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Full Length Research Paper

Description and evaluation of mycobacterium tuberculosis diagnosis

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Early diagnosis of tuberculosis (TB) and initiating optimal treatment would not only enable a cure of an individual patient but will also curb the transmission of infection and disease to others in the community. Of the several distinct components of TB control programmes, case-finding remains the cornerstone for effective control. However, there are no definite guidelines available as on date as how to use optimally the number of diagnostic tests ranging from simple AFB microscopy to complex molecular biological techniques which have become available over a period; to establish or rule out diagnosis of tuberculosis in a given patient. Therefore, there is need for description and evaluation of the existing important techniques available for the diagnosis of TB and drug susceptibility testing and their advantages and limitations which will help in the development of appropriate TB diagnostic guidelines for the implementation of TB control strategies.

Key words: Description, evaluation, techniques, diagnosis, *Mycobacterium tuberculosis*.

INTRODUCTION

Approximately one third of the world's population is infected with the TB bacillus, but a majority of infected people never develop active disease (Dye et al., 1999).

TB is a bacterial disease caused by *Mycobacterium tuberculosis.* The family mycobacteriaceae has over 60 members or species. Some species, including those in the *M. tuberculosis* complex, cause human disease, but most are not pathogenic to humans. Many of the non-pathogenic mycobacteria are found in the environment, e.g. in water and soil.

M. tuberculosis transmission commences when a person with active pulmonary TB coughs, sneezes or spits, launching TB bacteria into the air. Inhalation of these bacteria is the most common mode of infection. The risk of infection following exposure depends on several factors, including the concentration of bacteria in the air, the duration of exposure, the virulence of organism (debatable), and the immunocompetence of the exposed individual.

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Even in the absence of a competent immune system (that is in children) or under conditions that suppress immunity (Human Immunedeficiency Virus (HIV), diabetes, kidney failure, etc), most humans contain the bacilli and never become ill. This condition is referred to as latent TB infection (LTBI). Overall, there is only a 10% lifetime chance of a person with LTBI developing an active form of the disease (Rich, 1944).

When TB does outstrip the body's immune defences, active tuberculosis disease develops. Disease of the lungs (pulmonary TB) is the most common form of active TB and is the infectious form of the disease. A highly infectious person can transmit disease to 10 to 15 persons in a year, with household members being particularly at risk (Styblo, 1986). TB however, is not limited to the lungs but can affect virtually any organ of the body. Persons with extrapulmonary tuberculosis make up about 10 to 20% of all those with active TB. These forms of the disease are not infectious and are seen more commonly in children and persons co-infected with HIV.

The most important priority for TB control is the accurate diagnosis and prompts treatment of persons with active, infectious TB. Doing this both interrupts TB transmission and cures patients. In the absence of effective treatment, TB mortality is 50% or more

(Grzbowski and Enarson, 1978). This paper reviews the various techniques for the diagnosis of *Mycobacterium tuberculosis.*

ESSENTIAL DIAGNOSTIC PRIORITIES FOR THE DETECTION OF *MYCOBACTERIUM TUBERCULOSIS*

Since the discovery of TB, the basis for its definitive diagnosis has been detection of the bacillus in clinical specimens. Following microscopic detection of the organism in 1882, technical advances have allowed us to detect fewer and fewer organisms in a specimen. Unfortunately, however, the level of sophistication and cost associated with more sensitive techniques has, to date, made their general application unfeasible in developing countries.

Therefore, the basis for TB diagnosis in developing countries has continued to be the stained smear of expectorated sputum. Fortunately, this technique detects the most infectious patients and those most in need of treatment.

In contrast, in developed countries with lower rates of TB and greater human and financial resources, advanced techniques to complement sputum smear microscopy have been adopted and other tests to detect additional forms of disease, such as extrapulmonary and latent infection, have been applied. Thus, the quantity of financial and human resources has a direct impact on access to diagnostic tools and in part shapes a country's diagnostic priorities.

