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Diagnostics for pulmonary tuberculosis

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Abstract

Tuberculosis (TB) remains a leading cause of human suffering and mortality despite decades of effective treatment being available. Accurate and timely diagnosis remains an unmet goal. The HIV epidemic has also led to new challenges in the diagnosis of TB. Several new developments in TB diagnostics have the potential to positively influence the global campaign against TB. We aim to review the performance of both established as well as new diagnostics for pulmonary TB in adults, and discuss the ongoing challenges.

Keywords

Tuberculosis; pulmonary; diagnosis; HIV

INTRODUCTION

Tuberculosis (TB) continues to be a major cause of human suffering more than a century after its causative agent was discovered and more than six decades since effective treatments were developed. It infects a third of humanity and is a leading infectious cause of death worldwide, rivaling the impact of HIV/AIDS.[1] Much of the difficulty faced by tuberculosis care and prevention efforts has been the issue of rapid and reliable diagnosis, with many techniques from 90 years ago, when Postgraduate Medical Journal was first published, still in use. Timely diagnosis and initiation of therapy are also affected by patient delay and health system delays. Patient delay is the interval that occurs even before seeing a provider. There remains a substantial delay from the time of onset of first symptoms until most patients first present for clinical evaluation.[2] Patient delay has been associated with

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substance abuse, poverty, rural residence, less access to health care facilities, and little knowledge about TB.[3] Even after first contact with the health care system, it still takes on average more than 3 weeks for tuberculosis to be microbiologically diagnosed, even in wealthy countries.[2] Several factors are associated with delayed diagnosis once patients have presented for care. These include the coexistence of other lung disease, less severe symptoms including the absence of hemoptysis, extrapulmonary tuberculosis, poor health care infrastructure, and the lack of appropriate diagnostic tools.[3] Host factors such as age and immuncompetence also have a large effect on the ability to detect tuberculosis. Diagnosing tuberculosis in pediatric populations and in those with extrapulmonary tuberculosis is particularly challenging due the difficulties in obtaining sputum or the appropriate specimen for microbiological examination. In this review, we will focus on the spectrum of diagnostic tools for pulmonary tuberculosis among immunocompetent adults and those living with HIV, as well as recent advances and the challenges that remain.

Population Heterogeneity

When evaluating diagnostics for tuberculosis there are many potential pitfalls. Test performance can be affected by the choice of reference or gold standard, the local TB prevalence rate, and background HIV rate. Though the reference standard is most often culture, there are different culture media and techniques, possessing different performance characteristics.[4] Some studies instead rely on smear microscopy or clinical diagnosis and are less reliable or reproducible. The local burden of TB will also affect the interpretation of a test's predictive value. As an example, an apical chest x-ray infiltrate has different implications in Nebraska versus Namibia.

The HIV epidemic has also led to new challenges in the diagnosis of TB. Within the first year of infection, people living with HIV are at higher risk of acquiring latent TB, and progressing to active TB in both primary TB and reactivation of latent TB.[5,6] TB remains the leading cause of death for HIV-infected patients globally. The performance of most diagnostics tends to be poorer in those living with HIV due to more atypical presentations and lower bacillary burden of disease at presentation.

To present an overview of tuberculosis diagnostics, we first present the traditional tools of symptom screening, chest x-ray, sputum microscopy and solid media culture. We then examine newer tools that have been endorsed by the World Health Organization (WHO), and rolled out on a substantial scale, including interferon gamma release assays, fluorescence microscopy, liquid media culture, PCR and line probe assays.

TRADITIONAL DIAGNOSTICS

Symptom Screening

Many symptoms are associated with active pulmonary tuberculosis. In a recent meta-analysis of surveys done in low HIV and high TB incidence countries, the sensitivity for TB of a prolonged cough (>2 weeks) was 24% and specificity was 96%. A cough of any duration raised sensitivity to 56%.[7] In United States, with its low TB and HIV burden, The Infectious Disease Society of America (IDSA) and Centers for Disease Control and

Prevention (CDC) recommend that "TB should be suspected in any patient who has persistent cough for more than 2 to 3 weeks or other compatible signs and symptoms."[8]

