Relationship Between Estimated Pretest Probability and Accuracy of Automated Mycobacterium tuberculosis Assay in Smear-Negative Pulmonary Tuberculosis*

T. K. Lim, MBBS; A. Gough, PhD; Nyat-Kooi Chin, MD, FCCP; and G. Kumarasinghe, MD

Background: The AMPLICOR assay (Roche; Branchburg, NJ), a rapid direct amplification test for Mycobacterium tuberculosis, has only been licensed for use in smear-positive respiratory specimens. However, many patients with pulmonary tuberculosis (PTB) have smear-negative disease. The clinical utility of this test in patients with smear-negative PTB is unknown.

Objective: To evaluate the effect of pretest probability of PTB estimated by chest physicians on the accuracy of the AMPLICOR assay in patients with smear-negative PTB.

Design and methods: A prospective study of consecutive patients suspected of having smear-negative PTB. Two chest physicians estimated the pretest probability of active disease (high, intermediate, and low categories). Respiratory specimens were examined with radiometric broth medium cultures and with the AMPLICOR assay for M tuberculosis. The decision on a final diagnosis of PTB was blinded to the AMPLICOR results.

Results: Active PTB was diagnosed in 25 of 441 patients (5.7%). The AMPLICOR assay had an overall sensitivity of 44% and a specificity of 99%. Results of the assay were negative in seven patients with culture-negative PTB. The proportions of patients in the high, intermediate, and low pretest groups were 4.5%, 19.7%, and 75.7%, respectively. The incidence of PTB for each group was 95%, 3.4%, and 0.9%, respectively. The sensitivities of the AMPLICOR assay in the three groups of patients were 47%, 33%, and 33%, respectively, while the specificities were 100%, 98%, and 99%, respectively.

Conclusions: In patients suspected of having smear-negative PTB, the following conclusions were drawn: (1) the incidence of active PTB was low; (2) pretest estimates accurately discriminated between patients with high and low risk of PTB; (3) the risk of PTB was overestimated in the intermediate group; and (4) the utility of the AMPLICOR assay in the intermediate-risk group may be limited by the overestimation of disease prevalence and low test sensitivity. Further studies are needed on the role of the AMPLICOR assay in better selected patients with an intermediate risk of having smear-negative PTB.

Key words: AMPLICOR; Bayes theorem; Mycobacterium tuberculosis; rapid diagnosis; smear negative

Abbreviations: ATS = American Thoracic Society; MOTT = mycobacteria other than tuberculosis; MTB = Mycobacterium tuberculosis; PCR = polymerase chain reaction; PTB = pulmonary tuberculosis; TB = tuberculosis

The US Food and Drug Administration has licensed two commercially produced rapid direct tests (AMPLICOR Mycobacterium tuberculosis Test; Roche; Branchburg, NJ; and the Amplified Mycobacterium tuberculosis Direct Test; Gen-Probe Inc; San Diego, CA) for the purpose of confirming the presence of Mycobacterium tuberculosis (MTB) in smear-positive clinical specimens.1 This is in order

For editorial comment see page 574
per 100,000 population annually), most of the smear-positive specimens are collected from patients with active pulmonary TB (PTB) and are not associated with infections by MOTT.\(^2\) Moreover, infections due to MTB often can be distinguished by epidemiologic, clinical, and radiologic features from those associated with MOTT.\(^4\) Raviglione et al\(^5\) have predicted that, with the global resurgence of TB, the average number of new cases will increase to 163 per 100,000 in the year 2000. Thus, from a global perspective, there may be only a limited role for the approved use of these rapid tests in routine practice.

Between 25% and 60% of patients with PTB have smear-negative disease.\(^6\) The diagnosis of TB may be missed initially in \(\approx 50\%\) of these patients.\(^7\) This error may incur delays in treatment of \(\leq 12.5\) weeks until the return of positive culture results.\(^8\) The utility of rapid diagnostic tests in the early detection of smear-negative TB is, therefore, an important clinical consideration. It is also of considerable public health interest.

