Effect of Nucleic Acid Amplification for *Mycobacterium tuberculosis* on Clinical Decision Making in Suspected Extrapulmonary Tuberculosis*

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**Background:** Laboratory-based studies have suggested the nucleic acid amplification test (NAAT) for *Mycobacterium tuberculosis* may be useful in diagnosing extrapulmonary tuberculosis. We sought to determine how clinicians in one hospital used results of the NAAT in clinical decision making in cases of suspected extrapulmonary tuberculosis.

**Methods:** We performed a retrospective analysis of all patients who underwent the NAAT on at least one nonsputum sample, excluding cerebrospinal fluid, from 1999 to 2001 in one large urban hospital. For these patients, we reviewed the hospital course, with particular attention to date of the NAAT and its influence on days treated with antituberculous medications and days to final diagnosis.

**Results:** Thirty-five patients with suspected tuberculosis who had undergone the NAAT on extrapulmonary specimens were identified. From three patients, NAAT results were nondiagnostic because of inhibitors, and they were excluded from the analysis, leaving 32 patients. Tuberculosis was ultimately diagnosed in 14 of these 32 patients. NAAT findings were positive in specimens from 12 of 14 patients with extrapulmonary tuberculosis and in 0 of 18 cases in which tuberculosis was excluded (sensitivity, 86%; specificity, 100%; positive predictive value, 100%; negative predictive value, 90%). In only 2 of 19 patients treated with antituberculous medications was the NAAT result used to determine the onset or discontinuation of therapy. In no instance was a negative NAAT result used by clinicians as definitive evidence that a patient did not have extrapulmonary tuberculosis; in all but one case, patients were continued on antituberculous therapy until final culture results were available.

**Conclusions:** The NAAT proved to be a sensitive and specific test for detection of *M tuberculosis* in extrapulmonary specimens but did not weigh heavily in clinical decision making at our hospital. Judicious use of these tests may improve the accuracy and speed of diagnosis of extrapulmonary tuberculosis, while helping to eliminate unnecessary antituberculous treatment in patients without tuberculosis. (CHEST 2005; 128:102–107)

**Key words:** decision making; extrapulmonary tuberculosis; *Mycobacterium tuberculosis*; nucleic acid amplification testing

**Abbreviations:** AFB = acid-fast bacilli; AMTD = Amplified MTD; CI = confidence interval; MAC = *Mycobacterium avium* complex; NAAT = nucleic acid amplification testing

Extrapulmonary tuberculosis remains a diagnosis that is often difficult to establish immediately and conclusively. In many cases, it is not until culture results are available, up to 8 weeks after clinical presentation, that a diagnosis of extrapulmonary tuberculosis is definitely established or excluded. In addition, obtaining material for culture in extrapulmonary cases often requires invasive procedures. Finally, cases of extrapulmonary tuberculosis are more often culture negative than are pulmonary tuberculosis cases. At times, in order to avoid an invasive procedure, patients may be treated presumptively for extrapulmonary tuberculosis; if they appear initially to respond, efforts to confirm tuberculosis or exclude other diagnoses may be inappropriately deferred. As a result of these diagnostic challenges, the institution of appropriate antituberc-
nucleic acid amplification test (NAAT) is rapid to perform and produces results within hours. The NAAT detects *Mycobacterium tuberculosis* with extreme accuracy in respiratory specimens that stain positive for acid-fast bacilli (AFB), and can also detect organisms in a significant number of smear-negative specimens. The test has gained acceptance in a number of clinical settings for the diagnosis of pulmonary tuberculosis, and guidelines are available for its use. In the setting of extrapulmonary disease, the clinical utility of nucleic acid amplification assays is less clear. Multiple laboratory-based studies of the NAAT for *M tuberculosis* suggest the assay is both sensitive (73 to 100%) and specific (93 to 100%) in a wide array of extrapulmonary specimens. There is variability in results obtained in different studies and using different approaches to nucleic acid amplification. For example, Piersimoni and colleagues used strand displacement amplification and recombinant RNA amplification and found that the former approach was 74% sensitive and 100% specific for diagnosing extrapulmonary tuberculosis and the latter was 92.3% sensitive and 100% specific. Using the ligase chain reaction assay, Rantakokko-Jalava et al found a sensitivity of 73.3% and a specificity of 98%, using culture as a “gold standard.” Despite this evidence of accuracy in diagnosis, the NAAT has not gained widespread acceptance in the clinical diagnosis and management of cases of suspected extrapulmonary tuberculosis, and no guidelines are available to offer indications for its use in cases of suspected extrapulmonary tuberculosis. Moreover, the NAAT has not been officially approved by the US Food and Drug Administration for use in nonrespiratory specimens. Perhaps one reason the test is not used more widely for the clinical diagnosis of extrapulmonary tuberculosis is that most of the studies of the NAAT for extrapulmonary tuberculosis have been performed from the perspective of the laboratory and have provided little insight into how results are integrated into clinical practice. To address the actual clinical utility of the NAAT in the diagnosis of extrapulmonary tuberculosis, we sought to determine how the NAAT affected clinical decision making in cases of suspected extrapulmonary tuberculosis in our hospital.

