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SCIENCE WITH SOCIETAL IMPLICATIONS: DETECTING MYCOBACTERIUM TUBERCULOSIS IN AFRICA

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ABSTRACT:
Mycobacterium tuberculosis (Mtb) is one of the world's leading infectious killers and a disease that disproportionately affects the global poor. Diagnosing tuberculosis disease (TB) with confidence remains an elusive task, especially in resource-limited settings such as those throughout most of Africa. Most diagnostic protocols lack both speed and accuracy, as current methods rely on a combination of screening and confirmatory tests including symptom-based questionnaires, direct smear and microscopy, mycobacterial culture, and chest radiograph. The Xpert® MTB/RIF (Xpert) assay is a polymerase chain reaction (PCR)-based method for TB diagnosis. This assay presents an exciting prospect as a point-of-care (POC) or near-POC test; however, the assay cannot be implemented without changes in budgets, staffing, and infrastructure of existing primary care clinics in the region. This report provides a summary of the Xpert technology and both the benefits and challenges of implementation as a POC test in resource-limited settings.

BACKGROUND:
According to the most recent World Health Organization (WHO) report, Mtb infects an estimated 8.7 million people per year and kills 1.8 million of those infected (1). Unfortunately, fewer than 6 million cases of TB are reported and treated each year, suggesting that more than 2 million cases are not identified and left untreated (2). In terms of infectious diseases, this morbidity burden is a close second to HIV, though roles are reversed in terms of overall infection. Approximately 2 billion people are infected with Mtb, a much larger group than the 33 million infected with HIV (3). Though still more prevalent in men, TB is also emerging as a top killer of women and is one of the top ten causes of death in children worldwide (4, 5). The burden of disease falls disproportionately upon 22 'high burden' countries that constitute 80% of TB cases worldwide (6).

Nine of these 22 countries are in Africa (Democratic Republic of the Congo, Ethiopia, Kenya, Mozambique, Nigeria, South Africa, Uganda, Tanzania, and Zimbabwe) where infectious diseases remain common problems and resources are tightly constrained, making the diagnosis and treatment of the disease especially arduous. These countries are considered resource-limited, and some have a high prevalence of HIV infection. HIV infection is a confounding factor that sets the African region apart from that of Southeast Asia. The overall number of TB cases detected in 2012 was greater in Southeast Asia (1,993,614 vs. 1,282,355), but the number of deaths caused by TB among those persons who are HIV-positive is substantially lower than in the African region (51,000 vs. 250,000) (7). HIV co-infection is known to alter the natural course of TB, leading to lower proportions of smear-positive TB cases, higher rates of primary progressive TB upon initial infection, and higher case fatality rates (8). Many experts have called for a change in TB diagnostic protocols in such settings, citing the need for a straightforward, rapid test that can be used at the point of care (POC), similar to those widely adapted for HIV and malaria (9).

Current Protocol

In most developing nations, current tuberculosis diagnostic protocols rely on testing sputum samples with traditional laboratory methods such as acid-fast smear staining or prolonged culture growth, lacking accuracy and speed, respectively. The limitations of these routine laboratory methods include a lack of prompt, accurate diagnosis, lost chances to implement respiratory isolation, and delayed treatment initiation. All of these factors increase the risks of transmission, drug resistance, and case fatality in populations that are already fragile (8).

Chest radiograph (CXR), another traditional method of diagnosis, is limited by low accuracy due to the lack of a uniform radiographic appearance of TB, especially in those who are dually infected with HIV and have CD4+ counts <300 cells/μL. High rates of intra- and inter-observer variability present prominent barriers that further compromise reliability (6). Additionally, it is known that up to 22% of HIV-positive patients co-infected with culture-proven TB have normal chest radiographic findings (10). Confounded by other impediments such as cost and access, the utility of CXR in TB diagnosis has been increasingly questioned as the HIV epidemic has progressed, despite the fact that many National Tuberculosis Control Programs continue to rely on this method.