The utility of a new diagnostic test is dependent on both the intrinsic accuracy of the test itself and on the pretest estimates of disease probability in the patients being tested. A large number of studies have reported on the accuracy of rapid direct tests in the diagnosis of PTB.\(^9\) Most of these, however, are retrospective studies based on laboratory data. To our knowledge, no previous studies have examined prospectively the accuracy of these new molecular assays in relation to the pretest probability of smear-negative disease. The aim of this study was, therefore, to evaluate the effect of pretest probability on the accuracy of the AMPLICOR test in patients with smear-negative PTB.

**Materials and Methods**

**Patients**

Consecutive adult patients (age, \(> 14\) years) suspected of having PTB at the National University Hospital, Singapore, were studied prospectively between January and June 1998. The patients were identified when a specimen from the respiratory tract with a request for an MTB smear and culture was received by the microbiology laboratory. Respiratory tract specimens included induced or spontaneously expectorated sputum, tracheal aspirates, and those obtained from bronchoscopy and biopsies.

Patients were excluded from the study if any of the following conditions were met: (1) if the results of any smears were reported as positive by the laboratory; (2) if the patient was already receiving anti-TB drugs; (3) if all cultures were contaminated; (4) if only MOTT was isolated in the culture; (5) if only pleural fluid was processed; (6) if laboratory data were incomplete; or (7) if clinical data were incomplete.

**Routine Laboratory Mycobacterial Processes**

Respiratory specimens were decontaminated and digested by mixing 0.5% N-acetyl-L-cysteine and 2% NaOH with an equal volume of the specimen. After incubation at 36°C for 25 min, phosphate-buffered saline solution (pH 6.8) was added to the 25-mL mark and the contents of the tube were mixed gently prior to centrifugation at 3,500 g for 15 min. The supernatant then was discarded, and the sediment was resuspended in 0.5 mL phosphate-buffered saline solution. One hundred microliters of the decontaminated specimen was placed in a 2-mL safe-lock tube ready for processing. Direct smears were prepared for screening by fluorochrome staining (auramine O), and confirmation was made by Ziehl-Neelsen staining. The remainder of the specimen was inoculated into radiometric broth medium for culture and sensitivity testing (BACTEC; Becton Dickinson Diagnostics Instruments Systems; Sparks, MD).

**Direct Amplification Test (AMPLICOR Assay)**

**Specimen Preparation:** Sample preparation for polymerase chain reaction (PCR) was carried out according to the manufacturer’s recommendations. Briefly, 500 \(\mu\)L wash solution was added to 100 \(\mu\)L decontaminated specimen. The mixture was vortexed and centrifuged at 12,500 g for 10 min. The supernatant was removed, and the tube was vortexed to break up the pellet. One hundred microliters of lysis reagent then was added, and the mixture was vortexed to resuspend the pellet prior to incubation at 60°C for 45 min. After the addition of the 100 \(\mu\)L neutralization reagent, the specimen was ready for PCR amplification.

**PCR Amplification:** Fifty microliters of a working master mix was introduced into each amplification tube (one tube for each specimen, plus a positive and a negative control tube). The master mix contains an “internal control,” which allows for the identification of any specimens that contain inhibitors that could hinder target DNA amplification and, thus, potentially could produce false-negative results. Fifty microliters of each specimen and each control was placed into its appropriate amplification tube, which then was tightly closed. The specimens then were placed into the AMPLICOR analyzer (COBAS, Roche) for automated amplification and MTB detection.

**Purification of Specimens Containing Amplification Inhibitors:** Specimens that yielded negative MTB and internal control results were purified using the DNA kits (QIAamp; QIAGEN; Hilden, Germany).

