

Clinical Impact of Nucleic Acid Amplification Testing in the Diagnosis of *Mycobacterium Tuberculosis*: A 10-Year Longitudinal Study

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Background. Nucleic acid amplification (NAA) testing for *Mycobacterium tuberculosis* (MTB) offers improved diagnostic accuracy, compared with smear microscopy, in differentiating MTB from other mycobacteria. We aimed to evaluate the reliability and projected impact of NAA testing in patients with acid-fast bacilli (AFB) smear-positive respiratory samples.

Methods. We identified a retrospective cohort of all patients with AFB smear-positive respiratory specimens at Henry Ford Hospital from January 1, 2001 through December 31, 2011. We examined the association between patients' sociodemographic factors and clinical comorbidities with the likelihood of being diagnosed with MTB. We evaluated the projected change in duration of airborne isolation and unnecessary MTB treatment with introducing NAA testing into clinical decision making for AFB smear-positive patients.

Results. One hundred thirty patients had AFB smear-positive respiratory specimens, 80 of these patients had a positive NAA test result, and 82 patients grew MTB on culture. Nucleic acid amplification testing had a sensitivity and specificity of 97.6% and 100%, respectively. Integrating NAA testing into clinical decision making for patients with AFB-positive smears was associated with a significantly shorter time in airborne isolation (6.0 ± 7.6 vs 23.1 ± 38.0 , $P < .001$) and 9.5 ± 11.32 fewer days of unnecessary MTB treatment in patients with negative NAA test.

Conclusions. Nucleic acid amplification testing provided a rapid and accurate test in the diagnosis of MTB while significantly reducing the duration of isolation and unnecessary medications in patients with negative NAA test.

Keywords. diagnostic testing; *Mycobacterium tuberculosis*; nucleic acid tests.

The global disease burden caused by *Mycobacterium tuberculosis* (MTB) remains substantial with an estimated 10.4 million new cases and 1.4 million deaths in 2015 [1]. Early detection of MTB and prompt initiation of therapy is crucial for disease control. Historically, diagnosis of MTB relied on microscopic evaluation of the obtained sample, most commonly sputum, for the presence of acid-fast bacilli (AFB). This was then confirmed by isolating MTB in cultures. However, AFB smears have a low sensitivity and specificity [2] and do not distinguish MTB from nontuberculosis mycobacteria (NTM) [3]. The increasing incidence of NTM has further decreased the reliability of AFB smear as a rapid screening test for diagnosing MTB.

In 1995, nucleic acid amplification tests (NAATs) were initially approved to be performed on respiratory specimens from patients with smear-positive AFB and clinical features of tuberculosis (TB). Nucleic acid amplification test sensitivity and specificity in diagnosing MTB has been reported to be as high as 99% and 98%, respectively [4, 5], resulting in a higher MTB detection rate and shorter time to treatment initiation [6]. Nucleic acid amplification tests of respiratory specimens from patients with suspected pulmonary TB has been recommended by the US Centers for Disease Control and Prevention (CDC) [7]. The utility and cost effectiveness of NAAT for the rapid diagnosis of MTB have been evaluated in populations with high rates of human immunodeficiency virus (HIV) infection, in whom NTM is prevalent [8, 9]. However, the utility of NAAT has not been evaluated in other immunocompromised patient populations with high rates of NTM such as cancer patients and transplant recipients. We aimed to evaluate the reliability of NAAT, implemented in 2001 at our institution, among various hospitalized patient populations, and its expected impact on the duration of airborne isolation and unnecessary anti-MTB therapy.

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METHODS

Henry Ford Hospital is a 900-bed teaching hospital in Detroit, Michigan, which serves a predominately urban population with a high prevalence of HIV infection and also serves as a tertiary care center for cancer therapy and transplantation. We used our institution's medical and laboratory data repository to retrospectively identify the cohort of patients hospitalized from January 1, 2001 through December 31, 2011 with an AFB smear-positive respiratory specimen. The study was approved by the Henry Ford Health System Institutional Review Board, and the requirement for an informed consent was waived. All patient records and pertinent information were anonymized and deidentified before the analysis.