**Materials and Methods**

**Study Design**

We performed a retrospective analysis of all cases of suspected extrapulmonary tuberculosis in which an NAAT was performed on one or more nonspu tum specimens between 1998 to 2001 at Columbia Presbyterian Medical Center of the New York-Presbyterian Hospital. For the purposes of this study, we defined extrapulmonary tuberculosis to encompass both tuberculosis isolated outside of the lung (eg, scrofula, pleural tuberculosis) as well as tuberculosis with both pulmonary as well as extrapulmonary involvement. Growth of *M tuberculosis* in culture and/or clinical presentation strongly suggestive of extrapulmonary tuberculosis with documented response to antituberculous therapy was used as the “gold standard” to identify cases of extrapulmonary tuberculosis. Patients were eligible for inclusion in the analysis if the NAAT was performed on at least one nonspu tum specimen. We excluded from the analysis specimens for which the NAAT provided a nondiagnostic result, due to presence of inhibitors or other technical factors that did not allow the laboratory to clearly mark a test result as positive or negative. Eligible extrapulmonary specimens included ascitic fluid, pericardial fluid, pleural fluid, and tissue specimens; however, cerebrospinal fluid specimens were excluded from this study. Once patients were identified, data were abstracted from the medical charts, with particular attention to clinical presentation, date of NAAT and AFB stain, use of and timing of antituberculous therapy, culture results, and final diagnosis on hospital discharge or death.

**Laboratory Methods**

Specimens were digested and decontaminated using NALC/NaOH within 3 days of collection and stained for AFB using auramine 0 fluorescent stain. They were inoculated onto Lowenstein-Jensen, Middlebrook 7H11 selective biplate, chocolate agar, and BBL MGIT broth (Becton Dickinson; Sparks, MD) and incubated at 35°C in C2 for up to 8 weeks. The NAAT used was the Amplified MT (AMTD) [GenProbe; San Diego, CA]. Following the instructions of the manufacturer, the AMTD test on each specimen included a duplicate control that was seeded with *M tuberculosis* cells to detect nucleic acid amplification inhibition. It is laboratory protocol at our hospital to run the AMTD on all specimens that stain positive for AFB. In all other cases, however, the AMTD is restricted by the laboratory, requiring consultation with the Director of the Clinical Microbiology section as well as with an infectious disease or pulmonary attending physician to perform the test based on clinical suspicion of tuberculosis (Fig 1). Our analysis included subjects from both of these groups. Once a case has been approved for the NAAT, the laboratory runs the AMTD on all specimens received for that patient.

**Statistical Methods**

Unless otherwise indicated, sensitivity, specificity, and positive and negative predictive values of the AMTD were calculated on a per-patient basis, rather than on a per-specimen basis. The two-tailed Fisher Exact Test was used to compare results between groups. Bayesian calculations were used to determine...
confidence intervals (CIs) around positive and negative predictive values based on prior probability.

RESULTS

Patient Profile

A total of 35 eligible patients were identified for inclusion in the study: 16 patients with and 19 patients without a final diagnosis of extrapulmonary tuberculosis. Three patients were subsequently excluded from analysis because AMTD results on sputum samples were nondiagnostic due to the presence of inhibitors. The final analysis group of 32 patients consisted of 14 patients with tuberculosis and 18 without extrapulmonary tuberculosis. Characteristics of the two groups are shown in Table 1. Of the 14 patients with tuberculosis, 5 patients (36%) had pulmonary as well as extrapulmonary involvement. Of the 18 patients without tuberculosis, 8 patients (44%) were HIV positive and presented with either lymphadenopathy (6 of 8 patients) or abscesses (2 of 8 patients) that subsequently tested AFB stain positive. Ultimately, it was believed that all eight of these patients had Mycobacterium avium complex (MAC) infection rather than tuberculosis, although two of these eight patients never grew MAC on culture.

Specimens Tested

From these 32 subjects, the AMTD was performed on 55 extrapulmonary specimens. Fluid specimens comprised 36% of the specimens tested, including ascites, pleural fluid, and pericardial fluid. The other 64% of specimens tested were tissue specimens, and the majority (61%) of these were lymph nodes.

Results of AMTD Testing

AMTD results are shown in Table 2, stratified by final diagnosis of extrapulmonary tuberculosis. AMTD results were positive in specimens from 12 of 14 patients with extrapulmonary tuberculosis and in none of 18 cases in which tuberculosis was excluded. Overall, the AMTD for this patient sample had a sensitivity of 86%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 90% (95% CI, 77 to 103%). By contrast, AFB staining in the same patient population had a sensitivity of 50%, a specificity of 56%, a positive predictive value of 47% (95% CI, 21 to 72%), and a negative predictive value of 59% (95% CI, 35 to 82%) [Table 3]. The AMTD was significantly more specific than AFB stain (100% vs 56%) in cases of suspected extrapulmonary tuberculosis (two-tailed Fisher Exact Test, p < 0.003). There was a strong trend toward increased sensitivity of the AMTD compared to AFB stain as well (86% vs 56%, p = 0.04 in a one-tailed test, p = 0.10 in a two-tailed test).