**Estimate of Pretest Probability**

Patients were classified into three groups, high, intermediate, or low pretest probability of active PTB, by two respiratory specialists (N.K.C. and T.K.L.) who were blinded to all microbiological laboratory results. This was an intuitive estimate of disease probability based on a review of the presenting history, charts, other laboratory data, and, primarily, on relevant chest radiographs (Table 1). Every attempt was made to classify the patients as soon as they were identified (ie, usually within 2 to 3 days). The attending doctors were blinded to the AMPLICOR test results, and no management decisions were made on the basis of this test.

**Diagnosis of PTB**

The final diagnosis of PTB in each patient was determined by the two respiratory specialists based on all available clinical information, including response to anti-TB treatment. This determination also relied on computer records from the pharmacy.
in order to identify culture-negative patients. All patients were followed-up for at least 3 months (in the cases of patients who were receiving TB treatment) or until the return of cultures negative for MTB and an alternative diagnosis was established. The investigators were blinded to the AMPLICOR test results during this process.

Active PTB was defined as culture-positive disease, granuloma (with or without positive staining for acid-fast bacilli) that was identified in specimens of lung or thoracic lymph node biopsy, cavitary and nodal upper lobe disease consistent with PTB detected on either chest radiographs or chest CT, all of which conditions resolved or improved markedly after 3 to 6 months of treatment with anti-TB drugs. The group of patients receiving a final diagnosis of PTB included both those with culture-positive results and those diagnosed on global clinical grounds, as described above. The patients then were classified according to the American Thoracic Society (ATS) system for PTB, with ATS-3 denoting clinically active disease and ATS-4 defined as "old" PTB that is not currently active.10

Tuberculin skin testing was not used as a diagnostic criterion for PTB in this study. False-negative reactions are common (ie, ≥ 30% of reactions) among hospitalized elderly patients, while false-positive reactions may be seen in populations in which the local prevalence of clinically inactive PTB (ATS-4) is high and vaccination with bacillus Calmette-Guerin is universal.

Data Analysis

The data were analyzed and will be reported on a per patient basis. There were too few duplicated specimens to resolve discrepancies in results between different specimens from the same patient. The results of the AMPLICOR assay, cultures for MTB, and the final diagnosis were related to estimated pretest probability.

<table>
<thead>
<tr>
<th>Pretest Probabilities</th>
<th>Radiologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Upper lobe, patchy airspace infiltrates interpreted as &quot;active&quot;</td>
</tr>
<tr>
<td></td>
<td>Cavitary disease</td>
</tr>
<tr>
<td></td>
<td>Diffuse miliary pattern</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Middle or lower lobe abnormalities</td>
</tr>
<tr>
<td></td>
<td>Upper lobe abnormalities of indeterminate activity</td>
</tr>
<tr>
<td></td>
<td>Diffuse (nonmiliary) abnormalities</td>
</tr>
<tr>
<td>Low</td>
<td>Normal or near-normal chest radiographs</td>
</tr>
<tr>
<td></td>
<td>Multifocal, calcified nodules consistent with healed TB</td>
</tr>
</tbody>
</table>

Table 1—Radiologic Definition of Pretest Probabilities for PTB

<table>
<thead>
<tr>
<th>Pretest Probabilities</th>
<th>AMPLICOR +</th>
<th>Culture +</th>
<th>Final Diagnosis of PTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>20 (4.5)</td>
<td>9 (45)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>57 (20)</td>
<td>3 (3.4)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Low</td>
<td>334 (76)</td>
<td>4 (1)</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td>Total</td>
<td>441</td>
<td>16 (3.6)</td>
<td>18 (4)</td>
</tr>
</tbody>
</table>

*Values given as No. (%). + = positive.

Table 2—Results of the AMPLICOR Assay, Culture, and Final Diagnosis of PTB in Patients With Different Estimated Pretest Probabilities*

Twenty-one patients with a positive smear for acid-fast bacilli were excluded. In addition, 109 patients were excluded from the study for the following reasons: (1) 18 patients already were receiving anti-TB treatment; (2) all specimens were contaminated in 13 patients; (3) MOTT was isolated in 4 patients; (4) only pleural fluid was processed in 58 patients; and (5) there were incomplete data for 16 patients.