Isolation Policy

Our hospital's Infection Control Isolation policy, based on the CDC recommendations, requires that all patients with suspected MTB, or patients in whom an AFB smear and culture is ordered, be placed in airborne isolation. Placement in airborne isolation is continued until 3 sputum or respiratory specimens are AFB smear negative or an alternate diagnosis of the pulmonary illness is confirmed. If the sputum is AFB smear positive, the patient is started on empiric anti-MTB therapy until the mycobacterial cultures results are available. The AFB smear-positive patient is removed from airborne isolation after receiving at least 2 weeks of anti-MTB therapy, shows improvement of symptoms, and has 3 negative AFB smears. In addition, all AFB smear-positive patients are reported to the local health department for contact investigation. Since 2001, AFB smear-positive respiratory specimens were further evaluated with direct NAAT. Our Infection Control Isolation policy was modified in 2001 to permit the removal of AFB smear-positive patients from airborne isolation if the NAAT was negative for MTB.

Microbiology Methodology

Respiratory samples were decontaminated and concentrated for acid-fast smear (auramine rhodamine) and culture using standard procedures [10]. Both solid and liquid culture media were performed on all samples. Isolates from positive cultures were sent to the local health department for drug sensitivity testing and deoxyribonucleic acid fingerprinting.

Between 2001 and 2007, NAAT was performed daily using an in-house Amplified Mycobacterium Tuberculosis Direct Test ([MTD] Gen-Probe, San Diego, CA) on AFB smear-positive specimens. After 2007, all AFB smear-positive sputum and bronchoalveolar lavage (BAL) samples were sent to the Michigan State Tuberculosis Laboratory, where samples were analyzed using the MTD Gen-Probe assay. The turnaround time (TAT) for both the in-house and the state NAAT testing was comparable (24–48 hours). The TAT for both NAAT and cultures was measured as the time from obtaining a positive AFB smear to the time for corresponding final test result.

Clinical Data

Demographic variables including date of birth, gender, race, employment status, and housing conditions, and social history including drug use were abstracted from the medical records and contingent on patient self-reporting and physician documentation. Clinical variables evaluated included presenting signs and symptoms along with clinical risk factors associated with pulmonary TB (history of HIV, cancer, or transplant and positive tuberculin skin testing with purified protein derivative [PPD]).

For the AFB smear-positive group, we examined the association between patients' sociodemographic factors and clinical comorbidities with the likelihood of being diagnosed with MTB. The actual duration of airborne isolation and days of unnecessary MTB treatment (for AFB smear-positive patients with NTM) based on NAAT was compared with the expected duration of these endpoints had the culture results been used for removal from airborne isolation and clinical decision making as in the pre-NAAT period before 2001.

Statistical Analysis

Numeric study variables were summarized using means and standard deviations, whereas categorical study variables were summarized using frequency counts and percentages. The study groups were compared using 2-sample *t* tests for normally distributed numeric data, Wilcoxon rank-sum tests for nonnormally distributed numeric data, χ^2 tests for nonsparse categorical data, and Fisher exact tests for sparse categorical data. Categorical data sparsity was defined as the presence of expected cell counts less than 5. Statistical significance was defined as $P < .05$ for all comparison test results.

RESULTS

Patient Characteristics

Patient demographics and clinical characteristics are shown in [Table 1](#). Over a 10-year period, 130 patients with positive AFB smear of sputum or respiratory specimens including BAL samples had NAAT done and were included in our analysis. Only the index admission during which an AFB smear was positive and NAAT was performed was included in the analysis. Patients had a mean age of 55 ± 18.2 years. The majority were male (59%) and African American (69%). Of the 88 patients in whom HIV status was available, 22 (25%) were HIV seropositive. Fifteen patients (11.6%) had a history of malignancy and/or had undergone transplantation.

