

Changing Approaches to the Diagnosis of Tuberculosis

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Since this subject was last reviewed in the *American Journal of Respiratory and Critical Care Medicine* (1), new diagnostic tests for tuberculosis have been approved for use in the United States, Europe, and other industrialized regions, and there has been significant clinical experience with their use. In addition, several other new diagnostic assays have been studied and may be nearing approval by the Food and Drug Administration (or similar agencies in other countries) for routine clinical use. This commentary will review the need for new diagnostic tests for tuberculosis, examine the status of currently available tests and of several promising tests in development, and discuss potential scenarios in which such diagnostic tests might be used.

THE NEED FOR NEW DIAGNOSTIC TESTS FOR TUBERCULOSIS

In the absence of a truly effective tuberculosis vaccine (and perhaps even if such a vaccine were available), treatment of active cases remains the most important component of tuberculosis control programs. For such treatment activities to be efficient and effective, rapid and accurate diagnosis is a must. Both in developed industrialized nations and in resource-poor countries, a compelling case can be made that new diagnostic tests (both more rapid and more accurate than currently available approaches) for tuberculosis can have a substantial impact on tuberculosis control activities (2).

In many regions, including many parts of the United States for example, the value of the sputum smear examination for acid-fast bacilli has been diminished by the increasingly common phenomenon of *Mycobacterium avium* complex organisms being found in the sputum of patients in whom tuberculosis is a realistic diagnostic possibility, such as elderly persons with cough and abnormal chest radiographs, or patients with human immunodeficiency virus (HIV) infection. This has resulted in a marked decrease in the specificity and positive predictive value of the sputum smear, in some cases to as low as 50% (3). In clinical practice, the value of a test with a sensitivity of 50% and a specificity of 95% is vastly greater than a test with the same sensitivity but a specificity of only 50%. In the first case the positive predictive value in a population with a disease prevalence of 10% is 53%, whereas in the second case it is only 10%. Negative predictive values would be 94% and 50%, respectively. On the other hand, a test that rapidly distinguishes *Mycobacterium*

tuberculosis from *M. avium* complex in this setting can spare the health department from beginning unnecessary contact tracing activities for example, and can allow limited resources to be allocated to more useful endeavors. This may have happened to a certain degree with the widespread availability of nucleic acid amplification tests in the United States and other countries, although definitive utility of these tests in these settings has not been rigorously demonstrated.

In the absence of a rapid and accurate diagnostic test for tuberculosis, there are substantial economic costs related to isolation of patients and unnecessary empiric drug treatment (4). In addition, such treatment may have significant costs to patients in terms of adverse effects.

In resource-poor countries, emphasis has historically been on diagnosis and treatment of smear-positive cases of tuberculosis. This has occurred for several reasons. First, because of the expense involved, cultures for mycobacteria are not widely available in many parts of the world, and diagnosis rests mainly on clinical findings, radiographs, and sputum smear examination. Second, smear-positive cases account for the vast majority of instances of transmission of infection, and that treatment of such cases would have the greatest impact in reducing the spread of tuberculosis throughout the population. The limitations of this reasoning, however, are obvious. First, a substantial percentage of tuberculosis cases, even in poor countries, are smear negative, and delayed diagnosis of such cases undoubtedly has a harmful effect on individual patients, who are essentially being forced to develop more advanced disease (with undoubtedly more permanent sequelae, such as loss of pulmonary function) before treatment can be initiated. Second, although it is certainly the case that smear-positive patients are responsible for most transmission of tuberculosis through a population, recent studies using restriction fragment length polymorphism (RFLP) analysis indicate that smear-negative cases of tuberculosis contribute much more to ongoing transmission than has previously been believed (5), and this effect is likely greater as smear-negative cases progress. Thus, both in terms of individual patient outcomes and control of tuberculosis in a population, there is a significant cost incurred by restricting diagnosis to smear-positive cases alone.

In both resource-poor and resource-rich countries, clinical algorithms (based mainly on patient characteristics and radiographic findings) can raise the pretest likelihood for tuberculosis to 50% or more (6–8). This may help define a population in which more sophisticated diagnostic testing is warranted, but obviously clinical features alone cannot provide a sound basis for diagnosis of tuberculosis on a widespread basis.

NEW DIAGNOSTIC TESTS FOR TUBERCULOSIS

Tests for Active Tuberculosis

Broth-based culture systems. It is worth noting that broth-based culture systems such as BACTEC, MGIT (a nonradiometric

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method), MB/BacT, Septi-Check, and ESP, when combined with DNA probes for rapid species identification, are capable of producing positive results in 2 wk or less for the vast majority of sputum smear-positive specimens, and within 3 wk for smear-negative specimens (9–11). The value of culture is currently unsurpassed as it is the only widely available technology that allows for drug susceptibility testing.