A total of 441 consecutive patients were included in the study, and 639 specimens were examined. The types of specimens were as follows: sputum, 87%; bronchial and/or alveolar lavage, 6%; endotracheal tube aspirates, 5%; and biopsy specimens of either lung or thoracic lymph node, 2%. One specimen was processed in the majority of cases (73%), with an average of 1.45 specimens per patient (range, 1 to 4).

Three patients in the intermediate pretest group were anti-HIV antibody-positive. All three patients had presented with Pneumocystis carinii pneumonia and did not have PTB. Twenty-five percent of patients were diabetic.

Relationship Between Pretest Estimates and Incidence of Smear-Negative PTB

The overall incidence of PTB diagnosed by a positive culture was 4%, and that diagnosed by global confirmation of clinically active disease was 5.7% (Table 2). Only 4.5% of patients were deemed to have a high pretest likelihood of having PTB (high pretest group), while 95.5% of patients were estimated to have either an intermediate or a low risk of having active disease (Table 2).

Table 2 shows the relative incidences of positive AMPLICOR tests, positive culture for MTB, and final diagnoses of active PTB in the three groups of patients with different pretest probabilities. The incidence of the active PTB (12 patients were culture-positive and 7 were culture-negative) was 95% in the high pretest group. By contrast, the incidence of active TB (all three patients were culture-positive) was 0.9% in the low pretest group and 1.4% (all six
patients were culture-positive) in the 421 patients who were in the combined intermediate and low pretest groups.

Seven of 25 patients (28%) with a final diagnosis of PTB had smear-negative and culture-negative disease. All seven patients were in the high pretest group and had either granulomas on histologic examination of lung tissue and/or cavitary upper lobe disease on CT examination, which resolved after empirical anti-TB treatment. The AMPLICOR test was negative in all seven of these patients.

Sensitivities and Specificities of the AMPLICOR Test in Relation to Pretest Probabilities of PTB

The sensitivities of the AMPLICOR test for patients with culture-positive PTB in the high, intermediate, and low pretest probability groups were 75%, 33%, and 33%, respectively (Table 3). The sensitivities of the AMPLICOR test for patients with a final diagnosis of PTB in the three pretest probability groups were 47%, 33%, and 33%, respectively (Table 4). The overall sensitivity of the AMPLICOR test in patients with smear-negative PTB was 61% for culture-positive PTB, but it fell to 44% in the group with the final diagnosis of PTB, which included culture-negative patients.

The specificity of the AMPLICOR test was consistently high, ranging between 98% and 100% (Tables 3, 4). In contrast with the sensitivity, the specificity of the AMPLICOR test was much less affected by either grouping, according to pretest probability or by the definition of active disease.

Five of 416 patients (1.2%) (Table 4) had false-positive results for the AMPLICOR test. One patient had carcinoma of the esophagus, which presented with a mediastinal mass. The other four patients had old PTB that was not currently active (ATS-4). Three of these patients classified as ATS-4 were in the low pretest group. They had apical fibrocavitary PTB that had been treated 20, 8, and 5 years previously. One other patient classified as ATS-4 was in the intermediate pretest group and had apical nodules of indeterminate activity. There were no patients with false-positive AMPLICOR results in the high pretest group (100% positive predictive value).

The eventual diagnoses in 87 patients of the intermediate pretest probability group are shown in Table 5. The majority (59%) had either nontuberculous chest

### Table 3—Relationship Between Estimated Pretest Probability and Accuracy of the AMPLICOR Assay for Culture-Positive Patients

<table>
<thead>
<tr>
<th>AMPLICOR Assay</th>
<th>Culture</th>
<th>Pretest Probabilities for PTB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>AMPLICOR sensitivity, %</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>AMPLICOR specificity, %</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td></td>
<td>73</td>
</tr>
</tbody>
</table>

*See Table 2 for other abbreviations not given in text.