In developed countries with high income and low TB prevalence, their goal is the elimination of TB while in developing countries with low income and high TB prevalence, their goal is to identify and treat cases of TB.

In developed countries, higher diagnostic priority is placed on all forms of active TB disease, detection of multidrug-resistant TB and latent infection in high-risk groups. These measures are targeted at detecting and treating people with any form of TB (active or latent), with attention paid to high prevalence groups like socially marginalized people, people born in high-prevalence countries and also high-risk groups like HIV infected, close contacts of an active case, the immunocompromised and children under 5 years. Lower diagnostic priority is placed on latent infection in lower risk groups, identification of individuals found to be infected, or likely to be infected, including recent tuberculin skin test converters and individuals with certain medical conditions (diabetes, kidney failure).

In developing countries, higher diagnostic priority is placed on pulmonary tuberculosis highly contagious patients. Measures are targeted at the reservoir of highly contagious patients to intercept transmission by early diagnosis and treatment. Lower diagnostic priority is placed on pulmonary tuberculosis less contagious patients (pulmonary smear-negative), TB in other organs (extrapulmonary TB), latent infection (surveillance purposes) and multidrug resistant TB (surveillance purposes).

DETECTION OF *MYCOBACTERIUM TUBERCULOSIS*

In developing countries, screening for active disease is rarely conducted and case finding is therefore dependent on patients seeking care at a health facility (passive case finding). Those with active disease will present with a broad range of clinical manifestations, influenced by age, comorbidity, affected site (that is lungs, bones, lymph nodes, central nervous system), and severity of disease. Unfortunately, none of these manifestations points directly to TB as the definitive cause. And syndromic management is rarely recommended owing to the long, arduous treatment regimens and the social stigma associated with TB. At best, the history and clinical examination of a patient can establish "clinical suspicion", and laboratory and radiographic tests therefore play a critical role in therapeutic decision-making. TB patients coinfected with HIV, children, and those with extrapulmonary forms of the disease pose special challenges, and often a presumptive diagnosis based on clinical findings is justifiable owing to the limited availability and performance of the available diagnostic techniques.

There are special situations when age is a risk factor in infants, pre-school children and the elderly, the impact on clinical presentation is that the patient is at an elevated risk of developing active disease after primary infection, that is often a rapidly progressive, life-threatening disseminated disease. Children are often asymptomatic or have ill-defined symptoms in the early stages of illness. From the age of 67 to 70 years, people are at heightened risk of either reactivation or rapid progression of recent infection to disease.

In special situations where concurrent disease is a risk factor like in diabetes, cancer, drug abuse, nutritional status, alcoholism, or other conditions causing immunosuppression, the impact on clinical presentation is that these conditions may compromise the integrity of the immune system, resulting in progression from TB infection to disease. Symptoms and findings of the baseline disease state can obscure and delay the diagnosis of TB or result in misdiagnosis.

In special situations also where HIV is the risk factor, the impact on clinical presentation depends largely on the degree of immunocompromise.

When the immune system is strong, presentation does not vary greatly from the presentation of those who are HIV-negative. Conversely, with declining immune function, the clinical picture becomes uncharacteristic, with absence of cavitary disease, acute onset, progression and often dissemination of the disease.

The emergence of drug resistance to standard anti-

tuberculosis therapy has further challenged the limits of clinical diagnosis and heightened the importance of drug-susceptibility testing. The general approach to diagnosis and the current armamentarium of commercially available tools for screening and detecting active and latent TB infection are reviewed below. Of the tests described, conventional smear microscopy and chest X-ray are the most commonly used in both developing and developed countries.

RADIOGRAPHIC METHODS FOR DETECTION OF ACTIVE DISEASE

It is still widely believed that tuberculosis of the lung can be diagnosed by chest X-ray alone that is, patient visits once and spends one hour to undergo chest radiography and physician interpretes the radiographic image.