Nucleic acid amplification assays. There are currently two nucleic acid amplification assays available for commercial use in the United States: MTD (GenProbe) and Amplicor (Roche), and the MTD test has been approved for use in smear-negative cases in which the clinical suspicion is high. The MTD assay is an isothermal strategy for DNA amplification, and the Amplicor kit uses the polymerase chain reaction (PCR) to amplify unique nucleic acid targets that uniquely identify *M. tuberculosis* in clinical specimens. Despite these different approaches to amplification of target DNA regions of interest, substantial published experience indicates that the tests are roughly equivalent in clinical use (12, 13). Each test accurately diagnoses nearly every case of sputum smear-positive pulmonary tuberculosis, and each will also diagnosis about half the cases of smear-negative, culture-positive pulmonary tuberculosis. Based on these operating characteristics, the FDA in the United States initially approved these tests for the confirmation of a positive sputum smear in a case of possible pulmonary tuberculosis.

In an American Thoracic Society workshop it was recently asked, regarding the role of rapid diagnostic tests for tuberculosis, “What is the appropriate use?” (14). The published experience with currently available nucleic acid amplification (NAA) assays indicates that they perform well for the indications for which they were approved, namely the reliable confirmation that a smear-positive sputum specimen indeed represents *M. tuberculosis*. However, the literature also suggests that the use of these tests might be greatly expanded and allow more accurate diagnosis of pulmonary tuberculosis generally than is now accomplished (12, 13). In a recent comparison of both commercially available nucleic acid amplification assays with conventional smear and culture, the specificity of all techniques was extremely high, but the sensitivity varied widely. For smear, sensitivity was an expected 50%, and for culture it was nearly 100%. For the NAA assays, sensitivity was in the 80–84% range, also an expected value given the repeated demonstration that these tests detect all of the smear-positive cases of tuberculosis and about half of the smear-negative, culture-positive cases. Thus, the overall accuracy of the NAA assays ($[\text{true positives} + \text{true negatives}]/\text{all test results}$) was much higher than that of smear and not very much lower than that of culture. Newer versions of these tests also appear to have a significantly improved ability to detect smear-negative cases as well (15). In other words, if NAA assays are used to ask the question “Does my patient have active pulmonary tuberculosis?” the answer will be correct 92–95% of the time, as compared with 80% of the time if smears are used. (This will be true as long as the patient in question has no history of recently treated tuberculosis, a setting in which NAA assays may be less accurate.) In fact, if the cost of these assays were low enough, they could and should replace sputum smears for the purpose of making a diagnosis of pulmonary tuberculosis in previously untreated patients, if the specificity of the NAA was equal to or greater than that of the acid-fast bacillus (AFB) smear, which may in fact be the case in certain areas. At present, however, given their cost (\$50–\$100 per assay in most laboratories), such use of NAA tests would strain laboratory budgets unbearably. However, in certain settings such as centralized laboratories to which a large number of specimens

can be quickly and easily referred, these tests may in fact be economically feasible and clinically useful (16). Some analyses also suggest that there are scenarios in which use of NAA, at certain price points, might be cost effective in resource-poor countries (17).

The Centers for Disease Control and Prevention (CDC) recently updated its recommendations for use of nucleic acid amplification tests for the diagnosis of active tuberculosis (18). The CDC now recommends that AFB smear and NAA be performed on the first sputum smear collected. If smear and NAA are both positive, tuberculosis is diagnosed with near total certainty. If the smear is positive and the NAA negative, the statement recommends testing the sputum for inhibitors by spiking the sputum sample with an aliquot of lysed *M. tuberculosis*, and repeating the assay. If inhibitors are not detected, the patient can be presumed to have non-tuberculous mycobacteria (NTM). If a sputum is smear-negative but NAA positive, CDC recommends sending additional sputum samples. If positive, the patient can be presumed to have tuberculosis. If both smear and NAA are negative, an additional specimen should be tested by NAA. If negative, the patient can be presumed not to have infectious tuberculosis. The recommendations conclude by noting that clinicians must always rely on clinical judgment, and that, ultimately, definitive diagnosis rests on response to therapy and culture results. Although there is a certain logic to them, these recommendations are expensive, and, unfortunately, are based on few published data.