The WHO continues to recommend symptom-based screening questionnaires for control of tuberculosis in resource-limited settings. However, in light of the HIV epidemic, indicators such as productive cough, unexplained weight loss, prolonged fever, and night sweats have become less and less predictive adjunctive factors in the diagnosis of active TB (5). There are several methods, most commonly point-based scoring systems, used for this type of diagnosis, yet the correlation between the diagnostic scoring systems is poor. No scale has been shown to be effective with regards to sensitivity and specificity for the diagnosis of active TB (11).

Lastly, some Ministries of Health recommend a course of broad-spectrum antibiotics to differentiate TB from other causes of pneumonia or bronchitis, which can often present with similar symptoms. Interpretation of results is complicated by the fact that some symptoms of bacterial pneumonia or bronchitis will not resolve in a short time frame. Conversely, patients with active TB could experience respite from coughing during this time, and thus have their symptoms wrongly attributed to other bacterial causes while continuing to spread TB (6). Presumptive treatment of TB is an option, but this can be both wasteful and dangerous, especially if patients are concurrently taking anti-retroviral therapy (ART).

Considering that TB is a large cause of morbidity, it should be accurately diagnosed and treated as quickly as possible to reduce health and socioeconomic burdens and the risk of death. Early diagnosis and treatment is critical for reducing transmission in healthcare facilities and communities. TB is the most common opportunistic infection in HIV patients residing in areas where there is a high prevalence of co-infection (such as in Sub-Saharan Africa), and thus all ART programs should be able to efficiently and effectively screen for the

illness (12). The far greater numbers and potential severity of TB disease in HIV-negative patients necessitates reliable and efficient TB diagnostics in this patient population as well. A rapid, reliable POC diagnostic is urgently needed.

The Age of Xpert

The GeneXpert was developed by Cepheid Diagnostics (Sunnyvale, CA, USA) and was first intended for use in the anthrax outbreaks experienced by the United States postal system. The Xpert® MTB/RIF assays are designed specifically for tuberculosis diagnosis and were first endorsed for use by the WHO in December 2010, most notably as a first-line diagnostic test for suspected cases of TB in HIV-positive individuals (2). The Xpert, as it is often called for short, is a cartridge-based microfluidics system that purifies, concentrates, and detects specific nucleic acid sequences from minimally processed clinical samples. Samples are first treated with sodium hydroxide (NaOH) and an isopropanol-containing reagent (the 'sample reagent') to reduce biological hazards, then subsequently transferred to pre-loaded cartridges filled with liquid buffers and lyophilized reagent beads that are necessary for sample processing and performance of the PCR. Processing of samples is fully automated after the initial loading of the cartridge (1). Amplified target sequences created by PCR are detected in real time using molecular beacon technology that emits colored fluorescence (11).

Resistance to rifampicin, a drug commonly used in first-line therapy and a crucial identifier of so-called multi-drug resistant (MDR) TB, is detected by five *rpoB* gene probes. [Though not discussed in detail here, Xpert demonstrates high sensitivity for rifampicin resistance detection, but confirmatory drug sensitivity is often needed because of a high proportion of false-positive results (1).] The Infinity S platform, the largest of the GeneXpert family, can accommodate up to 48 different assays, allowing for concurrent yet independent runs of a large number of samples. The smallest platform loads a single cartridge at a time.

