-positive specimens in settings in which NTMs are common and (2) its ability to rapidly confirm the presence of *M. tuberculosis* in 50%–80% of AFB smear-negative, culture-positive specimens. Compared with culture, NAA tests can detect the presence of *M. tuberculosis* bacteria in a specimen weeks before culture for 80%–90% of patients suspected to have pulmonary TB whose TB is ultimately confirmed by culture[23-25]. Although NAA testing is recommended to perform the initial diagnosis of persons suspected to have TB, the currently available NAA tests should not be ordered routinely when the clinical suspicion of TB is low because the PPV of the NAA test is <50% for such cases[26].

The United States' Centers for Disease Control and Prevention (CDC) recommends that NAA testing should be performed on at least one respiratory specimen using a Food and Drug Administration (FDA)–approved test[23] from each patient that has signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established and for whom the test result would alter case management or TB control activities such as contact investigations. A single negative NAA test result should not be used as a definitive result to exclude TB, especially when clinical suspicion of TB is moderate to high. Rather, a negative NAA test result should be used as additional information in making clinical decisions, to expedite testing for an alternative diagnosis, or to prevent unnecessary TB treatment. Clinicians should interpret all laboratory results on the basis of the clinical situation[25]. The Korean guidelines for TB with intermediate setting recommend that when an individual is suspected of having pulmonary TB, NAA tests should be performed at least once in combination with AFB smear microscopy and culture[11]. NAA tests cannot replace culture and microscopy but should be interpreted along with conventional tests and clinical data

for diagnosing TB. NAA tests are also not useful for monitoring treatment progress since they can detect non-viable bacteria and give false-positive results; however, they can distinguish *M. tuberculosis* from NTM[25]. Smear microscopy or clinical presentation cannot differentiate between TB and NTM, and the recovery rate of NTM from AFB smear-positive sputum specimens is steadily increasing in Korea. Therefore, NAA tests could be a useful diagnostic test for patients with AFB smear-positive sputum for rapidly detecting pulmonary TB and differentiating from NTM[27].

2) Line probe assay: Conventional methods for mycobacteriological culture, identification of an *M. tuberculosis* complex and DST are slow and cumbersome, therefore, rapid DST of isoniazid and rifampicin or of rifampicin alone using molecular technologies is recommended over conventional testing in sputum smear-positive or culture proven cases at risk of multi-drug resistant (MDR)-TB, such as previously-treated patients[11,28]. Line probe assay (LPA) has generally been available for this purpose in rapid DST and is a type of molecular assay that can allow specific gene markers associated with rifampicin resistance alone or in combination with isoniazid to be detected. Resistance to isoniazid occurs primarily due to mutations in *kat*G, followed by mutations at the InhA active site (20%–35%) and in the promoter region of *ahp*C. Mutations in the *rpo*B region are found in about 96% of rifampicin -resistant *M. tuberculosis* isolates[29,30].

LPA technology is performed in the following steps, including extracting DNA from mycobacterial isolates or directly from clinical specimens, polymerase chain reaction (PCR) amplification of the resistance-determining region of the gene, hybridization of labeled PCR products with oligonucleotide probes immobilized on a strip, and colorimetric development that allows for lines to be seen where the probes are located. According to systematic reviews and meta-analyses to evaluate assay performance, results that compared conventional DST methods showed that LPA are highly sensitive (≥97%) and specific (≥99%) for detecting rifampicin resistance, alone or in combination with isoniazid (sensitivity ≥90%; specificity ≥99%), in *M. tuberculosis* isolates and in smear-positive sputum specimens[28]. However, LPA cannot replace conventional culture with DST, and mycobacteriological culture for smear-negative specimens with DST because second-line anti-TB drugs are still required.