In my view, a reasonable use of nucleic acid amplification assays for rapid diagnosis of tuberculosis is as follows: NAA assays should be used to confirm that a positive AFB smear does indeed represent *M. tuberculosis*. If smears are negative, but clinical suspicion is high (based on the impression of experienced observers [6]), NAA should be done on a sputum sample, either expectorated or induced. Although up to 50% of smear-negative, culture-positive cases may not be rapidly diagnosed in this way, the added value of rapidly diagnosing a sizable proportion of smear-negative cases is obvious. Such uses of the test in smear-negative cases have been approved by the FDA for the GenProbe MTD-2 assay. NAA should not be done on sputum samples from cases in which the clinical index of suspicion is low and the AFB smear is negative.

Several questions about the clinical use of NAA assays remained unanswered. Sputum smear examination is commonly used to gauge both infectiousness of a patient and the patient’s response to therapy. No data are available regarding the utility of NAA for these purposes. Also, the optimal number of sputum samples needed to exclude tuberculosis as a diagnostic possibility remains undefined, although some studies indicate that two negative NAA results on different sputum samples make pulmonary tuberculosis quite unlikely. Also, the utility of NAA in extrapulmonary forms of tuberculosis is incompletely defined. It appears that NAA assays are more sensitive than smears in these cases, although not sensitive enough to exclude tuberculosis as a diagnostic possibility if negative (19, 20). Thus, for example, although a cerebrospinal (CSF) sample with a positive NAA result can be considered presumptively to represent a case of tuberculous meningitis, a negative NAA on CSF from a patient in whom the diagnosis is considered likely from a clinical standpoint cannot reliably be used to exclude the diagnosis (21). At present, these tests certainly cannot replace cultures.

Rapid Detection of Drug Resistance

In parts of the world in which multidrug-resistant tuberculosis is a significant problem (Russia, for example), rapid detection of drug resistance would be greatly beneficial to tuberculosis

treatment and control efforts. In most instances, detection of rifampin resistance alone would suffice to signal the need for treatment with second line drugs. Rapid detection of rifampin resistance is technologically feasible by several approaches that examine either genotypic abnormalities (by identifying mutations in the region of the *M. tuberculosis rpoB* gene associated with rifamycin resistance) or actual phenotypic resistance (persistence of the organism in a rifamycin-containing medium) (22). One such approach, the line-probe assay, which detects rifampin resistance-related mutations in the *rpoB* gene of *M. tuberculosis*, is commercially available in Europe (23–25). Such assays may be particularly useful in countries such as the Russian Federation, where MDR-TB is common, or in situations in which there is a question about acquired drug resistance, such as cases of treatment failure or relapse following apparently successful completion of therapy. At present, however, these assays are not in clinical use in the United States, and they are likely to be at least as costly as available NAA tests.

Luciferase reporter gene assay. In this assay, a sample such as sputum is placed into medium and is then transfected with a luciferase-containing mycobacterial phage. If viable *M. tuberculosis* is present in the sample, it will take up the phage and the luciferase gene will function, producing visible light when luciferin is added to the assay. Drug susceptibility testing can be obtained by inoculating the clinical sample into antibiotic-containing medium (26).

Though the initial demonstration of this assay was impressive, clinical development of this assay has been slow. The developers of this approach have worked hard to create a technologically simple and inexpensive assay that can be used in countries that have limited financial and personnel resources. The current version of the assay, the “Bronx Box,” uses Polaroid film for the readout and has promise as a clinically useful tool. It is a self-contained unit that may be capable of detecting viable *M. tuberculosis* in as few as 2 d (27). Field trials of this assay are underway in the United States and abroad.

Molecular beacons. An assay for tuberculosis that employs the technology of molecular beacons has also recently been described. Molecular beacons are molecules that emit light when a chemical reaction occurs (28). This reaction will occur only when primers with DNA specificity bind their appropriate target region in PCR amplicons. In this way, rapid and sensitive diagnosis can be established. Recent studies by Piatek and colleagues demonstrated both the sensitivity and specificity of this assay not only in making a diagnosis of tuberculosis, but also in rapidly identifying mutations associated with antibiotic resistance (29, 30). However, as the authors of the study themselves note, “[t]he molecular beacon assay requires expensive equipment that is not yet widely available,” although they speculate that this equipment will soon become more widely available as sophisticated PCR assays for a variety of infectious diseases are developed and adopted by clinical laboratories. It seems apparent, however, that the widespread adoption of such sophisticated technology will be limited to wealthy nations.

Tests for Latent Tuberculosis Infection

Recent guidelines for testing for and treating latent tuberculosis infection have focused on targeted approaches: only patients who will be treated for a positive test should be tested in the first place. A more accurate method of detection of latent infection (e.g., one that does not cross-react with bacillus Calmette-Guérin (BCG) or NTM), would aid this targeted strategy greatly.