As indicated above, there were two patients who had AMTD results that were discordant with the final diagnosis; both results were finally classified as...
false negative. The characteristics of these patients are shown in more detail in Table 4.

We found that in our laboratory, the AMTD was more sensitive for tissue specimens (14 of 15 specimens from true tuberculosis patients; AMTD positive, 93%) than for fluid specimens (3 of 7 specimens from true tuberculosis patients; AMTD positive, 43%; two-tailed Fisher Exact Test, p = 0.02). Of note, in one of the two patients with AMTD false-negative results (Table 4), a high-quality surgical specimen, as indicated by the multiple granulomata seen on pathologic examination, was not sent for AMTD at all, leaving only the inferior quality fluid specimen on which to perform the AMTD.

Of 14 patients with a final diagnosis of tuberculosis, 7 patients (50%) had specimens that were AFB stain negative. The AMTD proved relatively sensitive (86%) even in these patients with AFB stain-negative specimens (six of seven AMTD positive vs zero of seven AFB positive; two-tailed Fisher Exact Test, p = 0.005). The AMTD was specific for M tuberculosis; there were no false-positive AMTD results in the six patients (nine specimens) that grew MAC but not M tuberculosis in culture. There were two patients who grew both M tuberculosis and MAC in culture; these patients both had appropriately positive AMTD results.

Effect of the AMTD on Initiation and Cessation of Antituberculous Therapy

Among 14 patients with true tuberculosis, all 14 received antituberculous therapy. Only once was a positive AMTD result the deciding factor in beginning treatment. By contrast, six of these patients (43%) were begun on antituberculous therapy when the patient was noted to be AFB stain positive, and three patients (21%) were begun on treatment based on a suggestive pathology result with granulomata or AFB positive stain. Among the 18 patients in whom tuberculosis was excluded, 5 patients received antituberculous therapy; in all five cases, treatment was initiated based on clinical suspicion alone (n = 2), positive AFB stain (n = 2), or suggestive pathology (n = 1); in each of these five cases, therapy was begun before the AMTD result was even available. In these same five patients, a negative AMTD result was used only once as the primary reason to discontinue antituberculous therapy.

Discussion

In our hospital, the AMTD for M tuberculosis in cases of suspected extrapulmonary tuberculosis is being performed with increasing frequency. However, clinicians in our hospital have not routinely used the AMTD result as a key factor in deciding to initiate or discontinue antituberculous therapy. While we found the AMTD compared to AFB stain to be significantly more specific (100% vs 56%, two-tailed Fisher Exact Test, p = 0.003) and to show a trend toward increased sensitivity (86% vs 50%, two-tailed Fisher Exact Test, p = 0.10; if there had been one additional patient with AMTD-positive, AFB-negative specimens, the two-tailed Fisher Exact Test p value would change to p = 0.05) for detecting the presence of M tuberculosis in nonsputum specimens from patients with suspected extrapulmonary tuberculosis, management decisions were far more often based on the AFB stain result than on the AMTD result. This frequent use of AFB stain as a major factor in clinical decision making likely stems from clinicians’ familiarity and comfort level with the AFB stain, which has been in use for many decades, as opposed to the relatively new
Because the positive predictive value of the AMTD was high (100%), a positive AMTD result for M tuberculosis should encourage clinicians to consider strongly the diagnosis of extrapulmonary tuberculosis and subsequent antituberculous therapy. Of course, a high positive predictive value depends not only on the characteristics inherent to the test being performed (sensitivity and specificity), but also on the prevalence of the disease in the population studied. We believe that the use of specialists to screen patients before the NAAT is done (Fig 1) serves to increase the prior probability of tuberculosis in the patients to be tested and therefore raises the positive predictive value. This has been demonstrated to be the case in previous studies.12

Extrapulmonary tuberculosis remains a challenging diagnosis for clinicians to make definitively. Because the NAAT for M tuberculosis is increasingly available, more rapid than culture growth, and more sensitive and specific than AFB stain, it is a useful test to expedite identification of cases of extrapulmonary tuberculosis. Though our sample size was small with limited power and our analysis restricted by the retrospective design, we believe our study provides some insight into how the NAAT for M tuberculosis has been used in clinical decision making in cases of suspected extrapulmonary tuberculosis and how it may be applied in the future. Our experience indicates that, when positive, the AMTD result should be used as strong evidence of likely tuberculosis and should influence clinical management accordingly. Improved sensitivity, as may be achieved through testing multiple specimens per patient including all surgical tissue specimens, is needed before clinicians can use a negative AMTD result to exclude definitively extrapulmonary tuberculosis.

REFERENCES
tuberculosis in various biopsy and body fluid specimens by the AMPLICOR *Mycobacterium tuberculosis* polymerase chain reaction test. Chest 1998; 113:1190–1194