Characteristics of Acid-Fast Bacilli Testing

The positive predictive values (PPVs) of AFB smears in diagnosing MTB across different clinical settings are shown in [Table 2](#). Overall, the PPV of AFB smear to detect MTB was 63.1% because MTB was isolated from 82 of the 130 specimens. The PPV of an AFB smear was significantly lower for HIV-seropositive patients (45.5%) and patients with history of

Table 1. Patient Demographics and Clinical Characteristics

Variable	Overall N (%)
Age in years (mean ± SD)	55.0 ± 18.2
Gender	
Female	53 of 130 (40.8%)
Male	77 of 130 (59.2%)
Race	
White	26 of 130 (20.0%)
African American	90 of 130 (69.2%)
Other	14 of 130 (10.8%)
PPD test positive	29 of 57 (50.9%)
HIV seropositive	22 of 88 (25.0%)
Cancer or transplant patient	15 of 130 (11.5%)
Drug abuse	32 of 130 (24.6%)
Homeless	9 of 130 (6.9%)
Healthcare worker	2 of 130 (1.5%)

Abbreviations: HIV, human immunodeficiency virus; PPD, purified protein derivative; SD, standard deviation.

malignancy or transplantation (26.7%). In contrast, the PPV of an AFB smear was significantly higher for patients with positive PPD test (86.2%) and patients with a prior history of TB (62.5%).

Characteristics of Nucleic Acid Amplification Tests

Among the 130 patients with positive AFB smear, 80 patients had a positive NAAT (61.5%) and the remainder were negative. All patients with positive NAAT and 2 of the 50 patients with negative NAAT had MTB isolated on culture. In the remaining 48 patients, the cultures grew *Mycobacterium avium* complex (60%), *Mycobacterium kansasii* (19%), *Mycobacterium chelonae* (6%), and *Mycobacterium xenopi* (14%). Based on the culture results, NAAT had a sensitivity and specificity of 97.6% and 100%, respectively, for the detection of MTB. The NAAT had a negative predictive value of 96.0% and PPV of 100%. Mean TAT for the NAAT was 1.43 ± 0.90 days, compared with 12.7 ± 7.6 days for the mycobacterial culture (Table 3).

Clinical Impact of Nucleic Acid Amplification Tests

Patients with positive AFB smear but negative NAAT spent a significantly shorter time in airborne isolation (6.0 ± 7.6 days vs 23.1 ± 38.0 days, $P < .001$). The estimated mean duration in airborne isolation was reduced by 6.1 days (difference between actual duration based on NAAT and expected duration based

on culture results) (Figure 1). Similarly, patients with negative NAAT had a significantly shorter hospital stay (12.0 ± 8.5 days vs 25.4 ± 38.2 days, $P = .044$) (Table 3). For the 48 patients with negative NAAT results, 20 were started on MTB therapy for an average duration of 2.6 ± 5.4 days. The mean duration of unnecessary anti-TB therapy was reduced by 9.5 ± 11.32 days (difference between actual duration of unnecessary anti-MTB therapy received and expected duration based on culture results) (Figure 1).

DISCUSSION

The implementation of NAAT of AFB smear-positive respiratory specimens at our institution had a significant positive impact on the clinical management of patients with suspected pulmonary TB. In our 10-year retrospective cohort, NAAT has proved to be a reliable test, especially in the immunocompromised patient population, in differentiating MTB from NTM with a sensitivity and specificity of 97.6% and 100%, respectively. In addition, NAAT allowed for rapid identification of patients with NTM, thereby resulting in significantly shorter duration of airborne isolation and decreased unnecessary exposure to anti-MTB therapy.

Our results highlight the accuracy of NAAT in the diagnosis of MTB in patients with AFB smear-positive specimens. The sensitivity and specificity of the test, when applied in our health system, were comparable to those reported in the literature [5, 8, 9, 11, 12]. Among patients with smear-positive respiratory specimens, AFB smears had an overall PPV of 63.1% compared with a PPV of 100% for NAAT. It is notable that the PPV of a positive AFB smear was much lower in our immunocompromised HIV-infected, cancer, and transplant subpopulations who have a high likelihood of NTM infection. Nucleic acid amplification tests will be most useful and have a greater impact in clinical and infection control decision making in these populations.

