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DIAGNOSIS AND MANAGEMENT OF TUBERCULOSIS

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Diagnosis and Management of Tuberculosis

Chapter 1

Association of Tuberculosis with HIV & Non Communicable Diseases

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1. Introduction

Tuberculosis is a contagious disease caused by the organism Mycobacterium Tuberculosis through adroplet mode of spread and a variable period of latency of infection. The organism had been identified in many specimens of almost 6000 years old as well as in various civilizations and amongst several prominent personalities too.

2. Diabetes mellitus [DM]

DM is an established risk factor for tuberculosis. Its prevalence is 2.1% to 16.4% in tuberculosis patients. Tuberculosis due to Diabetes Mellitus occurs because of impaired cell-mediated immunity, microangiopathy, kidney failure and micronutrient deficiency [2]. Diagnosis of tuberculosis is established by Oral Glucose Tolerance Test [2], Fasting Blood Sugar Level, and Glycosylated Hemoglobin. Regular screening of TB patients for DM in TB endemic regions is highly recommended as DM causes delayed sputum/culture conversion, increased case fatality, and treatment failure.

Clinical recovery in patients with DM-TB comorbidity shows slower improvement leading to a higher mortality during anti-tubercular treatment, BMI and hematocrit compared to those with TB alone at 2 and 5-months of follow-up.

Treatment of Diabetes Mellitus in Tuberculosis is parenteral Insulin, Oral Hypoglycemic Agents as the corner stones with the optimal goals of therapy being an HbA1c of <7%, RBS <180 mg/dL and FBS<120 mg/dl [2].

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3. Disorders of Calcium and Vitamin D Metabolism

Hypercalcemia is commonly associated with Tuberculosis. The prevalence of the disease is affected by the incidence of preexisting hypocalcaemia due to highly prevalent hypovitaminosis D. Nearly 12% of the patients show symptomatic hypocalcaemia, wanting treatment[2]. Higher skin pigmentation, immuno suppression like HIV, malabsorption, lower sunshine exposure, renal and liver dysfunction are common risk factors for hypovitaminosis D. A strong association between Vitamin D Deficiency and TB may be evidenced by increased production of cytokines by the immune cells. [An antimicrobial peptide], Calcitriol reducing the viability of Mycobacterium TB bacilli. There is an enhanced process of fusion of the phagosome and lysosome in infected macrophages showing an increase in intra cellular oxidative stress [2].

3.1. Adrenal Diseases

Patients with pulmonary tuberculosis present with refractory hypotension & hyponatremia have often been implicated to have adrenal involvement resulting in an ADDISONIAN CRISIS or disease in 40% cases.

The common clinical manifestations are refractory hypotension, fatigue, salt craving, myalgia, recurrent nausea vomiting, abdominal pain, hypotension & hyperpigmentation. on Lab Investigations these patients present with hyponatremia, hyperkalemia, hypoglycemia, and eosinophilia. Diagnosis of adrenal insufficiency is established by ACTH stimulation test [a 30-minutes cortisol level ≤414 nmol/L after a 1 μg ACTH stimulation test] and Early morning cortisol level. The adrenal gland is involved by tuberculosis directly or by cytotoxic inflammatory mediators.

Management of the Addison's disease requires daily low dose oral glucocorticoid, mineral corticoid replacement therapy with anti-TB therapy as per RNTCP guidelines and monitoring of Rifampicin doses [2].

3.2. Thyroid Dysfunction

It's commonly noted in patients with Tuberculosis with Sick euthyroid syndrome. Tuberculosis causes disruption of the hypothalamic-pituitary-thyroid axis, resulting in reduced stimulation of the thyrotropes and impairment of thyroid hormones release. Case reports of TB of the thyroid gland with subsequent destructive thyroiditis and thyroid dysfunction have also been documented [2].

Diagnosis of hypothyroidismis established by the decreased levels of free triiodothyronine (T3) concentration in the absence of clinical or biochemical features of primary or secondary hypothyroidism with a reduced thyroid stimulating hormone level. Thyroid diseases

coexisting with tuberculosis is associated with adverse clinical outcomes. Early and regular-Screening, and treatment of thyroid dysfunction among suspected TB patients is therefore very much advisable. On being diagnosed a hypothyroid the patient may be treated with appropriate thyroid replacement [2].