3) Xpert MTB/RIF (Cepheid): The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA; hereafter referred to as Xpert MTB/RIF) is a novel, rapid, automated, and cartridge-based NAA test that can detect TB along with rifampicin resistance directly from sputum within 2 hours of collection[31]. The GeneXpert cartridges are pre-loaded with all of the necessary reagents for sample processing, DNA extraction, amplification, and laser detection of the amplified *rpo*B gene target. A major advantage of the Xpert MTB/RIF test is that it can be accurately administrated with minimal hands-on technical time. The sensitivity and specificity of this test has been reported to be acceptable for TB detection[31,32]. The Xpert MTB/RIF test is a valuable, highly sensitive, and specific new tool for early TB detection and for determining rifampicin resistance. While it should be noted that mono-resistance to rifampicin is found in approximately 5% of rifampicin-resistant strains, a high proportion of rifampicin resistance is associated with concurrent resistance to isoniazid (~95%). Thus, detecting resistance to rifampicin can be used as a marker for MDR-TB with a high level of accuracy[9,31-33].

In a recent study of the performance of Xpert MTB/RIF, among the 561 culture-positive patients (561/1730), a single, direct Xpert MTB/RIF test identified 98.2% (551 out of 561) of the sputum smear-positive TB cases and 72.5% (124 out of 171) of those with sputum smear-negative TB. The test was specific in 604 of 609 patients (99.2%) not affected by TB. A second Xpert MTB/RIF test among patients with sputum smear-negative, culture-positive TB increased detection sensitivity by 12.6% and a third by 5.1%, to reach 90.2%. When compared to phenotypic DST, the Xpert MTB/RIF assay correctly identified 97.6% (200 out of 205) of patients harboring rifampicin-resistant strains and 98.1% (504 out of 514) of those with rifampicin-susceptible strains[34]. The WHO issued initial recommendations on Xpert MTB/RIF, especially for individuals suspected of having MDR-TB[33]. Xpert MTB/RIF has higher sensitivity for TB detection in smear-positive patients than in smear-negative patients; nonetheless, this test may be valuable as an add-on test following smear microscopy in patients previously found to be smear-negative[13]. Recently, the International Standards for TB Care recommended that Xpert MTB/RIF and/or sputum cultures should be performed in patients suspected of having pulmonary TB but that have negative sputum smears[12]. However, in settings or patient groups where rifampicin resistance is rare, the PPV of Xpert MTB/RIF is adversely affected, and is significantly lower when rifampicin resistance prevalence falls below 5%. Therefore, testing new TB cases that are not at risk of MDR-TB in low MDR-TB prevalence settings, including Korea, results in a low PPV, which requires rifampicin resistance confirmation by phenotypic DST or LPA (and not by a second Xpert MTB/RIF test) prior to initiating treatment[32]. However, a recently updated WHO policy recommends that a repeated Xpert MTB/RIF test on a fresh specimen can be useful when Xpert MTB/RIF detects *M. tuberculosis* with rifampicin resistance in patients considered to be at low risk of MDR-TB[35]. The tests are expensive, so careful consideration should be given in domestic circumstances to the resource implications of offering routine Xpert MTB/RIF testing. The current Korean guidelines recommend that the Xpert MTB/RIF test be used in patients suspected of having TB and are at high risk of MDR-TB, such as previously-treated cases or human immunodefieciency

virus–positive TB and severe cases of pulmonary TB[11].

Diagnostic Algorithms of Pulmonary Tuberculosis

1. Smear-positive pulmonary TB

People suspected of having TB should be referred for medical evaluation. A posterior-anterior chest X-ray should be taken, and a chest X-ray that appears suggestive of TB should be followed by further diagnostic investigation. Multiple sputum samples (at least two, possibly three samples) that allow at least, first one front-loaded sputum specimen should, if possible, be sent for TB microscopy and cultured for suspected respiratory TB before starting treatment[11]. Spontaneously produced sputum should be obtained if possible; otherwise, induction of sputum or bronchoscopy and lavage can be considered[36,37].

NAA tests can rapidly confirm TB diagnosis and distinguish *M. tuberculosis* from NTM in a sputum smear-positive person. Positive results for both the NAA and the AFB smear tests strongly suggest that the patient has TB, and anti-TB treatment should be initiated while awaiting culture results. The PPV of FDA-approved NAA tests for TB in the United States is >95% in AFB smear-positive cases. If the NAA result is negative and the AFB smear result is positive, a patient can be presumed to have an NTM infection[11,25]. Culture remains the gold standard for laboratory confirmation of TB and is required for isolating bacteria for DST and genotyping[11].

