

Update on the diagnosis of tuberculosis- Time for a Game Change

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Date Received: March 02, 2018

Date Accepted: May 10, 2018

Declaration of conflicting interests

The Authors declares that there is no conflict of interest.

ABSTRACT

Tuberculosis, particularly drug resistant tuberculosis (DR-TB), is a main problem globally especially in developing countries like Pakistan. Drug resistance in tuberculosis is basically a man-made phenomenon caused by inaccurate prescribing practices by physicians and disobedience on the part of patients. Timely diagnose and successful treatment of tuberculosis is a lifesaving intervention. Multidrug resistant tuberculosis (MDR-TB) has become an epidemiological issue and is the most important concern globally. Case management needs to be simplified and standardized as in many countries MDR-TB cases cannot receive individualized care from physicians. Tuberculosis tests are old fashioned and inadequate as the most widely test used is microscopy which misses half of all cases. Recent progress in molecular biology has brought major improvements in diagnosis of tuberculosis with introduction of some new diagnostic techniques. The new automated culture techniques have considerably reduced the time required for detection and antimicrobial susceptibility testing of tuberculosis. Introduction of new technique such as Xpert MTB/RIF test, a game changer, have also shown a lot of promise. However, most of these new techniques are too costly and sophisticated to be of any practical advantage to the common patients of TB living in developing countries for whom a timely and economical diagnosis as restrained as ever. In the present article we studied various existing modalities as well as the new advances in TB/MDR-TB diagnosis

Key Words

Mycobacterium tuberculosis; MDR-TB; Diagnosis; Diagnostic Techniques.

This article may be cited as: Khan AS and Ullah R. Update on the diagnosis of tuberculosis- Time for a Game Change . Pak J Chest Med 2018; 24 (2):101-107.

Introduction

Mycobacterium tuberculosis, causing tuberculosis, is probably the most important human pathogen. Annually about 9 million new cases are reported, with at least 3 million morbidity rate. World Health Organization (WHO) reported that one third of the world population is affected by Mycobacterium tuberculosis.¹ Tuberculosis is the leading cause of death worldwide due to any single infectious agent and 95% of these cases occur in the developing countries where diagnostic and treatment facilities are simple or nonexistent.¹ Annually incidence of TB is 267 and prevalence of all cases is 342 per 100,000 of population in Pakistan.¹ A drug Resistance survey, conducted in Pakistan showed that the estimated percentage of MDR-TB in new notified TB cases is 4.3% and in re-treatment cases is 19.4%.² In selected populations of Khyber Pakhtunkhwa, 3% of MDR-TB

is present in new cases, 26 % in previously treated cases and 17.4% in close contacts of MDR-TB patients^{3,4} while it is 4% in new and 19.4% in previously treated cases in Punjab, Pakistan.⁵

National TB Control Programs (NTCP) is mainly challenged by MDR-TB. Only 2% of the total occurred cases of MDR-TB are reported to the WHO and managed in accordance to international guidelines. The remaining cases are thought to be either mis diagnosed or improperly treated, resulting in further spread MDR-TB epidemic.¹

Early diagnosis and efficient treatment counts a lot, when it comes to disease control. Over past 15 years about 6 million deaths have been prevented by the global scale-up of tuberculosis therapy, which is definitely a great achievement in public health.⁶ But before a patient being treated actually have transmit-

ted the infection to his close ones, which is one of the reason of the disease spread every year,⁷ and is responsible for increasing infection every new year.⁶

In developing countries, diagnosis of tuberculosis is usually based on clinical presentation and chest x-rays. Clinical presentation alone is not sufficient for diagnosis because signs are mostly absent in early stages of disease, and symptoms are not very specific, and physical findings varies from patient to patient.^{8,9}

Chest x-rays was considered as the most efficient way of diagnosing TB, still it have important role, but x-ray alone is no more considered as a sufficient technique for accurate diagnosis. Although pulmonary tuberculosis almost always shows abnormalities on the chest x-ray⁹ radiological diagnosis alone can only be presumptive since the diagnostic criteria are non-specific.⁸ As clinical picture and chest x-ray pattern cannot always be used to distinguish between pulmonary TB¹⁰ and extra pulmonary TB the diagnosis can be misleading.¹¹ Association of HIV with TB has further complicated the issue. Both the clinical as well as radiological presentation in such cases can be atypical or even non-existent.^{8,9,12} Moreover, almost one-fourth of all TB cases are extra pulmonary^{13,15} and in such cases x-rays are of limit eduse while clinical presentations are erratic.

Laboratory processing is also an important aspect of TB diagnostics. Conventionally, laboratory diagnosis of tuberculosis has been based upon smear microscopy, tuberculin skin test and culture. However, many other different diagnostic techniques have become available in recent years. A brief discussion of these is given below.