4. Tuberculosis and the Kidney

There is a commonly observed association in between kidney and genitourinary diseases, with 14% to 41% prevalence [3]. The usual causative agents are the Mycobacterium tuberculosis complex and its symptomsare of conventional bacterial cystitis with poor response to the usual antibacterial agents. Biochemistry reveals sterilepyuria associated back, flank, suprapubic pain, hematuria, increased nocturnal frequency of urination which may or may not be associated with renal colic and also occasionally constitutional symptoms are seen.The diagnosis of urinary tract tuberculosis is based on the finding of pyuria in the absence of infection as judged by culture on routine media [3].

In early disease, it is often possible on intravenous urography to detect changes in a single calyx with evidence of parenchymal necrosis, and typically there is calcification on the plain film. In more advanced disease, urography will show calyceal distortion, ureteric strictures and bladder fibrosis [3].

Ultrasound examination of the urinary tract may reveal renal calyceal dilation and more overt evidence of obstruction which on histology reveals chronic tubulointerstitial nephritis with granuloma formation.

Usual complications of GU TB include multisystem spread of the tuberculosis, gradual fall in GFR, pyuria, amyloidosis, miliary tuberculosis and end-stage renal disease.

Contamination of the dialysis machine by environmental mycobacteria may occur causing a typical mycobacteriosis. In transplant patients, tuberculosis is a serious complicating factor in renal and other forms of transplantation, with an incidence, depending on geographic region, of 0.35% to 15.0%., the treatment for which is Isoniazid prophylaxis for 1 year.

5. Genital Tuberculosis

The epididymis is the most common site of tubercular infection in the genitourinary tract, commonly involved secondary to heamtogenous spread from a distant primary. The route of spread is usually heamtogenous.

Other parts of the GU system like the prostate, face testicular involvement by direct invasion from epididymis and prostatitis due to antegrade infection.

Diagnosis is supported byevident radiologic abnormalities in the urinary tract, Hyper-

calcemia, elevated levels of Calcitriol (1, 25- (OH) 2D3) [3].

Microbiologic diagnosis is done by isolation of the bacilli using inoculation or molecular methods. Acid-fast bacilli may be seen on microscopy of centrifuged urine or by Nucleic-acid amplification techniques, such as PCR. The morphology of the lesions depends on the site of infection,the virulence of the organism and the immune status of the patient [3].

Mode of spread of the tubercle bacilli is heamtogenous or by direct renal involvement, which occurs as a result of reactivation or relapse.

Renal tuberculosis are usually present bilaterally as compared to unilaterally presence. The renal medulla is the site of preference while the other pathological presentations are tuberculous pyelonephritis and “Cement” or “Putty” kidney. Scarring develops within the renal pelvis with calcification in 24% of cases, identifiable as renal or ureteric stones in up to 19% of cases. Infection frequently spreads down to the ureters into the bladder, producing cystitis associated with scarring [3].

The clinical consequences of an extensive renal lesion include auto nephrectomy. Destructive renal lesions may spread outside the renal capsule and produce a mass lesion, which can mimic a neoplasm. Ureteric involvement also may produce irregular ureteric strictures.

6. Tuberculosis in Hepatic diseases

A higher prevalence of tuberculosis is seen in cirrhotic as compared to the general population. Prevalence rate of tuberculosis is found to be 15 times higher than in the general population. Higher prevalence of tuberculosis was also noted in alcoholic liver disease [4].

In cirrhosis patients, extra pulmonary tuberculosis is more common. Ascites due to peritoneal tuberculosis may be difficult to diagnose in the setting of liver cirrhosis where portal hypertensive ascites is common. Patients with liver cirrhosis generally have impaired cellular immunity. A higher likelihood of false negative tuberculin test is seen. AFB smears are generally negative in such patients. Moreover, a high index of suspicion is required to exclude tubercular ascites and an ascitic fluid examination need to be done in all such cases [4].

Tubercular ascites in the setting of cirrhosis reveals anelevated SAAG [serum albumin ascites gradient] and high protein ascites with a lymphocytic predominant high cell count fluid. The ADA levels are usually more than PCR asMTB may be positive. The need for critical review of treatment of tuberculosis in cirrhotic arises because 3 of the 5 first line anti-tubercular drugs are potentially hepatotoxic. The administration of these drugs can lead to worsening LFT with decompensation of stable cirrhotic and sometimes cause fulminant hepatic failure with a high mortality. Current guidelines take a broad perspective regarding treatment of tuberculosis in liver cirrhosis. There is a recommendation that the more advanced the liver disease, the

lesser the amount of hepatotoxic drug that is used. It must be remembered that pyrazinamide has the highest hepatoxicity followed by Rifampicin and Isoniazid.Safer anti tubercular drugs are Ethambutol, Quinolones, Aminoglycosides and Cycloserine.