To prevent transmission of MTB in healthcare facilities, patients with suspected pulmonary TB are placed in negative-pressure airborne isolation rooms [13]. In areas with low TB prevalence, isolating all patients with pulmonary symptoms and chest radiograph suspicious for TB leads to overuse of airborne isolation rooms [14, 15]. Similarly, solely using an AFB

Table 2. Positive Predictive Value of AFB Across Different Clinical Characteristics

Clinical Characteristics	Total AFB Smear Specimens	Positive Culture for TB	Negative Culture for TB	AFB PPV
Total	130	82	48	63.1%
Immunocompromised	37	14	23	37.8%
HIV seropositive	22	10	12	45.5%
Cancer and/or transplant patient	15	4	11	26.7%
Previous history of TB	24	15	9	62.5%
Non-immunocompromised	102	71	31	69.6%
PPD positive	29	25	4	86.2%
Drug abuse	32	24	8	51.8%

Abbreviations: AFB, acid-fast bacilli; HIV, human immunodeficiency virus; PPD, purified protein derivative; PPV, positive predictive value; TB, tuberculosis.

Table 3. Difference in Turnaround Time for NAAT and Mycobacterial Culture, Duration of Isolation, and Length of Hospitalization

Variable	Overall (N = 130)	NAAT Negative (N = 50)	NAAT Positive (N = 80)	PValue
Turnaround time for NAAT in days, mean ± SD (median)	1.43 ± 0.90 (1.00)	1.38 ± 1.00 (1.00)	1.46 ± 0.83 (1.00)	.312
Turnaround time for mycobacterial culture, mean ± SD (median)	12.7 ± 7.6 (11.0)	12.1 ± 9.8 (8.0)	13.0 ± 6.0 (11.5)	.017
Airborne infection isolation days, mean ± SD (median)	16.8 ± 31.5 (7.0)	6.0 ± 7.6 (3.0)	23.1 ± 38.0 (10.0)	<.001
Days in hospital, mean ± SD (median)	20.5 ± 31.4 (11.0)	12.0 ± 8.5 (9.0)	25.4 ± 38.2 (13.0)	.044

Abbreviations: NAAT, nucleic acid amplification tests; SD, standard deviation.

smear-positive result to determine the need for airborne isolation may be unreliable given the increasing incidence of NTM [16]. Nucleic acid amplification tests resulted in a mean reduction of 6.1 days of airborne isolation among patients who had a positive AFB smear but negative NAAT.

Our study highlights that patients with positive AFB smears but negative NAAT receive unnecessary anti-MTB therapy for 2.6 days. This is significantly lower treatment than what would be expected if clinicians were to rely solely on culture results

(12.1 days). Accordingly, NAA testing provides both cost savings and decreases unnecessary exposure and potential adverse effects related to TB therapy.

There are multiple other benefits from performing NAAT in smear-positive patients. Several reports have evaluated the overall cost effectiveness of NAAT [9, 17], with a recent study reporting a cost reduction of up to \$2000 per NAAT performed [9]. The cost reduction in smear-positive patients was mostly related to shorter hospitalizations, shorter length of isolation