Test smear microscopy is the oldest technique used as a primary laboratory tool for diagnosis of TB, establish about 125 years before. Test smear microscopy usually misses half of all cases. Although really problematic, but still, such tests are practiced in underfunded and dysfunctional health care systems.^{16,18}

In developing countries like Pakistan, it is often the only diagnostic test available.¹⁷ WHO recommended this technique as the primary laboratory diagnostic test for the developing countries, as it is simple and inexpensive, and can detect the most infectious cases of TB, thus breaking the cycle of transmission.¹¹ Advantages of the technique include low cost rapidity and simplicity, and its high specificity. It detects the most infectious subsets of patients and is useful in monitoring response to treatment.^{17,19} However, the technique is hampered by several drawbacks: it fails

to differentiate between dead and viable bacteria, speciation is not possible and most importantly, its sensitivity may vary from 33% to 75%.^{9,17,19} The reason for this reduced sensitivity is that a large number (at least 5000 AFB/ml) of bacilli must be present in a specimen for the smear to be positive.^{9,19} This lack of sensitivity has limited the utility of the technique. The sensitivity of the test can be increased by examining multiple specimens^{9,10,20} and by processing the specimens by centrifugation and liquefaction techniques using sodium hypochlorite²¹ chitin²² or N-acetyl-L-cysteine in 1% sodium hydroxide solution.⁹ An adequate volume of sputum must be processed to get better results; at least 5 ml has been recommended. Sometimes it is not possible to obtain adequate amount of sputum, especially in children who cannot expectorate. In such cases sputum induction can be of use in obtaining the required amount of sputum.⁹

It is estimated that two billion persons around the world have latent tuberculosis infection and about 10% of these would develop active disease.^{23,24} Another test, known as tuberculin skin test, established in the 19th century and still in practice in the clinical setup. The tuberculin skin test, although not 100 percent sensitive for infection with *M. tuberculosis*, but is useful in latent infection which can be readily identified with the help of it.¹⁵ Despite the fact that antigen used in this test are not very sensitive and specific, it leads the run, as no better and alternative choice is available.⁹ Even among patients with proven tuberculosis without apparent immune suppression, 10 - 20 % will have negative skin reactions. Sensitization to tuberculin can also be induced by infection with non-tuberculous mycobacteria (NTM) and BCG vaccination. The immune reaction caused by true infection and by BCG can't be differentiated.²⁵ Repeated usage of tuberculin tests can seriously question its efficiency in infection detection in high risk population, e.g. as initially tuberculin-negative contacts of active cases and workers with occupational exposure. Random variability due to differences in administration, reading or biologic response, immunologic recall of preexisting delayed type hypersensitivity to mycobacterial antigens (boosting phenomenon), or new infection (conversion phenomenon) can increase or decrease (reversion phenomenon) the size of tuberculin reactions.

Several factors contribute to the results of Tuberculin skin test, which includes prevalence of TB in facility, risk factors, environmental and epidemiological potential of infection for each patient. The tuberculin test, like all medical tests, is subject to variability, but many of the inherent variations in administering and

reading tests can be avoided by careful attention to details. The preferred skin test for Mycobacterium tuberculosis infection is the intradermal or Mantoux method.²⁵ In high-risk groups (intravenous drug users, mycobacteriology lab workers, children less than 4 years of age and populations in high prevalence settings like Pakistan), an induration of 10mm or more is considered as positive. However, in immunocompromised patients including HIV positive persons, an induration of 5 mm is considered as positive. In persons with no known risk factors for tuberculosis, an induration of 15 mm is taken as positive.²⁶

By using culture technique, about 80-85% sensitive and 98% specific result can be obtained. However efficient diagnosis still requires isolation of Mycobacterium tuberculosis from culture.^{9,15,27} The increased sensitivity of the technique is due to its ability to give a positive culture even when small numbers (10-100 AFB/ml) of bacilli are present in the specimen. Culturing makes pathogen species identification and drug susceptibility testing possible.⁹ The main drawback of the culture technique is the long time required for mycobacterial growth to occur due to their slow doubling time of 18-24 hours. It can take up to 8 weeks for visible growth to occur on solid medium and another 3 weeks might be required for anti-microbial susceptibility testing, which is unacceptably a long time in clinical practice.⁹ It has been recommended by the United States Centers for Disease Control (CDC) that both M. tuberculosis identification and determination should be performed within 30 days.²⁸

In molecular biology, last two decades are considered as a revolutionary era. The technical advances have led to the introduction of several new diagnostic tools for tuberculosis as well as improvement of the existing techniques. Several new automated culture systems have been commercially introduced in the last few years, which have reduced the detection time significantly. These include the radiometric BACTEC 460 TB system, the colorimetric BacT/ALERT MB Susceptibility Kit,^{29,30} Mycobacterial Growth Indicator Tube (MGIT) systems^{31,32} with an oxygen quenching-based fluorescent sensor and the ESP (Extra Sensing Power) Myco-ESP Culture System II based on the continuous monitoring of pressure changes due to the consumption or production of gas resulting from metabolic activity of microorganisms growing in a liquid medium.³³ These liquid systems basically help in detection of mycobacteria because they support rapid growth, and thus are useful for antimicrobial susceptibility testing.⁹ The high cost and technical

sophistication of these systems, however, precludes their use outside reference laboratories.