Assays of interferon production by peripheral blood mononuclear cells. T lymphocytes, both CD4⁺ and CD8⁺, are capable of producing the proinflammatory cytokine interferon- γ in

response to stimulation with *M. tuberculosis* (31). Recently, attempts have been made to exploit this aspect of the immune response for the diagnosis of tuberculosis infection. Peripheral blood mononuclear cells (PBMCs) separated from blood samples drawn from patients with known infection with *M. tuberculosis* can be simulated *in vitro* with purified protein derivative (PPD) and production of interferon- γ by PBMCs can then be measured easily by enzyme-linked immunosorbent assay (ELISA). The few published studies of this assay (commercially sold in Australia and elsewhere as Quantiferon, although not yet available in the United States) indicate that this may be an accurate method of detection of latent tuberculosis infection. Compared with tuberculin skin testing, Quantiferon does not require a return visit by the patient for interpretation of test results, although it is more expensive and technically complex.

Studies of this approach to the diagnosis of tuberculosis have been limited by the lack of a gold standard for the detection of latent tuberculosis infection. The initial experience with the tuberculin-stimulated interferon- γ assay was in patients with possible or probable latent tuberculosis infection. In an early report, the assay had a sensitivity of 90% and a specificity of 98% when compared with tuberculin skin test results. (32).

In a recent American trial of this assay, a cohort of HIV-negative and HIV-positive intravenous drug users was evaluated (33). Of 300 HIV-negative patients, 59% were positive for interferon (IFN) release, whereas only 24% had positive tuberculin skin test results. Of 167 HIV-positive patients, IFN release was positive in 19% and the TST in 9.6%. The κ statistic (coefficient of agreement) was low in both groups: 26% for HIV-negative patients and 28% for HIV-positives.

A large trial done in the United States, sponsored by CDC, in which skin testing and interferon release were compared in populations well characterized for actual risk of tuberculosis infection, history of BCG vaccination, and likelihood of exposure to *M. avium*, has recently been completed (34). In this carefully done trial, great efforts were made to classify patients according to clinical risk of latent infection. A total of 1226 patients were included, and overall 390 had positive tuberculin skin tests and 349 had positive IFN release results, with agreement of 83.1% and a κ statistic of 0.6. Striking was the finding that multivariate analysis revealed that TST-positive, IFN release-negative patients were six times more likely to have received BCG vaccine than TST-positive, IFN release-positive patients, suggesting that this assay may be able to discriminate between true infection and BCG vaccination. In addition, the assay displayed some ability to distinguish between infection with *M. tuberculosis* and NTM.

Increased specificity for IFN release assays may also be provided by stimulating cells with early secreted antigen-6 (ESAT-6), a mycobacterial product that distinguishes *M. tuberculosis* from other mycobacteria, including BCG (35, 36).

The definitive study of the utility of IFN release assays for the diagnosis of latent infection might be to apply both the TST and IFN assay to a cohort at high risk for developing tuberculosis and then to observe which test more accurately predicts the development of active disease. The feasibility and ethics of this approach are questionable, however.

CONCLUSION

In the past few years, several new technological approaches to the diagnosis of tuberculosis have been developed. Many of these are better tests (in terms of overall sensitivity, specificity, and statistical accuracy) than AFB smears for the diagnosis of pulmonary tuberculosis although only one, nucleic acid

amplification, has come into general use. By and large, the use of this assay has been limited to wealthy countries such as the United States, which together account for less than 5% of the world's tuberculosis burden. Although these tests may represent a significant advance in overall accuracy compared with AFB smear, and are much faster than mycobacterial cultures, cost and technician requirements have limited their adoption in many clinical settings. In places in which the cost of these assays can be borne by laboratories and hospitals, they should come into more widespread clinical use. Tests for the rapid detection of antibiotic susceptibility clearly work in the research laboratory setting, and clinical use should be feasible. It should be noted again, however, that none of the new tests has been rigorously evaluated for its ability to monitor response to therapy and infectiousness of the patient, two current uses of the AFB smear.

New product development for tuberculosis diagnostics faces the same challenge as new drug development: cold economic considerations weigh heavily against bringing products to clinical use in countries that have limited resources to pay for them. Even in wealthy countries such as the United States, laboratory directors often find their budgets severely taxed when they attempt to use new tests on a widespread basis. Future trials of novel diagnostics for latent infection and active disease should incorporate evaluation of clinical practice algorithms and assessments of cost effectiveness.

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