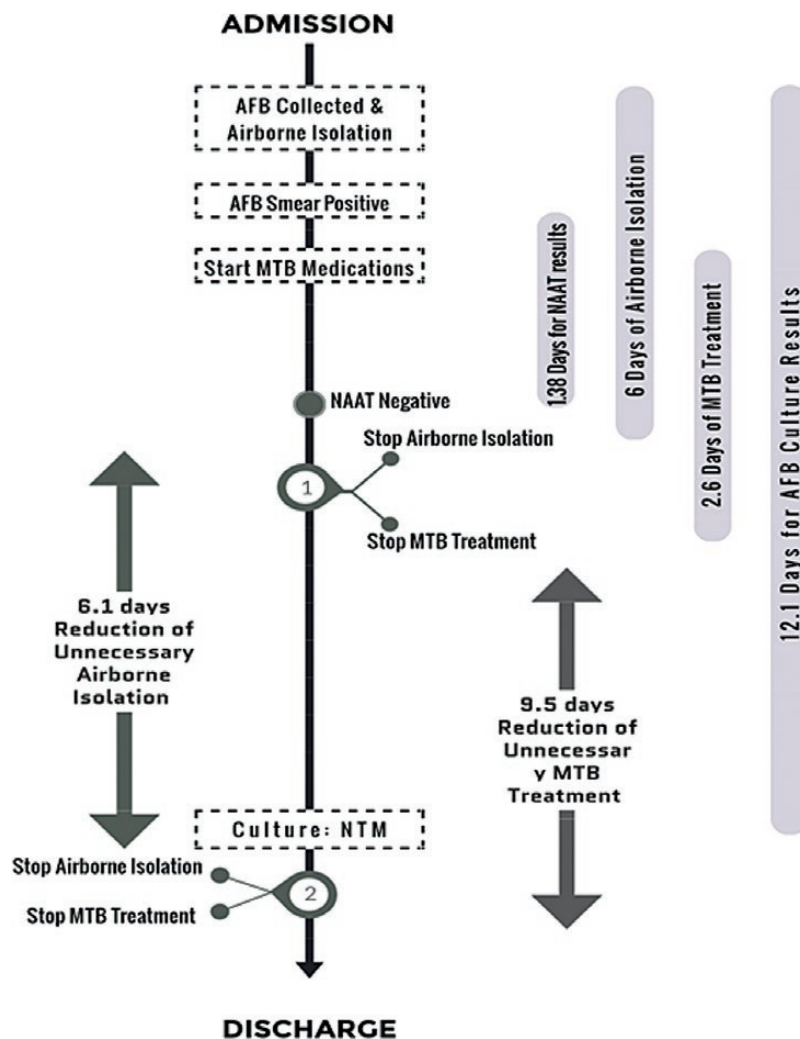


Figure 1. Projected impact of nucleic acid amplification test (NAAT) on reducing duration of airborne isolation and duration of unnecessary *Mycobacterium tuberculosis* (MTB) treatment for patients with nontuberculous mycobacteria (NTM). 1 denotes that actual time of discontinuing airborne isolation and stopping MTB treatment. 2 denotes the expected time discontinuing airborne isolation and stopping MTB treatment, if NAAT was not available. AFB, acid-fast bacilli.

time, patients taking fewer MTB medications, and fewer contact and exposure investigations [9].

Our study has several limitations. We estimated the impact of NAAT in reducing length of airborne isolation and duration of unnecessary MTB therapy based on a hypothetical algorithm that reflected the clinical management before the implementation of NAAT in 2001. Because our study was a retrospective chart review, the variables studied, particularly those related to social history and drug use, were dependent on patient reporting and proper documentation. In addition, we did not have information regarding the HIV status for all of the subjects. Because our institution does not perform NAAT on smear-negative samples, we were unable to assess the clinical impact of NAAT under these conditions.

CONCLUSIONS

Through this 10-year longitudinal study, we highlight multiple advantages NAAT has introduced in the routine management of AFB smear-positive patients, especially in the immunocompromised patient populations. The availability of the rapid and accurate NAATs resulted in a shorter period of airborne isolation and reduced duration of unnecessary anti-MTB medications. Recent advances in molecular testing for MTB include Expert MTB/Rif assay, which allows for both MTB detection and rifampin resistance testing. Although patients with negative Expert MTB/Rif assay results can avoid prolonged airborne isolation and MTB treatment, those with positive assay can be started on effective treatment much sooner than waiting for culture-based drug susceptibility results [18].

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