Cirrhotic patients with essentially normal baseline liver function tests may be treated with standard 4 drug regime for two months followed by two drugs for remaining four months (total 6-months treatment). Since Pyrazinamide is potentially the most hepatotoxic drug, it may be completely avoided and a nine month, three drug regime may be used. Regular monitoring of LFT is recommended [4].

Patients with varying degrees of cirrhosis can be treated with two hepatotoxic drugs - nine months of Isoniazid, Rifampin and Ethambutol (until or unless isoniazid susceptibility is documented) - two months of Isoniazid, Rifampin, Ethambutol and Streptomycin followed by six months of Isoniazid and Rifampin. Category A patients may be treated with one hepatotoxic drug for two months of Isoniazid, Ethambutol and Streptomycin followed by 10 months of Isoniazid and Ethambutol.

In severe liver dysfunction, no hepatotoxic drugs but 18-24 months of Streptomycin, Ethambutol and Quinolones can be administered.

Regular LFT monitoring should be done in all cirrhotic patients receiving anti-tubercular treatment and drug therapy may be stopped /altered as per the LFT reports.

Hepatotoxicity due to antitubercular treatment is more commonly observed in patients with hepatic cirrhosis [4]. In the general population, the criteria for stopping anti tubercular treatment is AST / ALT >three times upper limit of normal and symptomaticor AST/ALT >t five times upper limit of normal even if asymptomatic.

As a general principle a rising trend of liver abnormalities on two consecutive testing may be an indication for stopping treatment. The absolute level of transaminases cannot be used as the sole criteria in cirrhotic. Any rise in S Bilirubin should be treated with great caution and hepatotoxic drug treatment stopped immediately [4].

Treatment should be stopped and re-started after serum bilirubin and transaminase return to near normal. Drugs are re-started in a sequential fashion starting with rifampin first followed by isoniazid and lastly pyrazinamide which may be avoided altogether.

The prognosis of patients with hepatic cirrhosis depends on the stage of disease and associated complications. The 1-year mortality is 34% whereas patients with complications admitted in ICU have 1-year mortality rate of 69% [4]. The mortality rate in patients with tuberculosis who have not received treatment (or delayed treatment) is >50%. The prognosis in patients of liver cirrhosis who develop tuberculosis is poorer compared to either disease alone.

The 30-day case fatality rate was found to be 27.3% and one year case fatality rate was found to be 47.7% [4].

7. Tuberculosis in Patients with Hematological Malignancies

Tuberculosis (TB) is an infectious disease that causes more than 1 million deaths worldwide every year. In addition, it is estimated that one-third of the world population is infected with M. tuberculosis in a latent state, which involves an eventual risk of progressing to active TB disease. Patients with immunodeficiency, such as those suffering from hematological malignancies, have a greater risk of progressing to TB disease once infected. It is estimated that the Relative Risk of TB disease in patients with hematologic malignancies is 2 to 40 times that of the general population. The diagnosis of TB in these patients is often challenging as they often present clinical characteristics that are distinct to those of patients without any other underlying disease. Mortality due to TB is higher. Therefore, it is recommended to diagnose latent TB infection and consider preventive therapy that could avoid the progression from a latent state to active TB disease. There are currently two methods for diagnosing latent TB infection: The Tuberculin Skin Test (TST) and the Interferon-Gamma Release Assays (IGRA). Due to the lack of sensitivity in patients with immunodeficient conditions, a combined TST-IGRA testing is probably the best way for latent TB diagnosis in order to gain sensitivity. Treatment of latent TB infection and TB disease is based on same principles as treatment of routine tuberculosis cases.

Patients with Hematological Malignancies have immunodeficient status which facilitates the emergence of infections [6]. Alteration in the Th1 cell response of the HM itself or that caused by antineoplastic chemotherapy or hematopoietic stem cell transplantation (frequently associated to the administration of high doses of corticosteroids) lead to an impaired immune response that particularly promotes the progression from LTBI to active TB.

The risk of developing TB can vary depending on the type of Blood Dyscrasia. Acute Myeloid Leukemia, Chronic Lymphocytic Leukemia and Chemotherapy were also associated to a higher risk of developing the disease [6]. Patients with HM have a higher risk of TB reactivation than the general population [7].