Mycobacteria contain large amounts of lipids. A specific compound, which is known as mycolic acids (component of cell wall) is synthesized by each mycobacterial species. Mycolic acids are present in very specific configurations and the type of mycolic acid can be used to distinguish different mycobacteria.⁹ Techniques like, thin layer chromatography (TLC), gas chromatography and high performance liquid chromatography (HPLC) can be used to detect these mycolic acids. More than 50 mycobacterial species can be differentiated via HPLC. Moreover, it requires only a few hours and a limited number of organisms from pure cultures. HPLC is costly, therefore its availability is limited.^{9,19}

One of the dramatic achievements in science during the last decade has been the advancement in molecular biology. The ability to detect small amounts of DNA or RNA by amplification techniques has led to the development and introduction of rapid and accurate diagnostic techniques in microbiology. Nucleic acid techniques for detection and identification of bacteria fall into three basic groups: (1) target amplification by polymerase chain reaction (PCR), transcription media amplification, nucleic acid sequence based amplification (NASBA), etc. (2) probe amplification using ligase chain reaction or Q-beta replicase; and (3) signal amplification, as in branched DNA assay.^{34,35} Mycobacteria can be detected directly from specimen by several molecular techniques like direct amplification tests (DATs), drug testing and direct detection in cultures, thus reducing the time frame for diagnosis from months to days.³⁵ Several assays for detection of mycobacterial nucleic acids by the PCR are now commercially available. PCR assays have made diagnosis and specific identification of Mycobacterium tuberculosis possible within a day. The test is rapid, sensitive and specific and can detect fewer than 10 organisms in clinical specimens, compared to 105 bacilli/ml necessary for AFB smear positivity. PCR is highly specific but less sensitive than culture technique.^{9,10,19}

In case of AFB smear positive, clinical respiratory specimens the sensitivity and specificity of PCR are 95% and 98% respectively.^{9,35} However, sensitivity for smear negative cases has varied from 40% to 77% although the specificity has remained approximately 95%. The conjugative usage of DATs with microscopy and culture, and then doing result interpretation in accordance to clinical data is strongly suggested by The American Thoracic Society.⁹ Another problem with

the DATs is that they cannot differentiate between dead and viable organisms.^{8,34} Thus, the response to chemotherapy cannot be assessed by PCR assays. Molecular amplification tests are also quite expensive and technically sophisticated.¹⁰ Several biochemical and phenotypic tests are used to attain species level identification of mycobacterial isolates traditionally. Still, different isolates of same species respond differently to reactions, even the same isolate response deviates with increasing time duration, which affects the species identification. Laboratories are adopting molecular techniques because of less efficiency and more time consumption of biochemical testing for species identification.³⁵ Molecular techniques are highly reliable and reproducible, they can even detect accurately in contaminated mixtures. With the advent of modern molecular biology, it is now possible to investigate the drug resistance mechanisms and genes involved in *M. tuberculosis* *gpoB* gene allied rifampicin resistance.³⁵ Epidemiologic studies and TB outbreaks investigations can also be performed with the help of DNA fingerprinting (Restriction Fragment Length Polymorphism (RFLP)), along with detection of laboratory cross contamination, exogenous re-infection and reactivation.^{8,10}

Tuberculosis can be controlled with timely diagnosis and treatment. However, regions with high prevalence of tuberculosis also lack the resources to institute effective control measures. It is estimated that three quarters of all TB cases in Pakistan are never diagnosed.⁸ The diagnosis is mostly based upon clinical suspicion or on the therapeutic response to anti-tuberculosis drugs, rather than on the basis of culture isolation. This results in appropriate use of anti-tuberculosis drugs. Further more, compliance with treatment remains poor. All these factors are responsible for the development of drug-resistant tuberculosis and spread of infection. Despite TB having been declared a national emergency in 2001, implementation of the national TB control program in Pakistan has been hampered by the underdeveloped health facilities, lack of resources and poor management.³⁶