However, practical experience and numerous studies have shown that no radiographic pattern is diagnostic of tuberculosis (Nyboe, 1968).

Many diseases of the lung have a similar radiographic appearance that can easily mimic tuberculosis (Nakamura et al., 1970).

Similarly, the lesions of pulmonary tuberculosis can take almost any form on a radiographic picture (American Thoracic Society, 2000).

In developed countries and other settings where facilities and resources permit, patients with signs and symptoms of pulmonary TB are screened by chest X-ray. Films can be helpful in localizing abnormalities in the lung. However, to establish the tubercular aetiology of an abnormality, further examination is necessary and only bacteriology can provide the necessary proof. The advantages of radiographic methods are that it is convenient, fast and highly sensitive in those uninfected by HIV. It's limitation is that it is nonspecific resulting in overdiagnosis when used alone, relatively expensive, limited availability of equipment in most high burden countries, requires specialized equipment and power source and is particularly unreliable in settings of HIV.

DIAGNOSTIC LABORATORY METHODS FOR DETECTION AND CONFIRMATION OF *MYCOBACTERIUM TUBERCULOSIS*

Smear microscopy

Worldwide, the most common diagnostic test use to detect tuberculosis is microscopic examination of stained sputum or other clinical material on a glass slide. When present in sufficiently high concentrations, the bacteria can be readily identified by a trained technician using this technique, which has changed little since it was invented over 100 years ago. Microscopy is cheap to perform, specific enough to indicate treatment in countries where TB is prevalent, and can be completed within hours if necessary. Sputum smear microscopy involves sputum collection, smears preparation (fixation and staining), smears air-drying, microscopic examination, micrograph of acid-fast bacilli and reporting of results. Total time is two hours and patient visits two or three times.

Microscopy requires a large number of bacilli to be present in order for the result to be positive (5,000 - 10.000 per ml of sputum), and identifies the most infectious subset of patients (Starke and Taylor-Watts, 1989). However, this requirement limits its sensitivity, especially for less advanced disease.

Certain groups of patients, such as those with advanced HIV coinfection, people with tuberculosis outside the lungs, and children, are usually sputum smear –negative.

The inherent low sensitivity of the test is compounded by the conditions under which it is commonly performed, poor equipment, heavy workload, and inexpert or unmotivated staff. The proportion of cases detected by microscopy is often as low as 20 - 30% of all cases (Correa, 1977). Duplicate or triplicate sputum examinations are requested to help overcome this problem.

This need for multiple tests, each of which requires sputum collection, drying, staining and meticulous examination, results in delays in reporting and a relatively large number of patients do not complete the testing or are lost to the health care system despite having a positive test. Several methods are in use that may increase the speed or sensitivity of microscopy somewhat, including the use of fluorescence microscopes (Khan and Starke, 1995). Because of the greater cost of the necessary equipment, fluorescence microscopes are used primarily in industrialized countries.

The advantages of smear microscopy is that it detects the most infectious cases, highly specific in high prevalence settings, inexpensive and widely established while the disadvantages are its difficulty to be maintained in the field, it requires well-trained and motivated technicians, it is insensitive (35-70%) especially in HIV infection, children and extra-pulmonary disease (Chow et al., 2003; Ferrer, 1996; Perez-Rodriguez and Jimenez, 2000), it cannot distinguish between drug- resistant and drug –sensitive *Mycobacterium tuberculosis* and it also requires repeat visits (Rich, 1944; Styblo, 1986) by the patients.

Culture by phenotypic and morphological characterization

Bacteriological culture, considered the diagnostic gold standard, can identify the *M. tuberculosis* organism in over 80% of TB cases with a specificity of over 98% (Fourie et al., 1998; Mandalakes and Starke, 2005; World

Health Organization, 2006). As few as 10 to 100 viable bacilli per ml may be detected, although the sensitivity of cultures varies substantially depending on the specimen-processing method and the culture medium used. Compared to smear microscopy, culture is more expensive and requires more highly trained personnel but allows detection of more forms of disease, including less advanced cases.