2. Smear-negative pulmonary TB

Current TB diagnostics are still suboptimal in performance, especially for smear-negative TB. Because TB can present with many different symptoms, the first obstacle in diagnosing smear-negative TB is discerning the varied clinical presentations, to determine which conditions are highly suspicious and should be included in the differential diagnosis. A diagnostic approach to an AFB smear-negative patient with possible TB includes, where available, a detailed medical history and clinical examination as well as radiological, microbiological, molecular, and histological investigations[38,39]. In patients suspected of having TB with smear-negative, culturing remains essential for diagnostic testing and should be included when evaluating patients suspected of having TB with negative sputum smears. When performed correctly, culture increases diagnostic sensitivity, which ideally results in earlier case detection. The state-of-the-art diagnostic path often uses the tests mentioned above in combination, and additional diagnostic tools/procedures such as induced sputum[37], chest CT imaging[14,17,40], bronchoscopy with lavage[36,41], and lung tissue biopsy[42,43] are sometimes performed.

NAA testing is recommended to perform the initial diagnosis of patients that are suspected to have TB, even in smear-negative patients. If the NAA result is positive and the AFB smear result is negative, the clinician must consider the case as TB positive and begin anti-TB treatment while awaiting culture results[11,25]. If sputum smears and NAA tests are negative, and TB is still suspected, cultures are the most sensitive tests for TB. Culture is therefore very useful for diagnosing both smear-negative TB and drug-resistant TB. Although new diagnostic methods can decrease turn-around time with considerable diagnostic accuracy and convenience, they are not a replacement for culture and conventional DST[25,44-46]. However, in certain TB cases that are caused by pauci-bacillary disease, where only a small number of *M. tuberculosis* organisms are present, cultures can be negative; in addition sampling error, or technical problems can occur. In cases where the culture is negative, the standard used to compare with the diagnostic test could be response to treatment, clinical features, or a positive culture in the future. A TB diagnosis in this population would likely be achieved on a case-by-case basis and as such, would probably not be reported in many studies[9,38,39].

Diagnosing TB worldwide still consists of methods that are intended to isolate the pathogen, which is a major limitation when the mycobacterial load is low or the site of infection is not easily accessible. For these reasons, diagnosis of smear-negative TB is often delayed, and such a diagnosis is often made based on the clinical response to empiric anti-TB treatment without microbiological confirmation[38,39,44]. Diagnostic algorithms have been recommended in the absence of rapid, simple, and accurate diagnostic tools for smear-negative pulmonary TB[11,38,39,47]. Diagnosing smear-negative pulmonary TB is achieved with at least two adequate expectorated multiple sputum samples, with negative smears, chest radiography findings consistent with TB, and a lack of response to a number of days of broad-spectrum antibiotics while awaiting culture results. For such patients, sputum cultures should be obtained and active follow-up is needed[25,44]. There are several limitations to using diagnostic steps based on antibiotics response. Because the fluoroquinolones and aminoglycosides are active against the *M. tuberculosis* complex and may cause transient improvement in people with TB, they should be avoided in this diagnostic phase. Strict adherence to the sequential steps of the algorithm could delay appropriate treatment in patients with an illness that is worsening rapidly. Finally, studies have shown that patients with TB might respond, at least transiently, to empirical broad-spectrum antibiotics, a frequent element of diagnostic algorithms[7,48]. Recently, the WHO revised the TB definition to include clinically-diagnosed TB instead of smear-negative TB. A clinically-diagnosed TB case is one that does not fulfill the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. Clinically-diagnosed cases subse-

quently found to be bacteriologically-positive (before or after starting treatment) should be reclassified as bacteriologically confirmed[13].

Clinicians who manage patients with suspected TB should ensure that their diagnostic practices align with the guidelines for TB, and use sputum smear microscopy and culture to investigate adult patients with suspected active TB[3,9,11]. Novel methods allow for rapid diagnosis of active TB in patients with negative sputum smears for AFB and enable prompt, highly accurate identification of drug-resistant strains of *M. tuberculosis* directly from respiratory specimens. Some of the structural limits of current TB diagnostics are likely to be overcome by such new tools, but research is still needed. The success of implementing new technologies and the development of additional diagnostic approaches must take into account the diagnostic needs of each context (point-of-need testing approach) together with the logistic, economic and technical constraints that are present in the majority of intermediate-TB-burdened settings in Korea.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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