Xpert has been shown to be highly specific for Mtb, with 20 different non-tuberculosis *Mycobacterium* species exhibiting no cross-reactivity (11). The analytical limit of detection of the Xpert® MTB/RIF assay is 131 CFU/mL, giving it a sensitivity two orders of magnitude more sensitive than smear microscopy and nearly equivalent to solid agar culture. Liquid culture has a limit of detection of 10-50 CFU/mL, slightly better than the Xpert assay. The pooled sensitivity of Xpert when used to replace smear microscopy as an initial diagnostic test was 88% while specificity approached 99% (98.4% using meta-analysis) (13) . Sensitivity for smear-positive cases is greater than that of smear-negative cases—98.7% vs 67.0% respectively—which is not surprising, and shows a great deal of promise for Xpert as a preliminary screening tool in place of smear microscopy (13). In HIV-positive persons, a 31% increase in sensitivity (52.8% vs 84.0%) was documented when Xpert was used instead of smear testing (1). Additionally, Xpert has been shown to have superior sensitivity when compared to MTBRplus (Hain Lifescience, Nehren, Germany) and LightCycler Mycobacterium Detection (LCTB) (Roche, Basel, Switzerland), other nucleic acid amplification tests (NAATs) developed for similar purposes (14). It has been estimated that a sputum-based assay with the ability to detect active TB with 85% sensitivity and 97% specificity could save 400,000 lives per year. Of the aforementioned NAAT assays, Xpert is the only one that can achieve these targets (14).

Making Strides

The largest difference among the three methods is the time to detection of disease. The Xpert returns results within 2 hours, whereas definitive smear microscopy results can require multiple samples and up to 8 days and culture results typically require as long as 42 days (1). It has been shown that without the use of Xpert results to direct therapy, the median time to treatment for smear-negative, culture-positive cases of TB is 56 days. With the implementation of Xpert, the median time-to-treatment was reduced to only 5 days and rates of untreated smear-negative, culture-positive TB were nearly halved (9). These statistics are especially important when considering an opportunity to decrease the amount of patients lost to follow-up, as a shorter time to diagnosis and treatment allows for more patients to receive medical intervention. The rapid return time also provides for real-time TB contact identification in partners or relatives that may accompany a TB-positive patient to the clinic. Testing among contacts can be initiated immediately, greatly reducing the burden of contact-tracing (15). The assay has also been preliminarily validated using a variety of samples including gastric lavage, nasopharyngeal aspirates, stool, urine, and cerebrospinal fluid. This eliminates the necessity of sputum collection that can greatly hinder pediatric diagnoses, a large problem for a population vulnerable to TB (16, 17).

In terms of biosafety, Xpert far exceeds sputum microscopy, especially for use in settings where bio-containment facilities are not available. Prior to testing on the GeneXpert platform sputum is incubated in sample reagent for 15 minutes, reducing the viability of Mtb by more than 8 logs (1). Bioaerosols are not generated during sample processing for Xpert, a stark contrast to standard smear microscopy (11). The need for biosafety cabinets is eliminated with the use of Xpert. Furthermore, hazardous rifampicin-resistant strains of the bacteria are quickly and easily identified in high-risk patients (9).

Given the automated and cartridge-based approach used in the GeneXpert platform, diagnostic outcomes are less dependent on user skills than those obtained with smear microscopy (9). Monthly variation in test performance is less likely to differ between sites, and staff persons are noted to have increased morale at study sites where Xpert was implemented. When Xpert was implemented in a primary clinic setting in Africa, additional human resources were required in order to obtain and process samples, return results, and initiate treatment all in one visit. One study found that 2.5 additional staff members were needed to manage 16 TB patients per day, in addition to increased management responsibilities (1). Although the concern over staff training for Xpert use is often raised, previous studies have found that proficiency with Xpert can be achieved in 1-3 days with operators that do not have previous molecular biology or computer skills. This is especially important when considering the lack of skilled personnel, often cited as a barrier to healthcare and diagnoses in remote regions.

Analysis of Cost-Effectiveness

All other factors aside, cost is always a pressing issue when implementing new technologies in resource-limited settings. FIND Diagnostics (Geneva, Switzerland) negotiated a discount pricing structure for Xpert assays that is applicable to 145 developing countries, including all of those considered 'high burden' TB countries, and most of the other countries on the African continent. The platform and associated machinery, including a computer, can be purchased for $17,000 USD, and supplemental funding from the Bill and Melinda Gates Foundation, USAID, UNITAID, and PEPFAR has dropped the cost of each cartridge to $9.98 USD for the next 8 years. Procurement of cartridges occurs through the "Global Drug Facility," which requires a median lead time of 40 days, and the payment of a shipping cost ranging from $0.33 USD to $1.44 USD per cartridge (2). These burdens place the cost of one Xpert MTB/RIF test very close to that of liquid culture, though still greatly exceeding that of smear microscopy (13). However, projects such as TB REACH—a global initiative of the Stop TB Partnership—have supported implementation of Xpert technology through competitive grant making processes, helping to further offset costs in low and middle income countries (LMICs).