The utility of screening for other symptoms, or combinations of symptoms, is harder to characterize. Studies have been done mainly in high burden countries in Asia and sub-Saharan Africa, which are difficult to generalize to low-burden countries with lower rates of HIV, such as the US and Western Europe. The main drawback to symptom screening is its poor sensitivity. Among patients with microbiologically confirmed pulmonary tuberculosis in Los Angeles County, 75% had a cough of any duration, and 52% had a cough for more than 2 weeks, 58% had fatigue, 50% had a subjective fever, 46% reported sweats, 43% reported weight loss, 41% reported chest pain and 23% reported hemoptysis.[9] In a different setting of a large community survey in China there was also a low sensitivity of cough and hemoptysis for TB diagnosis.[10] When looking at all comers, such as a study in Birmingham, Alabama homeless shelters, residents reported classic TB symptoms of cough (7%), weight loss (4%), night sweats (2%), and fatigue (2%). However only 3% had microbiologically confirmed pulmonary TB with only 1 of the 4 TB cases reporting a history of cough.[11] Similarly in Zimbabwe, a community survey of 7121 randomly selected adults found that 9.9% of them had at least one symptom of tuberculosis while only 0.43% were diagnosed with pulmonary TB.[12]

In regions with a high burden of HIV, a prolonged cough was more suggestive of TB (49%) than in low incidence regions (24%); furthermore, all symptoms screened had a higher sensitivity and lower specificity in HIV-infected individuals [12] People with HIV can progress to active disease more quickly, leaving less time with no or mild symptoms, but also are at risk for other opportunistic diseases that can cause generalized symptoms.[7] The WHO does recommend that all patients with HIV be screened for active tuberculosis regularly at each visit[13], but finding a screening rule to reliably rule out tuberculosis has been difficult. Cain et al interviewed 1748 Cambodian patients with HIV for symptoms of TB and then generated 80 million combinations of symptoms to try and find the best performing set of questions. They found a 3 symptom combination of cough or fever of any duration, or more than 3 weeks of drenching night sweats in the past 4 weeks had a sensitivity of 93% and specificity of 36%.[14] Getahun et al incorporated this study and several others in a meta-analysis and found that asking about current cough, fever, night sweats or weight loss had 78.9% sensitivity for active TB. However the performance varied widely based on what setting the patients came from (clinic, community or occupational).[15] There is no perfect universal symptom screen, and symptom combinations may differ depending on the patient population's immune status. The current WHO recommendation for TB screening is to evaluate either for 1) cough lasting more than two weeks or 2) for cough of any duration, fever, night sweats, hemoptysis and weight loss depending on the resources available and background rate of TB[13]. In patients living with HIV, WHO recommends a slightly different screen for cough of any duration, fever, night sweats and weight loss.[16]. A small proportion of patients with HIV and positive sputum cultures may be asymptomatic. Whether this represents "active" tuberculosis disease has been the subject of ongoing debate. Some have reported that high proportions of these patients will progress to symptomatic TB disease, while some remain asymptomatic.[17,18]

Chest X-ray

The chest x-ray has been a part of TB diagnosis for over a century. In immunocompetent individuals, it is rare to have a normal chest x-ray with active pulmonary TB. In a review of all the cases of culture confirmed pulmonary tuberculosis in Saskatchewan from 1988 to 1997, 4.8% had a normal chest x-ray as judged by both a TB specialist and a board-certified radiologist.[19] This sensitivity is offset by poor specificity. Some patterns of chest x-ray abnormality are considered more “typical” of TB disease such as upper lobe infiltrates or cavitary lesions.[20] However, even when limited to these typical chest x-ray infiltrates, specificity remains low. One survey of TB suspects admitted to a large urban hospital in the US found a specificity of only 63% for typical chest x-ray changes. By limiting suspects to those with typical infiltrates, the sensitivity dropped to 73%.[20] In a study from the UK, Amsterdam and Rotterdam looking at newer digital chest x-ray technology, the sensitivity of radiologists and chest physicians for detecting culture positive TB on chest x-ray was a similar 77%.[21] HIV coinfected TB patients are less likely to have typical chest x-ray infiltrates, especially with declining CD4 cell counts.[22] In a community survey in high TB and HIV prevalent South Africa, the specificity for any abnormal chest x-ray was 67%, and when limited to typical changes it was 83%.[23] Additionally, older studies reported that atypical chest x-ray patterns suggested a diagnosis of primary TB as opposed to reactivation of latent TB. This was based on the use of recent tuberculin skin test (TST) conversion as evidence of a primary TB infection.[24,25] However newer molecular epidemiology studies that rely on clusters of TB to identify primary infections have not supported this association.[26,27] The subjective nature of chest x-ray interpretation also presents a challenge. Even amongst experienced radiologists and chest physicians there are high levels of inter-observer variability.[28] Thus, while certain chest x-ray findings can be indicative of tuberculosis, it remains an insensitive and nonspecific test.