### Table 4—Relationship Between Estimated Pretest Probability and Accuracy of the AMPLICOR Assay for Final Diagnosis of PTB

<table>
<thead>
<tr>
<th>AMPLICOR Assay</th>
<th>Final Diagnosis of PTB</th>
<th>Pretest Probabilities for PTB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>AMPLICOR sensitivity, %</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>AMPLICOR specificity, %</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

*See Tables 2 and 3 for abbreviations not given in text.
The pretest probability estimates were reliable when the suspicion of PTB was either very high (ie, 95% incidence of PTB in the high pretest group) or very low (ie, 0.9% incidence of PTB in the low pretest group). Most likely, this is an indication of the proficiency of the investigators, who need to make prompt decisions regarding the activity of TB based on simple clinical and radiologic information, and is comparable to the performance of binary probability models that have been designed for the prediction of PTB based on epidemiologic, clinical, and radiographic data.15,19 The emphasis in clinical practice and the purpose of prediction models are to quickly identify patients with active disease for isolation and prompt treatment. This study was not designed to test the accuracy of such pretest predictions. The investigators who made the final classification of PTB were blinded to the AMPLICOR test results but not to the estimates of pretest probability. However, the pretest probability estimates and final diagnostic classification of PTB were conducted at least 3 months apart and were based on straightforward clinical and radiographic features. This probably would have reduced, though not eliminated, any bias in this aspect of the study.

Patients in the low pretest probability group experienced very low incidence of active disease (ie, <1%). This was the largest subgroup of patients (76%) investigated for PTB. We estimate that, assuming an incidence of 1% (Table 2) and a sensitivity of 33% (Table 4), 300 patients will need to be tested with the AMPLICOR assay in order to diagnose PTB in 1 patient in this group. In addition, the diagnosis of PTB in two other patients will still be missed. This strategy is unlikely to be very cost-effective. We suggest, therefore, that, despite the high specificity and high negative predictive values of the AMPLICOR test in this subgroup, patients who are not immunocompromised, who return negative results for sputum smears, and in whom the clinical suspicion of PTB is low should not be investigated further. Our results, thus, concur with those of Divinagracia et al,13 which suggest that screening by specialists can reduce the need for microbiological testing of PTB by >50%.

Patients deemed to have a high pretest probability for PTB should either receive empirical anti-TB treatment or undergo further investigations to confirm the diagnosis. Early empirical treatment is probably the more expedient option unless the risk of missing a serious disease, such as a malignancy, is also present. The role of the AMPLICOR assay and other direct amplification tests in avoiding delays and invasive testing in such patients needs further evaluation. Jouveshomme et al20 have shown, in a retrospective study of 32 patients with high clinical infections or cancer. The results of the AMPLICOR assay were positive in one of three patients with culture-positive PTB (ATS-3) in this group. The results of the AMPLICOR test were, however, negative in two other ATS-3 patients in this group. One patient had apical infiltrates that were initially interpreted as of indeterminate activity, while the other patient had miliary PTB that was interpreted as diffuse interstitial disease on the plain radiograph.

**DISCUSSION**

The incidence of smear-negative PTB in this study was low (5.7%). It was, however, comparable to that reported among patients being investigated for PTB in California (range, 3.6 to 8.5%),11,12 New York (6.2%),13 and France (3.9%),14 and in patients isolated for suspected PTB in New York (4.8%).15 Thus, the action threshold for the clinical suspicion of PTB appears to be comparable between different institutions despite wide differences in the local prevalence of the disease.16 This also is consistent with the notion that PTB may be overinvestigated in routine hospital practice.13

To our knowledge, this is the first prospective study that relates pretest probability with the performance of the AMPLICOR assay in consecutive patients suspected of having smear-negative PTB. Explicit expression of diagnostic uncertainty is a key step in using Bayes theorem to predict the impact of new tests on patient management. It is recommended by the ATS Workshop Report on rapid diagnostic tests for TB and also by most other experts.17,18 However, in this study, we found that the performance of the AMPLICOR assay was influenced by the accuracy of the pretest estimate itself (Table 4).

