Initial experience with GeneXpert MTB/RIF assay in the Arkansas Tuberculosis Control Program

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BRIEF REPORT

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ABSTRACT

Background

Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent. Rapid and accurate diagnosis of pulmonary and extra-pulmonary tuberculosis (TB) is still a great challenge. The GeneXpert MTB/RIF assay is a novel integrated diagnostic system for the diagnosis of tuberculosis and rapid detection of Rifampin (RIF) resistance in clinical specimens. In 2012, the Arkansas Tuberculosis Control Program introduced GeneXpert MTB/RIF assay to replace the labour-intensive Mycobacterium Tuberculosis Direct (MTD) assay.

Aims

To rapidly diagnose TB within two hours and to simultaneously detect RIF resistance.

Objectives

1. Describe the procedure used to introduce GeneXpert MTB/RIF assay in the Arkansas Tuberculosis Control Program.
2. Characterise the current gap in rapid *M. tuberculosis* diagnosis in Arkansas.
3. Assess factors that predict acid fast bacilli (AFB) smear-negative but culture-positive cases in Arkansas.
4. Illustrate, with two case reports, the role of GeneXpert MTB/RIF assay in reduction of time to confirmation of *M. tuberculosis* diagnosis in the first year of implementation.

Method

Between June 2012 and June 2013, all AFB sputum smear-positive cases and any others, on request by the physician, had GeneXpert MTB/RIF assay performed as well as traditional *M. tuberculosis* culture and susceptibilities using Mycobacteria Growth Indicator Tube (MGIT) 960 and Löwenstein-Jensen (LJ) slants. Surveillance data for January 2009–June 2013 was analysed to characterise sputum smear-negative but culture-positive cases.

Results

Seventy-one TB cases were reported from June 2012–June 2013. GeneXpert MTB/RIF assay identified all culture-positive cases as well as three cases that were negative on culture. Also, this rapid assay identified all six smear-negative but *M. tuberculosis* culture-positive cases; two of these cases are described as case reports.

Conclusion

GeneXpert MTB/RIF assay has made rapid TB diagnosis possible, with tremendous potential in determining isolation of TB suspects on one hand, and quickly ruling out TB whenever suspected.

Key Words

Tuberculosis, GeneXpert MTB/RIF assay, AFB

What this study adds:

1. What is known about this subject?

Mycobacterium tuberculosis is one of the most important causes of death from an infectious agent. Rapid and accurate diagnosis remains a significant challenge.

2. What new information is offered in this study?

Laboratory confirmation of *M. tuberculosis* diagnosis within two hours at a single office visit is now possible with GeneXpert MTB/RIF assay, which includes determination of RIF drug resistance.

3. What are the implications for research, policy, or practice?

There is tremendous potential to determine isolation of suspects, and also to quickly rule out TB whenever suspected. Early diagnosis that is made possible by a rapid assay would likely lead to better patient outcomes and reduced risk of TB transmission in the community.

Background

The Arkansas Tuberculosis Control Program implemented the GeneXpert MTB/RIF assay in June 2012. This assay replaced the MTD. The current protocol is to perform the assay on all index sputum AFB smear-positive specimens. Our prior experience was that for the period January 2009 to March 2012, 50 of 140 (35.7 per cent) culture confirmed cases were in fact AFB smear-negative initially. There is need, therefore, to expand use of the test to include AFB smear-negative specimens; the sensitivity of the assay in detecting *M. tuberculosis* in such a scenario is 72.5 per cent with one specimen and 90.2 per cent with three sputum specimens.[1] In AFB smear-positive, culture-positive patients the sensitivity of the assay is 98.2 per cent with a single specimen and specificity is above 99 per cent compared with the gold standard of culture.[1] The challenge, however, is to identify a group of suspected TB cases on which it would be cost effective to perform the assay since expansion of rapid testing to cover all smear-negative specimens is expensive. The cost is US $70 per test.