Microscopy

Staining for acid fast bacilli (AFB) has been the cornerstone of TB diagnosis since its discovery by Koch in 1882.[29] Smear microscopy has the benefit of being inexpensive with low technical requirements, though does require substantial technician training and is time consuming. The value of smear microscopy is that it is able to identify the most infectious patients.[30] However, there are several factors that can affect the yield of a smear such as time of collection, sputum processing and using standard (Ziehl-Neelsen) versus fluorescent (auramine) stains. Overall it is estimated that a TB burden of 1×10^4 bacilli per ml can be detected with a smear.[28] One systematic review found two processing techniques that led to higher sensitivity, either 1) treating sputum with bleach or sodium hydroxide and centrifugation or 2) treatment with ammonium sulphate or bleach and subjecting to overnight sedimentation; however neither improved specificity compared to direct smears.[31]

Overall the sensitivity of a single sputum smear compared to culture is approximately 60%, though it varies widely depending on how advanced disease is at time of presentation.[28] There does appear to be good inter-observer agreement on AFB smears, even under field conditions by rapidly trained technicians.[28] HIV-infected patients have been reported to have lower rates of sputum smear positivity, attributed to lower rates of cavitary disease and

overall less bacillary burden.[32] Traditionally three smears on different days have been collected early in the morning when the yield is thought to be highest. However a recent meta-analysis of same day "spot" sputum collections compared to a mix of spot and early-morning collections showed no difference in sensitivity or specificity.[33] This has led the WHO to change its recommendations to two "spot" collections to sufficiently evaluate for tuberculosis.[34] There has also been a traditional emphasis on obtaining three samples to increase the sensitivity but the incremental yield of a third smear is low (2–3%).[35] In one study of patients who already had two negative smears, it took between 122 and 796 3rd smears to find one additional patient with smear positive TB, depending on the setting. [36] The US has not changed its guidelines yet, with the CDC still recommending 3 consecutive sputum smears, with at least one being an early morning specimen.[37]

Solid Media Culture

Mycobacterial culture has traditionally been performed on solid media, usually Lowenstein-Jensen. Culture has increased sensitivity over AFB smear, with the ability to detect on the order of 1×10^2 bacilli per ml, though requires 4–6 weeks, limiting utility.[28] It can also distinguish between non-tuberculous and tuberculous mycobacteria, which are indistinguishable on microscopy. Historically, in large TB control programs in high-burden countries, culture played a smaller role as patients with smear-negative but culture positive TB are less infectious, have a more indolent course of disease, and were therefore not seen as major contributors to the ongoing epidemic and considered lower priority.[38] However in high HIV burden countries, smear negative patients have been shown to have poor outcomes and have therefore become a higher priority.[39,40]

Drug Sensitivity Testing

Solid media culture is also used for testing the drug sensitivity of isolates. Once growth of M. tuberculosis (*Mtb*) is detected on standard media it can be subcultured onto media containing antibiotics. In the absolute concentration method, the isolates are inoculated onto different media containing progressive concentrations of antibiotic to find the minimum at which 99% of growth is inhibited.[41] In the resistance ratio method, the minimal concentration that inhibits growth is divided by the minimal concentration that inhibits a standardized strain of *Mtb*. The most common technique is the simpler proportion method where two media are made, one with and one without antibiotic and the proportion of *Mtb* colonies growing in the presence of antibiotic is compared to those growing without antibiotic. Predetermined cutoffs determine whether the isolate is considered sensitive or resistant.[42]

NEW DIAGNOSTICS

Interferon gamma release assays

The tuberculin skin test (TST) has been used for decades to detect latent tuberculosis. It utilizes a heterogeneous mix of protein antigens present in both tuberculosis and other mycobacteria to assess immune activation in the form of induration at the site of injection. Interferon gamma release assays (IGRAs) were developed to find a more specific test that would not contain antigens from bacille Calmette-Guerin (BCG) used in TB vaccines.