Although bacterial culture is routinely applied in industrialized countries, access to culture facilities is limited in countries with fewer resources and its use in the public sector is restricted to smear-negative TB and to cases of suspected drug resistance. As *M. tuberculosis* grows slowly, conventional culture with visual detection of bacterial colony formation usually requires 2 to 6 weeks. Recently, a number of growth indicators have been used, often involving liquid media and automated systems that shorten the detection period to 1 to 3 weeks in most cases.

Mycobacterial culture involves sputum collection, sputum decontamination, inoculation on solid or liquid media, incubation on solid and liquid media, reading culture results, Mycobacteria species identification using molecular or biochemical methods and reporting of results. Total time involved is 2 to 6 weeks and patient visits 2 or 3 times.

We have the manual culture media which consists of the solid egg-based or agar based Lowenstein - Jensen and Middlebrook 7H10 or 7H11 respectively and the liquid synthetic Middlebrook 7H9 broth, locally prepared or commercial with growth indicators (MGIT, MBRedox ®). The advantage of the manual culture media is that it may be locally prepared, low cost, long refrigeration tolerated and individual colonies are identified while its disadvantages are that it takes 2 to 6 weeks detection time, less sensitive than liquid and has labour- intensive read-out.

The automated solid media which consists of DioTK (Salubris) and automated liquid media Bactec 460 (Becton Dickinson), MGIT ™ 960 (Becton Dickinson), MB/BacT (bioMerieux), SeptiChek- AFB ™ (Becton Dickinson) and ESP Culture II System (Trek Diagnostics). The advantages of the automated culture media are that it takes 1 to 4 weeks detection time, speeds drug – susceptibility testing, speeds species identification and decreases workload while its disadvantages includes the fact that it is expensive, requires more infrastructure, equipment purchase and maintenance is needed.

Nucleic acid amplification by molecular techniques

Nucleic acid amplification constitutes a rapidly evolving improvement in the detection and identification of *M. tuberculosis.* Bacterial DNA (or ribosomal RNA transcribed into DNA) is enzymatically amplified and detected with an appropriate reading system via a signal-generating probe. Several enzymatic amplification processes have been developed and introduced into commercial products; the most widely used are PCR (polymerase chain reaction), TMA (transcription mediated amplification) and SDA (strand displacement amplification). Tests based on nucleic acid amplification are usually highly specific for *M. tuberculosis* (close to 100%), although some commercial products require a 2-step diagnostic procedure (initial test for mycobacteria genus, followed by tests which differentiate *M. tuberculosis* from non-tuberculous mycobacteria). Positive results can be obtained with less than 10 bacteria/ml; therefore sensitivity is much better than smear microscopy, but slightly less than culture.

Currently, nucleic acid tests are used primarily for confirmation of smear- positive results or for primary case finding in combination with other methods. The most outstanding feature of nucleic acid amplification methods is the short time-to-result (between a half and one working day, including sample preparation) paired with a high level of diagnostic accuracy. Because of their price and complexity, the use of these methods is still limited to developed countries.

The nucleic acid amplification test steps for Gen-Probe's MTD Test involves using the test kit, lysis of cells and release of the nucleic acid target. Subsequently, a specific sequence of the mycobacterial nucleic acid is amplified, resulting in million-fold increased target DNA concentration, labeled DNA probe is added, resulting in a concentration- dependent complex of labeled DNA probe and DNA and detection of signal originating from the labeled probe bound in complex, using an automated reader. Total time taken is 2.5 - 3.5 h and patient visits the laboratory only once.