Objectives

This study had four main objectives:

1. Describe the procedure used to introduce GeneXpert MTB/RIF assay in the Arkansas Tuberculosis Control Program.
2. Characterise the current gap in rapid *M. tuberculosis* diagnosis in Arkansas.
3. Assess factors that predict AFB smear-negative but culture-positive cases in Arkansas.
4. Illustrate, with two case reports, the role of GeneXpert MTB/RIF assay in reducing time to confirmation of *M. tuberculosis* diagnosis in our first year of implementation.

Method

The Arkansas TB Control Program acquired GeneXpert MTB/RIF assay system at the beginning of 2012 through a grant from the United States' Centers for Disease Control and Prevention (CDC) to replace the MTD assay for rapid diagnosis of *M. tuberculosis* that we have used the past 10 years. After a validation period, GeneXpert MTB/RIF assay was deployed in the State TB Laboratory in June 2012. The MTD assay was limited to AFB sputum smear-positive specimens to differentiate Non-tuberculous mycobacteria (NTM) from *M. tuberculosis*, and was labour intensive.

GeneXpert MTB/RIF assay uses real-time polymerase chain reaction (PCR) technology. The cartridge design for mixing the specimen and reagents allows full sample preparation and after loading in the machine, it becomes a closed system. GeneXpert MTB/RIF assay produces results within two hours; that is, confirmation of *M. tuberculosis* and susceptibility of organism to RIF by detecting the rpoB gene1, which determines resistance.

Between June 2012 and June 2013, all sputum AFB smear-positive samples, and any others, on request by the physician, had GeneXpert MTB/RIF assay performed as well as traditional *M. tuberculosis* culture and susceptibilities using Mycobacterial Growth Indicator Tube (MGIT 960) and Lowenstein–Jensen (LJ) slants.

Population-based surveillance data from the Arkansas TB Registry for the period January 2009 to June 2013, was used as historical data to construct a predictive model for AFB smear-negative samples that eventually grow *M. tuberculosis* on culture. This is critical to us because we are not using GeneXpert MTB/RIF assay on all suspects at this time, which would translate into testing about 900 samples a year.

Two case-reports that were rapidly diagnosed using GeneXpert MTB/RIF assay are presented to illustrate the utility of assay; the AFB smears were negative in both cases but eventually *M. tuberculosis* culture confirmed results of GeneXpert MTB/RIF assay.

Results

The Smear-Negative, Culture-Positive Group: Factors for Consideration

A predictive model for smear- negative, culture-positive cases is shown in Table 1.

The model shows that race-ethnicity is an independent predictor of TB suspects that would benefit from the rapid GeneXpert MTB/RIF assay. Persons who are non-Caucasian are 2.3 times more likely to present with an AFB sputum smear-negative, but eventually culture-positive disease. Cavitation is also significant, in that persons with a cavity on a chest radiograph are likely to have smear-positive and culture-positive disease.

Table 1: Odds Ratios from a Multivariable Logistic Regression Model Assessing Factors Associated with Sputum Smear-Negative, Culture-Positive Tuberculosis Cases vs. Sputum Smear-Positive, and Culture-Positive Cases, Arkansas, January 2009–June 2013 (n=225)

Factor	Odds Ratio	95% CI	P-Value
Age (years)			
Less than 35	1.06	0.50–2.24	0.87
35+	1.0		
Sex			
Female	1.47	0.78–2.79	0.23
Male	1.00		
Race, Ethnicity			
Non-Caucasian	2.29	1.10–4.78	0.02
Caucasian, Non-Hispanic	1.00		
Country of Birth			
USA	1.77	0.81–3.85	0.15
Foreign- born	1.00		
Chest X-Ray			
Abnormal	1.14	0.43-3.02	0.79
Normal	1.00		
Cavity Chest X-ray			
Yes	0.35	0.17-0.69	0.002
No	1.00		

Table 2: Tuberculosis Cases by Sputum Smear Status and Culture, Arkansas, June 2012–June 2013 (n=71)

Sputum Smear	*M. tuberculosis* Culture		
	Positive, n(%)	Negative, n(%)	Total
Positive	22(81.5)	3(6.8)	25
Negative	5(18.5)	41(93.2)	46
Total	27	44	71

Table 2 shows that 5 of 27 (18.5 per cent) of the culture-confirmed cases were initially sputum AFB smear-negative. Our earlier findings for the period January 2009–March 2012 showed a substantially higher proportion, 50 of 140 (35.7 per cent) of the cases were sputum smear-negative but culture-positive.

