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.[1] 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 antituber-

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culous therapy is often delayed in patients who do in fact have tuberculosis, while patients without tuberculosis may be treated unnecessarily.[2] Because of the morbidity and mortality associated with delaying treatment for true tuberculosis and the significant added expense and potential toxicity of antituberculous therapy in patients without tuberculosis, a diagnostic tool to expedite identification of patients with true extrapulmonary tuberculosis could both improve patient care and decrease costs of unnecessary hospitalization and medication.

The 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.[1] The test has gained acceptance in a number of clinical settings for the diagnosis of pulmonary tuberculosis, and guidelines are available for its use.[3] 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.[4–11] There is variability in results obtained in different studies and using different approaches to nucleic acid amplification. For example, Piersimoni and colleagues[9] 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[11] 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 nonsputum 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 nonsputum 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 CO_2 for up to 8 weeks. The NAAT used was the Amplified MTD (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