The advantages of nucleic acid amplification is that results are available in several hours , specificity 98-100% (17), sensitivity is greater than 95% in sputum that is acid-fast bacilli (AFB) smear- positive, and 60-70% in smear –negative, culture-positive specimens (18-20). Recently developed amplification tests may have better sensitivity in smear –negative specimens while retaining the same high degree of specificity (World Health Organization, 2006; 21 and 22). It also shows promise for materials other than sputum (blood, lymph, bone marrow, gastric aspirate, cerebrospinal fluid, urine, bronchial aspirate, cerebrospinal fluid, urine, bronchial aspirate and lavage), although results have considerable variability (23-24).

The disadvantages include the cost, complexity, lower specificity (higher proportion of false-positives) under field conditions and in-house tests may be less expensive but are more time-consuming.

Serology

Serological antibody tests that could detect diagnostic

response to TB have been pursued for over 100 years. The serological tests do not require live cells or culture and are able to adapt to multiple assay formats (ELISA, lateral flow and dipstick) that can be integrated into laboratories at different levels of healthcare systems and in resource-poor settings. Although the technology of serological tests for other diseases has been mature and is able to achieve accurate detection, the efforts to device serological test for TB include a vast amount of confusing and contradictory literature over the past decades. In most patients, progressive TB does generate easily detectable levels of antibody directed at a variety of *M. tuberculosis* protein and non-protein antigens.

However, serological responses to TB disease are quite heterogenous in human, and many confirmed TB patients do not have detectable antibodies against the individual antigens that have been included in commercial assays. Therefore, although dozens of companies, many of them small, have developed lateral flow or other serological tests for TB, the clinical performance of these tests has proven poor, especially in HIV co-infected patients.

Modern technologies have been applied to expand the pool of target antigens and have shown that novel antigens may offer improved detection of serum antibodies. Microarray is one of these technologies to generate multiplexed assay platform using fractionated native or recombinant M. tuberculosis proteins and other types of antigens that allow examination of the diagnostic potential of large number of antigenic targets.

Interrogation of the microarrays with large numbers of well characterized patient sera may make it possible to identify a set of diagnostic antigens (composite biomarkers) that together show high sensitivity and specificity to be incorporated in an assay for point-of-care diagnosis for TB control in high burden countries.

In contrast to many infectious diseases for which serodiagnosis or detection of antibodies or antigens in blood which involves preparation of supplies, sample collection, application of blood sample to immunochromatographic strip, reading the IC strip and positive results are when control and patient bars are visible) is used, technology has so far largely failed to provide and adequately sensitive, specific and practical method as a first-line screening tool for clinical use in TB [25,26] Specificity is hampered by antibodies in the sample that cross-react with environmental mycobacteria, leading to false-positive results. Also, the lack of reproducible methods for purifying antigens means that results are variable. Currently available serological tests, therefore, offer little compared to standard smear microscopy, but their superior operational characteristics hold some promise. Since none of the assays is yet approved by regulators in North America, Europe, or recommended by the international TB community, their use is restricted to the private sectors of countries lacking diagnostic regulatory bodies. The advantages of serological tests are that they are more convenient when obtaining specimens from extrapulmonary cases and children suspected of having pulmonary disease. They have high negative predictive value, results are available within 1 hour, involves simple technology and relatively inexpensive.

Its disadvantages includes that its sensitivity is highest in patients with smear-positive, but more lower in children, patients with extrapulmonary disease, human immunodeficiency virus (HIV) –infected individuals, and smear –negative cases. It cannot reliably distinguish active tuberculosis disease from latent infection with *M. tuberculosis* and also cannot distinguish *M. tuberculosis* from other species of mycobacteria.

DIAGNOSTIC LIMITATIONS IN SPECIAL SETTINGS

Diagnosis of TB in HIV-seropositive individuals, in children, and in persons with extrapulmonary forms of TB, remains an unmet challenge in both high-prevalence and low-prevalence settings. Persons with extrapulmonary TB do not transmit disease and those immunocompromised by HIV and/or of paediatric age generally transmit less owing to the lower frequency of cavitary disease and /or expectoration (children). While this makes them less of a public health threat, they remain a dilemma of nightmare proportions for physicians in clinical practice for the reasons outlined in Table 1.