Table 3: Comparison of Sputum Smear, Culture, and GeneXpert MTB/RIF assay Results in 44 Tuberculosis Cases, June 2012–June 2013

Sputum Smear	GeneXpert		*M. tb* Culture	
	Positive	Negative	Positive	Negative
Positive	24	0	21	3
Negative	6	14	6	14

Of the 44 cases that had results on AFB smear status, AFB culture and GeneXpert MTB/RIF assay, there was discordance on three cases; GeneXpert MTB/RIF assay was positive but *M. tuberculosis* culture was negative in these three cases.

Illustration of GeneXpert MTB/RIF assay diagnosis in two case reports

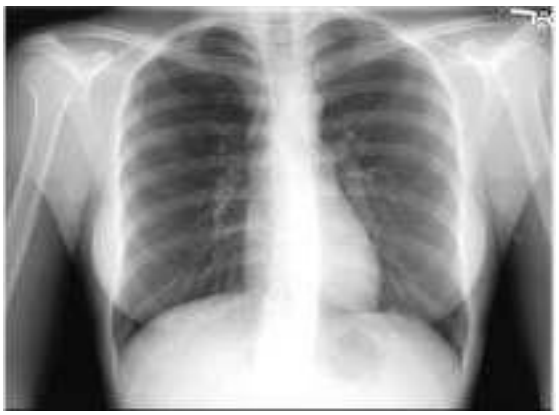
Case Report 1

A 41-year-old African American male presented with a history of human immunodeficiency virus infection (HIV) diagnosed in 2005, and had previously been on highly active antiretroviral therapy (HAART). He complained of cough, fevers, night sweats, and weight loss over the previous four weeks. His CD4 was 130/mm^3 and viral load was >500,000 copies/ml. The chest X-ray was normal. He had a T-SPOT.TB (Oxford Immunotec Inc, Marlborough, MA), an Interferon Gamma Release Assay (IGRA) test a year earlier that was positive, but no further evaluation was done. Sputum collected during the current work-up was smear- negative but the GeneXpert MTB/RIF assay was positive and showed no RIF resistance. Laboratory work-up including complete blood count and liver function profile were normal. The patient was started on four drug anti-tuberculous therapy. His culture grew *M. tuberculosis* after four weeks and sensitivities showed the organism to be susceptible to all primary TB drugs.

Case Report 2

A 21-year-old Caucasian female was seen in the clinic with complaints of cough, weight loss of 10 pounds, and fatigue, all of which had been present for about four weeks. She had no other medical problems and had been previously healthy. Her chest X-ray showed right upper lobe opacity (Figure 1) and a tuberculin skin test (TST) was positive at 12mm. Her TST was negative two years earlier. She had an extensive travel history, most recently to Uganda and other countries, including Honduras, Ukraine, and Mexico. Her travels were part of missionary activities that included direct patient care. Sputum collected during work up was smear-negative but the GeneXpert MTB/RIF assay was positive and showed no RIF resistance. Laboratory work including complete blood count and liver function profile were normal. The patient was started on four drug anti-tuberculous therapy. Her culture grew *M. tuberculosis* after six weeks and sensitivities showed the organism to be susceptible to all primary TB drugs.

Figure 1: Chest X-ray of a 21-year-old Caucasian female showing right upper lobe opacity



Discussion

The invaluable key in bringing utility of the rapid GeneXpert MTB/RIF assay to TB diagnosis is a reasonable index of suspicion. The proportion of AFB smear-negative, culture-positive specimens is a function of time to diagnosis, with delayed diagnosis being associated with a relatively higher proportion of AFB smear-positive disease. Our trend data on smear-negative culture-positive TB, comparing 18.5 per cent currently to 35.7 per cent in the earlier period, seems to suggest that there has been an increase in delayed TB diagnosis in Arkansas. It may also be because of the small numbers in the one-year period.