Patient cells are incubated with *Mtb* specific antigens and interferon-gamma release is measured as a marker of activation.[41] Two main commercial tests have been developed and are in current use, the T-SPOT.TB, which uses isolated blood mononuclear cells and the QuantiFERON-TB Gold In-Tube (QFT-GIT), which uses whole blood.

There is interest in utilizing IGRAs to assess for the presence of not only latent but also active tuberculosis. However, in a systematic review of studies of patients suspected of active tuberculosis, the QFT-GIT had a pooled sensitivity of 69% and specificity of 52% while the T-SPOT.TB had a pooled sensitivity of 83% and specificity of 61%. HIV co-infection decreased the sensitivities to 60% and 76% respectively.[43] Based on this data, the WHO advises that "neither IGRAs nor the TST should be used for the diagnosis of active TB disease."[44] The sensitivity is too low to reliably detect disease while the specificity is too low for a negative test to reliably exclude it.

Fluorescence

Fluorescent AFB stains for tuberculosis have been available since the 1930s but historically required specialized microscopes with fragile mercury vapor light sources. They also needed regular maintenance and a dark reading room, all of which limited its utility. The benefit of fluorescence has been the ability to use a lower power objective lens that allows a microscopist to look at a larger portion of the smear. Newer light emitting diode (LED) fluorescent microscopes are more robust and function in a standard light room.[45] A systematic review found that fluorescence was 10% more sensitive than standard (Ziehl-Neelsen) AFB stains with a similar specificity. Use of fluorescent staining also reduced reading time to 1 minute for a single smear compared to 4 minutes for a conventional smear.[46] The WHO recommended in 2009 that conventional microscopy be replaced by fluorescent LED microscopy, leading to more widespread use in recent years.[45]

Liquid Media Culture

TB culture has also seen incremental improvement in techniques. Newer liquid media systems initially were based on radioactive growth indicators but now use fluorometric growth indicators. Their advantage over traditional solid media is improved sensitivity with an increased yield of about 10% but more importantly, they are quicker to identify *Mtb*, resulting in a faster time to positivity.[4] False positives can occur due to cross-contamination in the lab, with reports from developed nations of up to a 3% false positive rate.[47] Liquid media culture systems are also able to perform drug sensitivity testing by a proportional method of comparing growth in a critical concentration of antibiotics versus antibiotic-free media. Liquid culture systems have become the standard of care in developed nations and are recommended by the WHO in middle and low income countries as well for *Mtb* detection as well as for drug sensitivity testing.[48]

Polymerase Chain Reaction

The most exciting development in TB diagnostics has been the use of polymerase chain reaction (PCR) for rapid diagnosis. PCR has the advantage of a turn-around time within hours, high specificity, and the potential for high sensitivity. Initially a variety of both in-house non-commercial and commercial assays were developed. Studies of their ability to

detect TB show a large degree of heterogeneity, which may be explained by the use of different cut-off values, different primer targets and different sample collection and preparation protocols. However even when controlling for these factors there is still a large variation in the reported sensitivity.[49,50] Additionally, many PCR assays require highly trained staff and sophisticated laboratory infrastructure.