Although advances in diagnostic mycobacteriology have been, at times, spectacular, we still have not come up with the ideal diagnostic test for TB; one which is simple, rapid, inexpensive and accurate. The automated culture and molecular techniques are sensitive and have reduced the detection time, but they are too expensive and sophisticated for wider use in the resource-poor third world countries with the highest burden of tuberculosis.¹⁷ The newly introduced phage assays have the potential of wider utility in

under developed countries like Pakistan as they are sufficiently sensitive and specific, rapid, simple and relatively cost-effective. Used in conjunction with smear-microscopy, they have shown excellent results.^{37,38}

WHO have endorsed the extensive use of several diagnostic tests for tuberculosis.^{39,40} Automated PCR test can even make a simple lab technician to detect TB infection and antibiotic resistance within less than two hours.⁴¹ Such techniques have spark to raise TB diagnosis. Rapid diagnosis and timely treatment will hopefully control TB epidemic in near future. In a large, well-conducted, multi country study, Boehme et al. (2010) evaluated the efficiency of automated tuberculosis assay (Xpert MTB/RIF) for the presence of *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF) has been evaluated, with the efficiency of 98% infection detection and resistance to rifampicin accurately.⁴¹

Automated tuberculosis assay (Xpert MTB/RIF) is thought to be more reliable than conventional nucleic acid amplification tests because it is simple, safe for contamination, highly sensitive to smear negative samples and do not require high safety level lab. MTB/RIF assay also have some concerns like high cost, confined only to rifampicin resistance treatment monitoring, limited mutations detection, infection-control intervention and incompetence to specify the patients' sputum smears which are positive for reporting.

On the plus side, the MTB/RIF assay promises to decentralize molecular diagnosis, since it potentially can be used at the point of treatment in a microscopy center or in a tuberculosis or HIV clinic. However, because the studies conducted by Boehme et al. are limited to reference laboratories, therefore it can't be deduced clearly that this test could be used in such settings. Extensive studies are required to evaluate and scale up this test, in order to analyze its usefulness and afford ability to patients. We will be able to save more than 15 million lives by 2050, in case we modify rapid nucleic acid amplification test to more efficient levels.⁴² The availability of the diagnostic test to the patient actually marks its impact, no matter how an efficient a test is, if it is not available it has no worth. The system in which a test/intervention is used is actually increasing or decreasing its impact. We must focus on improving and strengthening our health systems, to insure rapid diagnosis and accessibility of proper treatment on time. Health-system should adopt and manage new technologies openly. Although the burden on health systems will be

reduced by a simple dipstick like, point-of-care assay, such tests are not likely to be available in the short term.⁴⁰ Large scale innovations and deliveries should be supported by stake holders in order to understand the true worth of advanced technologies. It is strongly recommended that researchers and industries should work in proper collaborations to facilitate innovation in more economical way, especially for the countries with less resource. Researcher should work to quick identification, and to deal with full spectrum of issues more critically, to find more operational and implementable solutions for improvement. Proper policy making should be done by policymakers and regulators to ensure the indulgence of national programs to rapidly incorporate new tools to transform scientific evidence into best possible outcomes. New technology should be extensively funded to facilitate innovations. Delivery system should be promptly modified and special attention should be given to projects focused upon tuberculosis control. Proper awareness should be spread and patient advocates and activists should hold everyone accountable and ensure that communities drive demand for improved systems and tools.

Great deals of advancement have been observed in diagnostics despite of all the hurdles and challenges. Further improvements are also being extensively researched in order to give the better deal of facilities to common man's life, more funding is required to establish better health care systems, and develop better technologies. We are particularly optimistic about the potential role of governments, product developers, and companies in emerging economies with high tuberculosis burdens, such as China, India, Brazil, South Africa and Pakistan. Now they can modernize novel, low cost assays and incorporate them in both National Tuberculosis Control programs and private laboratories, supported by successful public-private partnerships. Emerging economies have the potential to become global leaders in innovative product development and delivery. The eradication of TB at the end of 2050 could be a reality of these countries manage their TB control programs efficiently. However, they need to be further evaluated and improved before their validity can be firmly established. At present no single test is adequate for the diagnosis of tuberculosis. Recent advances in diagnosis of TB are really attractive but still have limited real time impact because of affordability issues for common man. There is a dire need for development of newer diagnostic techniques and refinement of existing ones to cater for the particular needs of the resource-poor third world. If the general trend in scientific advancement is any indicator, the

future looks promising. In the meantime, we have to make the best use of what we already have, relying on a judicious combination of the existing laboratory tests in conjunction with x-rays and clinical presentation for the diagnosis of TB. The medical community must combine the best possible use of the tools already in hand with increased awareness of the magnitude of the problem, a high index of suspicion, early case identification and prompt and optimum treatment.

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