CONCLUSION

In conclusion, the advantages and limitations of each available TB diagnostic method are evident and no test is yet available that meets target specification. These characteristics of current TB diagnostics are based on two factors: Ease to use and Performance. Performance is a compilation of sensitivity, specificity and speed while ease to use is a compilation of safety, number of steps, cost, robustness and training simplicity.

In terms of ease to use, serology and microscopy methods of diagnosis are easier to use and more desired than X-ray and culture methods. In terms of performance, culture is more desired and performs better than the X-ray, microscopy and serology methods.

Microscopy and X-ray methods perform better and are more desired than serology method. Serology method is least in terms of performance and less desired than the X-ray, microscopy and culture methods (World Health Organization, 2006).

Furthermore, the quality of test results with existing methods is dependent on the availability of sufficient human and financial resources, training of laboratory personnel and monitoring of performance.

New methods that overcome limitations and respond to the challenges posed by special populations will be well

Table 1. Challenges of diagnosis.

Diagnostic approach	TB-HIV	Paediatric TB	Extrapulmonary TB
Clinical	Onset can be acute rather than chronic. Less likely to have copious sputum production or haemoptysis. Coinfection with other bacterial, viral or parasitic pathogens adds complexity. Extrapulmonary and disseminated forms of TB are more common.	Less than 6 years of age- acute onset and dissemination common. Nonspecific constitutional symptoms, with up to 50% asymptomatic during the initial stages. Diagnosis often confused with pneumonia, asthma or congenital pulmonary abnormalities. Extrapulmonary forms common (26%). Association with an adult source and failure to thrive may still be the best available predictors.	Nonspecific swelling, pain, with or without loss of function, at one or more of the following sites: -Pleural space -Lymph node -Peritoneum -Kidney/bladder -Uterus -Central nervous system -Bone, joint space. Fever, weight loss and appetite loss may or may not be present.
Radiographic	Variable; cavities less common.	Hilar adenopathy is the hallmark of primary TB. However, abnormalities are often subtle and easily missed.	General nonspecific findings with the exception of advanced spinal or vertebral TB (Pott's disease)
Tuberculin Skin test	Does not distinguish between infection and disease. HIV+individuals are often anergic.	May take up to three months to develop a positive test following exposure. False-positives (BCG vaccination, environmental mycobacteria) and false-negatives (serious viral, bacterial or tuberculous infection, immunosuppression) occur.	Cannot distinguish between infection and disease.
Laboratory	Low bacillary load in clinical specimens leads to negative acid-fast stain and culture. Requires sputum induction, bronchoscopy and/or culture to confirm diagnosis.	Unable to expectorate, therefore swallow bacilli-this is the rationale for testing gastric contents for acid-fast bacilli. However, sensitivity is notoriously low, that is <10% (Starke and Taylor-Watts, 1989). Furthermore, optimal sputum and gastric aspirate cultures are sensitive in <50% of cases (Correa, 1977; Khan and Starke, 1995).	Invasive procedure required to obtain clinical specimen (fluid or biopsy). Low bacillary load in clinical specimens leads to negative acid-fast stain and culture. Chemical analysis of pleural or peritoneal fluid inconclusive (exudate, lymphocytic, high protein, very low glucose) (Chow et al., 2003; Ferrer, 1996). Adenosine deaminase in pleural fluid –variable results (Perez-Rodriguez and Jimenez, 2000).
Scoring systems-attempt to produce Diagnostic standardization	Research under way.	Seventeen different scoring systems published. No one method adapted for general use in all settings. Modifications required in areas of high HIV prevalence, limited resources, malnutrition and high burden. Diagnosis continues to rely primarily on clinical findings and in association with an adult source. Failure to thrive may still be the best available predictor (Fourie et al., 1998; Mandalakes and Starke, 2005).	

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