More recently the Xpert MTB/RIF system has generated considerable interest. Xpert probes for the *rpoB* gene of mycobacterium tuberculosis, which encodes for the majority of rifampin resistance. With this it is able to simultaneously identify the presence of *Mtb* as well as rifampin resistance. Resistance to rifampin is a strong indicator of concurrent isoniazid resistance and thus multi-drug resistant (MDR) TB.[51] The Xpert has been studied in multiple large multi-center randomized control studies with a pooled sensitivity in sputum samples of 89% compared to culture and a specificity of 99%, while AFB smear had a sensitivity of 65%. Among subjects who are AFB smear negative, the sensitivity of Xpert decreases to 67%.[52] The ability to rapidly detect smear negative patients leads to more rapid initiation of therapy, cutting the delay from 56 days to 5 in one large study.[53] There may also be a role for Xpert in reducing the time for rule out of tuberculosis in hospitalized patients[54]. Cross-reactivity with other non-tuberculous mycobacteria has been seen in only 0.6% of cases. As with other diagnostics, the sensitivity of Xpert is lower in HIV-infected patients with a pooled sensitivity of ~80% compared to culture, but can detect 61% of cases missed by smear microscopy.[52]

Xpert has a sensitivity of 95% and specificity of 98% for rifampin resistance compared to standard culture and drug susceptibility testing (DST) based on a meta-analysis that included studies with both solid and liquid culture as the reference standard. The cutoffs for detection of rifampin resistance have been adjusted in several software upgrades leading to minor variations in the sensitivity and specificity.[52] Detection of rifampin resistance is reduced from 106 days with traditional drug sensitivity testing to 1 day.[53] Furthermore, though Xpert can identify rifampin resistance, which is a strong marker for MDR TB, Xpert is limited in its inability to provide additional drug susceptibility testing, including INH. This full DST is necessary for determining the exact diagnosis including identifying extensively drug resistant TB (XDR TB), and for designing the optimal therapeutic regimen. Another limitation is that GeneXpert detects the DNA of nonviable *Mtb* so is not recommended for treatment monitoring.

Overall, the excellent performance characteristics led it to be endorsed by the WHO in 2011, leading to rapid uptake in TB control programs globally. However, assessment of the impact on overall TB program success has been mixed. Two recent randomized studies of Xpert versus microscopy in real-world settings showed the Xpert had better sensitivity and quicker time to starting TB therapy but had no impact on morbidity or mortality at 6 months.[55,56] The authors speculated that part of this could be due to high amounts of smear negative patients being treated for tuberculosis empirically independent of the smear results. Thus the benefit observed from increasing TB case detection with Xpert testing is attenuated by the large number of smear negative patients being treated, potentially unnecessarily, for TB. Another potential reason is that the major impact on mortality may be in identifying rifampin resistance, and these studies had few patients with drug resistant TB.

Line Probe Assay

PCR has also been used in conjunction with a new class of diagnostics called line probe assays (LPAs). Sample DNA is PCR amplified with biotinylated primers. The products are then run down a strip of nitrocellulose paper that contains DNA probes that when bound to target DNA activate a colorimetric indicator. Much as the Xpert MTB/RIF looks for evidence of resistance in the *rpoB* gene to predict phenotypic resistance to rifampin, the DNA probes on LPAs can also conjugate with either wild type or resistance mutated DNA. Their main use has been for the rapid detection of drug resistant tuberculosis, either directly from AFB smear positive sputum, or indirectly from cultures of patient samples. As opposed to conventional phenotypic DST, which can take several weeks to result, LPAs report results in a matter of hours. LPAs have not been FDA approved in the US but have been widely used globally and are endorsed by the WHO for the detection of drug resistant TB.[57]

The two main commercial LPA tests in use for first line resistance are the INNO-LiPA and the GenoType MTBDRplus which both have probes for the *rpoB* gene. They demonstrated comparable sensitivity of about 95% for rifampin resistance and specificity of 98% compared to phenotypic DST.[58] The GenoType MTBDRsl has additional probes for mutations in *gyrA*, which encodes DNA gyrase and is a target of fluoroquinolones, and *rrs* that encodes the 16S ribosomal subunit, and is a target for aminoglycosides and capreomycin. It also has probes for *embB*, which encodes for a portion of arabinosyltransferase that is the target of the first line drug ethambutol. The performance for second line DST is not as good as for rifampin, with the MTBDRsl only able to identify 85% of fluoroquinolone resistance and ranging from 66 to 87% for the injectables (amikacin, capreomycin and kanamycin). Still its specificity was good at greater than 95%.[59] In HIV-infected individuals there is little data, with one study of 90 TB patients showing reduced sensitivity for fluoroquinolone resistance of 55.6% and as low as 9.4% for kanamycin resistance.[60]