The first case report was a known HIV positive patient and had a prior positive T-SPOT.TB test. Clinicians should also know the strong link between HIV and progression to TB, such that presentation with AFB smear-negative sputum, and a seemingly normal chest X-ray should not deter the pursuit of a TB diagnosis in this scenario. The presentation here may be indicative of early pulmonary disease or advanced immunosuppression. Thus, early diagnosis that is made possible by a rapid assay would likely lead to better patient outcomes and reduced risk of TB transmission in the community.

The second case report illustrates an important factor in the epidemiology of TB in the United States, namely that history of travel and residence in a TB endemic area is a big risk factor in acquiring TB. Not all clinicians initiate TB treatment based on a clinical diagnosis. So, even if a suspicion of TB was entertained in this case, it took six weeks for the cultures to grow *M. tuberculosis*. Such a delay in initiation of treatment could have resulted in poor outcomes.

TB is a major public health problem. TB infection affects up to one-third of the world's population,[2] and TB is responsible for the death of almost two million people each year.[3] The International Standards for Tuberculosis Care (ISTC) recommends that patients suspected of having pulmonary TB should submit at least two sputum specimens for bacteriological examination. One of these samples should be obtained early morning, because the sample would have the highest yield at that time.[4]

The rapid and accurate diagnosis of pulmonary and extra-pulmonary TB in children and adults continues to be a challenge. Ineffective TB detection and transmission of drug-resistant TB strains endanger TB control activities.[3] The case detection rate of TB worldwide was just 66 per cent in 2012[5] and the lack of rapid and accurate diagnostics remains a critical issue that jeopardises TB control and elimination goals.[6,7] The increasing incidence of drug-resistant TB cases is also a great threat to its control.[8] An estimated 450,000 cases of multidrug-resistant TB (MDR-TB) occur worldwide each year and yet only about 84,000 cases were diagnosed and notified to the WHO in 2012.[9] This low rate in detecting new cases of MDR-TB points out to a critical deficiency in diagnostic laboratory capacity.[8]

Currently, culture and molecular-based TB drug susceptibility testing is limited due to insufficient resources and high cost. Recent years have seen a number of new assays developed to improve the diagnosis of TB and also multiple assays to rapidly detect drug resistance. Some of these assays have been approved by the WHO and one of these, Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) has been described as a potential "game changer" for TB control, especially in TB endemic countries.[10-12] GeneXpert MTB/RIF assay was approved by the Food and Drug Administration (FDA) in the United States in June 2013. Current nucleic acid amplification methods used to detect *M. tuberculosis* are complex, labour intensive, and require technical expertise. In the United States, the case for involvement in global TB control efforts is compelling; 63 per cent of TB cases are reported among foreign-born persons. Support for deployment of GeneXpert MTB/RIF assay technology globally will be strategic and cost effective.[13,14] The challenge is to identify a group of TB suspects for whom the GeneXpert MTB/RIF assay will be beneficial yet cost effective. From the logistic regression model, non-Caucasian cases were more likely to present with AFB smear- negative but culture-positive disease after controlling for several factors, including country of birth. This is particularly helpful because the index of suspicion of TB in this group tends to be relatively lower in the US. Nevertheless, it is quite possible that the predictors that clearly identify a set of cases for

GeneXpert MTB/RIF assay have not been measured in this study; for example, duration of cough.

Conclusion

Arkansas has successfully deployed the GeneXpert MTB/RIF assay, a tool that has the potential to bring standardised, sensitive, and very specific diagnostic testing for both TB diagnosis and drug resistance. Our experience shows that Xpert MTB/RIF assay is an accurate, sensitive, and specific test for the rapid detection of pulmonary TB. The case reports illustrate two important factors that need to be considered, namely HIV status and history of travel to TB endemic countries. Thus, an index of suspicion has to be formed by reasonable documentation of medical and social history, and physicians should be aware and consider these issues when evaluating TB suspects.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ETHICS COMMITTEE APPROVAL

Patients have been de-identified and appropriate approval has been taken for this publication.