A recent cost-effectiveness study evaluated Xpert MTB/RIF implementation in five countries—Bostwana, Lesotho, Namibia, South Africa, and Swaziland—using smear microscopy as the reference standard

in the diagnostic algorithm. Though system costs were projected to increase by $460 million USD over 10 years, it was hypothesized that 132,000 TB cases and 182,000 TB deaths would be averted in this same period. A substantial portion (58%) of the projected increase in costs was slated for antiretroviral medications for HIV-positive patients who would live longer with earlier TB diagnosis by Xpert. This equated to a cost-effectiveness of $959 USD per disability-adjusted life-year (DALY) over 10 years, with approximately 500,000 DALYs averted. To put this data in perspective, it is important to consider that the average GDP per capita in the assessed region is $6,850 USD—far more than the cost of each averted DALY. The estimates for cost-effectiveness ratios over a 20-year period were 20% less than those assessed for the 10 year period, suggesting the importance of longevity considerations in the assessment of new diagnostic tools (8).

Challenges for Implementation

Many experts agree that while reports of Xpert implementation show great promise in the quest for better TB diagnostics, implementation comes with a higher price in reagents, staff, maintenance, and upkeep. The maintenance and upkeep problems faced in LMICs are often irrelevant in developed countries. For example, an uninterrupted supply of electricity is a large concern in LMICs. One must budget for a back-up generator in case of a power outage, a common occurrence in resource-limited communities. Without such provision, daily power outages are known to destroy the GeneXpert instrument or send calibration askew. Power outages can also cause problems for cartridge storage, as 28°C is the threshold of heat that these products can withstand (1). With temperatures in many African countries often exceeding 35°C, a refrigerator is a necessary purchase to ensure cartridge usability. Additional provisions for security measures against theft of the expensive machine in poor areas are also necessary, and areas with adequate ventilation (preferably outdoors) need to be created for sputum collection (15).

Another drawback of working with DNA samples is the need for a clean environment. Many primary care clinics are set up in rural areas that do not have concrete and are built mainly on dusty land. Even minor contamination can yield false results with a technique as sensitive as PCR. Additionally, quality control is generally overlooked in these resource-limited settings and is rarely mandated by regulating bodies. Reliable, adequate personnel to operate this equipment and perform routine quality control checks can be problematic, owing to inadequate training and education programs and barriers to receiving higher education in many resource-limited areas. In developed countries, even the most basic POC machines are checked often and controls are performed. Trends and shifts in data are monitored closely and action is taken immediately should something go amiss, a luxury that is not always easy to maintain in many African countries.

Preliminary POC Study Findings

Shortly after the WHO endorsement in 2010, South Africa was the first country to roll out Xpert technology for TB diagnosis. The initial program placed instruments at centralized microscopy centers and reference laboratories, but the country has recently transitioned its model to attempt Xpert implementation as a POC test in primary care settings. Preliminary studies of Xpert's use in such settings have produced expected results, including an accelerated time to diagnosis and treatment and the need for more staff and changes in clinic infrastructure to accommodate Xpert instruments in-house. Namely, provisions for same-day results required that staff were ready to register new cases and initiate treatment at any time of the day, rather than just in the mornings as had been done when smear microscopy was used (clients were told to return to the clinic for a morning appointment to receive results a few days after their sputum sample was submitted) (15). Additionally, the clinic and staff had to provide for instrument insurance, decentralized billing, stock control of cartridges, and the linkage of results to the national TB database—all tasks not previously performed at the primary care level.