Other Technologies

Several additional technologies for diagnosing tuberculosis are in the pipeling, but have not been sufficiently validated or endorsed by the WHO to realize widespread uptake. Colorimetric redox indicator (CRI), nitrate reductase assay (NRA) and microscopic observation drug susceptibility (MODS) assays have been reviewed by the WHO but only recommended “as an interim solution while capacity for genotypic or automated liquid culture and DST is being developed.”[61] Other technologies such as thin-layer agar, bacteriophage assays and loop mediated isothermal amplification (LAMP) have been insufficiently evaluated to gain a WHO recommendation.[62]

CONCLUSION

While these new diagnostic tools have generated considerable interest and carry a great deal of potential to affect incidence, mortality, and transmission, their full impact on the global campaign against tuberculosis has yet to be evaluated. Overall there has been progress by one important measure, the case detection rate. This is the ratio of reported cases to those expected based on incidence surveys. In 1995 the TB case detection was as low as 38 to 41%

worldwide and is now estimated to be at 63%. This still remains short of the WHO goal of 70%[1].

To reach the remaining 37% of tuberculosis patients who are not diagnosed will take both new diagnostic technologies as well as strengthening of health care systems. While the new diagnostics discussed here have improved TB detection, the ideal diagnostic test will 1) be point of care, immediately providing a diagnosis of TB in the presence of the patient and 2) provide rapid drug susceptibility testing to identify drug resistance immediately, so that patients can initiate appropriate care without delay.

Identifying TB patients earlier, optimizing diagnostic algorithms to integrate technologies, initiating patients on treatment quickly, integrating with antiretroviral therapy for those who are HIV-infected, implementing widespread preventive treatment, and keeping patients retained in care are all important parts of a comprehensive TB care and prevention program. This is embodied in the WHO's new End TB Strategy, which also recognizes the need for political will, engagement of communities and civil society, and health care systems and policies that support TB treatment and regulation, and innovative research supporting new therapeutics and implementation.[1, 63] In the end, while new diagnostics are crucial, TB elimination will require a comprehensive, multifaceted approach.

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3 Research Priorities

- Is a point of care test for pulmonary and extrapulmonary tuberculosis feasible, effective at reducing mortality, and cost effective?
- Is a rapid diagnostic test to identify the full spectrum of TB drug resistance feasible, effective at reducing mortality, and cost effective?
- What is the optimal combination of symptoms and rapid diagnostics that is most effective and cost effective for diagnosing pulmonary and extrapulmonary tuberculosis in resource-limited settings?

Table 1

Sensitivity & Specificity of Diagnostic Tools

Diagnostic Tool	Sensitivity	Specificity	Pros	Cons
Symptom Screening	Variable, up to 93%	Variable	Cheap, easy to implement	Large tradeoff between sensitivity and specificity, performance depends on setting
Chest X-ray	73–95%	63%	Already implemented in many centers, cheap to perform	Requires expertise to interpret, high inter-observer variability
Sputum Microscopy	60%	95%	Cheap	Time consuming, low sensitivity in HIV coinfection
Solid Media Culture	Reference standard	Reference standard	Cheap	Requires significant lab infrastructure, requires up to 8 weeks
IGRA *	69–83%	52–61%	Does not require sputum	Poor performance for active disease
Fluorescence Microscopy	70%	95%	Higher sensitivity and faster than conventional microscopy	Requires specialized equipment
Liquid Media Culture	10% more than solid media	Reference standard	Faster than solid media but still requires weeks	Specialized equipment and highly trained technicians
PCR *	10–100%	5–100%	Rapid turn-around, most are highly specific	Expensive, highly variable performance, need highly trained personnel
Xpert MTB/RIF	89%	99%	Rapid turn-around, good performance in HIV positive, moderately priced, rifampin resistance testing included	Unclear impact on mortality
LPA *	66–95%	99%	Can be performed directly on patient samples	Expensive

* IGRA – Interferon-gamma release assay, PCR – Polymerase chain reaction, LPA - Line Probe Assay