![](http://journal.publications.chestnet.org/pdaccess.ashx?url=/data/journals/chest/21952/)
suspicion of PTB, that the use of the ligase chain reaction could hypothetically reduce invasive procedures by 29% and the incidence of delayed therapy by 21%. Because both the AMPLICOR assay and ligase chain reaction retain high specificities for smear-negative PTB, a positive result with either test would confirm the diagnosis of PTB in such patients and would obviate further investigations. Thus, we agree with the suggestion that performing one of these tests may be an appropriate intermediary step before proceeding to invasive diagnostic procedures in high-risk patients. However, these tests have low negative predictive values in high-risk patients and should not be used to rule out active disease (Table 4). The cost-effectiveness of this approach in comparison with early empirical treatment, however, needs to be evaluated prospectively.

Twenty percent of patients in this study were considered to be at intermediate risk for PTB. A reasonable estimate of the incidence of PTB would be between 20% and 50% for this subgroup of patients. However, the actual incidence of PTB in the intermediate-risk group in this study was only 3.4%. The sensitivity and positive predictive value of the AMPLICOR assay in this group of patients were low and comparable to the patients with low pretest prevalence. We had anticipated, according the Bayes theorem, that a diagnostic test such as the AMPLICOR assay, which has moderate sensitivity and high specificity, would provide useful additional information in patients with intermediate pretest probabilities. However, in this study, the risk of PTB was overestimated and was associated with low AMPLICOR assay test sensitivity and low positive predictive value. This is an error that may limit the clinical utility of the AMPLICOR assay.

The initiation of microbiological investigations for MTB by house staff rather than by specialists was probably an important reason for the overinvestigation of PTB in this study. Most patients in the intermediate-risk group presented with diagnoses such as acute community-acquired pneumonia, lung cancer, or bronchiectasis (Table 5). These patients probably should have been categorized as having a low rather than an intermediate risk of PTB. However, our risk-stratification scheme was based mostly on objective radiologic features and allowed little room for individual judgment (Table 1). More accurate selection criteria for the intermediate-risk category are needed. One such strategy has been described by Catanzaro and colleagues, who reported active disease incidence in 29% of the patients in their intermediate group. In contrast with our study, all patients were recruited by specialists with experience in the evaluation of TB. However, these specialists recruited only a relatively small number of smear-negative cases. Thus, the role of specialist opinion in improving the accuracy of the AMPLICOR assay in this subgroup of smear-negative patients needs further evaluation.

Most studies comparing different commercial amplification kits for the rapid identification of MTB report no significant differences in test performance. The overall sensitivity of the AMPLICOR assay in this study was low (culture, 61%; clinical PTB, 44%) but was within the range of 33 to 87% reported in the literature for smear-negative PTB. It is higher than the sensitivity of 37% reported by Al-Zahrani et al in a prospective study of 357 patients suspected of having smear-negative PTB using an in-house PCR. Like the ligase chain reaction (the results of which were negative in all six smear- and culture-negative patients), however, the AMPLICOR assay also failed to detect smear- and culture-negative PTB. The clinical utility of the AMPLICOR assay and other direct amplification tests for MTB may be enhanced by combining them with alternative diagnostic approaches such as serologic testing, bronchoscopy with lavage, or CT scanning. The optimal combination and/or sequence of tests for the rapid diagnosis of PTB needs further definition.

We conclude that in patients suspected of having smear-negative PTB, the overall incidence of disease was low, the pretest estimates of high and low risk for PTB were generally accurate, but the incidence of PTB was overestimated in the intermediate-risk group. The accuracy of the AMPLICOR assay was influenced by the accuracy of pretest estimates of disease prevalence. The utility of the AMPLICOR assay in patients with intermediate risk of PTB needs further study.

References