Figure 1: Dr. Tim Meade and patients, Tiny Tim and Friends Pediatric Clinic, Lusaka, Zambia.

One study highlighted that it took longer than the anticipated two hours to return Xpert results to clients. Clients had to be registered and educated on providing quality sputum samples, samples had to undergo processing (though minimal) and transport to the clinical laboratory on-site, and results had to be printed and filed. Each of these tasks was necessary for the full client experience, and increased the two-hour time frame estimated for Xpert run time. Additionally, any sample collected 2-3 hours before the end of the workday had to be run overnight, and loss to follow-up occurred among clients that had to return the next morning for their results (15). Another study with a high proportion of HIV-positive patients noted that a single POC Xpert only identified 43% of TB cases as diagnosed using culture or radiological grounds (18). These small observational studies highlight the empirical treatment of patients that continues even when Xpert is a POC assay. Forty-one percent of patients with negative Xpert results at one South African site were started on treatment on the basis of radiological or clinical findings, highlighting the complexity of TB diagnosis and the interface between diagnostic tests and clinical decision-making (18).

These findings represent only small, observational studies, and thus generalization is uncertain. A recent randomized control trial conducted at five sites in South Africa, Zambia, Zimbabwe, and Tanzania tested 1,502 patients using either a POC smear microscopy test or POC Xpert test administered by a clinic nurse, greatly expanding the breadth of Xpert POC literature. Epidemiological data comparing the two tests was similar to previous findings, as Xpert out-performed smear by 33% in sensitivity (83% vs. 50%) and produced similar specificity values (95% for Xpert, 96% for smear) (19). Eighty-one percent of patients who were culture positive were diagnosed within 1 day when using the Xpert test, whereas only 43% of such patients were diagnosed within 1 day using smear microscopy. Xpert, performed by a clinic nurse at the POC, was just as accurate as that performed in a laboratory, with similar sensitivity and proportion of unusable results. Patients tested with Xpert at the POC showed reduced dropout rate, meaning they were more likely to initiate treatment and promptly inhibit tuberculosis transmission. Ninety-seven percent of Xpert positive patients across the five sites were started on treatment within one week and 71% of those started treatment on the day of presentation (19).

The overall number of patients on treatment in both groups did not differ, likely because chest radiographs were also available and treatment was offered on empirical grounds despite POC assay results. Additionally, it was found that using Xpert does not affect clinically important long-term changes in morbidity as assessed using the TBScore

tool for symptom-based assessment (20). Thus, this trial—the first of its kind—implies that predicted long-term epidemiological effects of Xpert may be overestimated.

CONCLUSIONS:

While the Xpert provides an efficient method of diagnosing TB in a timely manner, skepticism remains regarding the implementation of Xpert as a POC test. The most appropriate measures of feasibility for POC assays will come from investigations that assess specific locations where these tests will be used. Evaluations of Xpert use in primary care settings have expanded the body of literature, but further data needs to be collected and analyzed before the assay can be validated as a true POC test (21). South Africa took a bold step in adopting the technology as the initial diagnostic test for suspected cases of pulmonary tuberculosis, but a wider scale of implementation is needed to provide the clinical and programmatic data necessary for further expansion. Data-driven studies in resource-poor settings with diverse diagnostic practices should be a priority for future research (22).

The need for a rapid, accurate, and efficient diagnostic test for tuberculosis is urgent, with vast implications for health improvement across the African continent. Continued investment in the development and implementation of technologies such as Xpert has the potential to truly change healthcare in some of the world's most underserved, resource-limited areas. If funding for such research can remain a priority, there is likely a better future for many Africans living in TB-endemic areas, with improved care of affected patients and decreased transmission in the community.

The authors of this article participated in a Lusaka (Zambia) based study designed to compare methods of TB diagnosis in HIV-positive pediatric patients at a primary care clinic (Tiny Tim and Friends Pediatric Clinic). The results of screening using a symptom-based screening questionnaire were compared to results from smear microscopy, liquid culture, and Xpert, with the goal of improving screening methods and diagnosis of TB in this specific (and notoriously difficult to diagnose) population. In our experience, Xpert at the POC is difficult to implement in this setting. Primary care clinics do not have the resources, staff, or time to implement extra tests due to high volumes of patients each day and limited budgets. Even orchestrating the transport of samples for this study (once per week to an offsite laboratory facility 10 minutes away) proved difficult to organize and execute, though not impossible. New costs in terms of transport and staff time did indeed arise, and losses to follow-up were observed due to the lack of same-day results. The Zambia AIDS-Related Tuberculosis (ZAMBART) project, one of our close partners, is beginning to implement Xpert at the POC in some Zambian government clinics, though data from these trials has not yet been published. Dr. Timothy J. Meade of Tiny Tim and Friends has been instrumental in this research (Figure 1), as well as clinic staff and volunteers (Figure 2).

Figure 2: Clinic staff and volunteers at Tiny Tim and Friends Pediatric Clinic, Lusaka, Zambia. From right to left- Riemke Mars, Sobe Makomo Mbalaso, Aubri Carman, and Judith van der Kraats.

REFERENCES:

1. Lawn S.D., et al. 2013. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. The Lancet infectious diseases 13:349-361.
2. Creswell J., et al. 2014. Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries. BMC infectious diseases 14:2.
3. Mirlekar, B., S. Pathak, and G. Pathade. 2013. Mycobacterium tuberculosis: approach to development of improved strategies for disease control through vaccination and immunodiagnosis. Indian journal of leprosy 85:65-78.
4. Swaminathan S., and B. Rehka. 2010. Pediatric tuberculosis: global overview and challenges. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 50 Suppl 3:S184-194.
5. 2012. WHO Policy on Collaborative TB/HIV Activities: Guidelines for National Programmes and Other Stakeholders, Geneva.
6. Keeler E, et al. 2006. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature 444 Suppl 1:49-57.
7. Organization WH. 2013. Global Tuberculosis Report 2013.
8. Menzies N.A., et al. 2012. Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF: a dynamic simulation and economic evaluation. PLoS medicine 9:e1001347.
9. Boehme C.C., et al. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377:1495-1505.
10. Sterling T.R., P.A. Pham, and R.E. Chaisson. 2010. HIV infection-related tuberculosis: clinical manifestations and treatment. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 50 Suppl 3:S223-230.
11. Van Rie A., et al. 2010. Xpert((R)) MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert review of molecular diagnostics 10:937-946.
12. Kapata N., et al. 2012. Scale-up of TB and HIV programme collaborative activities in Zambia – a 10-year review. Tropical medicine & international health: TM & IH 17:760-766.
13. Steingart K.R., et al. 2013. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. The Cochrane database of systematic reviews 1:CD009593.
14. Scott L.E., et al. 2011. Comparison of Xpert MTB/RIF with other nucleic acid technologies for diagnosing pulmonary tuberculosis in a high HIV prevalence setting: a prospective study. PLoS Med 8:e1001061.
15. Clouse K., et al. 2012. Implementation of Xpert MTB/RIF for routine point-of-care diagnosis of tuberculosis at the primary care level. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde 102:805-807.
16. Zar H.J., et al. 2012. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55:1088-1095.
17. Nicol M.P., et al. 2013. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 57:e18-21.
18. Hanrahan C.F., et al. 2013. Time to treatment and patient outcomes among TB suspects screened by a single point-of-care xpert MTB/RIF at a primary care clinic in Johannesburg, South Africa. PloS one 8:e65421.
19. Theron G., et al. 2014. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet 383:424-435.
20. Wejse C., et al. 2008. TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scandinavian journal of infectious diseases 40:111-120.
21. Drain P.K., et al. 2013. Diagnostic point-of-care tests in resource-limited settings. The Lancet infectious diseases.
22. Van Rie A., et al. 2013. Point-of-care Xpert(R) MTB/RIF for smear-negative tuberculosis suspects at a primary care clinic in South Africa. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease 17:368-372.