8. Tuberculosis & Cardiovascular Diseases

Tuberculosis and non-communicable diseases have an established association [7]. Several Infections have been identified which may have a causative role in causing CVS disorders like Chlamydiapneumoniae, *Helicobacter pylori* , Influenza virus and Human immuno deficiency virus (HIV). Infections due to hepatitis B virus, hepatitis C, Epstein Barr virus, cytomegalovirus (CMV) and periodontal bacteria have also been associated with atherosclerosis and CVD through chronic systemic inflammation and other mechanisms. Latent tuberculosis

infection (LTBI) is associated with chronic inflammation.

The possible effects of tubercular infection on cardiovascular disease are an increase in inflammation leading to coronary artery plaque formation and/or plaque rupture, autoimmune disease. The most common cross reaction of antibodies from infection, autoantibodies in atherosclerosis centers on the heat shock protein (HSP) system. M. tuberculosis may not only affect the coronary vessels, but also the myocardium.The potential effects of tuberculosis disease do not appear to be limited to coronary heart disease (CHD) but extend to other atherosclerosis-mediated vascular diseases such as stroke. The possible mechanism of action ismyocarditis, arteritis, and excessive cytokine release [7].

A potential link between tuberculosis and Takayasu's arteritis is seen, but causative role of either diseases in this interaction is yet to be established conclusively [7].

9. Relation between tuberculosis & Cerebrovascular accidents

Tuberculosis in age and gender-matched subjects has shown to have a high likelihood of ischemic stroke, nearly 1.52-times greater among tuberculosis patients in the initial follow up period [5]. Almost 6.0% of the tuberculosis patients had an ischemic stroke, which is higher than that of the general population [5]. The actual mechanisms behind this association is not very clear. Infection causes the activation of a persistent inflammatory response that starts a shower of cytokines and chemokines and acts as a link between infection and atherosclerosis. Recent respiratory tract infections increase the risk for cardio embolic and large-vessel athero-thromboembolic strokes. Association between tuberculosis and stroke may be partially explained by the synergistic effect of tuberculosis and smoking on vessel pathology [5].

10. Tuberculosis & COPD

Tuberculosis (TB) and chronic obstructive pulmonary disease (COPD) carry a significant burden in terms of morbidity and mortality worldwide [8].

Susceptibility of an individual to the development of active tuberculosis and COPD is poorly understood [8].

A study from Turkey, which reviewed 5480 cases of active pleuropulmonary TB found that COPD was the second most common co-morbid condition [8]. No changes were noted between the two groups in symptoms of dyspnea and cough but a higher frequency of hemoptysis was noted.

11. Tuberculosis & HIV

TB & HIV CO INFECTION is a major challenge to medical resources of countries in the African & Asian sub continents where by an estimated 33.3 million people are co infected

[9]. TB is the most common opportunistic infection among HIV-infected individuals and co infected individuals are at high risk of death.

The spectrum of radiographic manifestation of pulmonary TB is dependent on the relative level of HIV-related immunodeficiency [9]. During the early phase of HIV, when individuals are not immunosuppressed, the radiographic pattern is similar to HIV uninfected individuals with more typical lesions - upper lobe infiltrates with or without cavities. With the advancing of immunosuppression, extra pulmonary involvement, intra-thoracic/mediastinal lymphadenopathy, lower lobe infiltrate and miliary TB become more common [9].

The most commonly used method of TB detection involves microscopic examination of sputum for acid-fast bacilli (AFB). Microscopy has the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. However, to be considered smear positive a specimen needs to contain approximately 105 mycobacteria per milliliter.

Culture of Mycobacterium tuberculosis is much more sensitive than smear microscopy and has been recommended to assist in the diagnosis of TB in HIV-infected individuals. Culture also allows subsequent strain characterization and drug susceptibility tests. The traditional method of inoculating solid medium such as the Lowenstein-Jenson (L-J) medium or Middlebrook medium is sensitive but slow, as growth may not be visible until after 6-8 weeks of incubation. The WHO endorsed the use of Gene pert-Rif for the rapid diagnosis of TB as well as rifampicin resistance among HIV-infected individuals with clinical suspicion of TB [9].

GeneXpert is a TB-specific automated, cartridge-based nucleic acid amplification assay, having fully integrated and automated sample preparation, amplification and detection using real-time PCR, providing results within 100 minutes. Xpert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with 100% specificity.

New diagnostic techniques are developed to detect M. tuberculosis MPB-64 (TAUNS) antigens in peripheral blood, early secreted antigenic target 6 in the cerebrospinal fluid, lipoarabinomannan (LAM) in the urine, etc. by ELISA–based commercial assays. Urine LAM assays tend to perform better in HIV-infected compared to HIV uninfected TB patients. The combination of urine lipoarabinomannan testing and sputum smear microscopy needs further evaluation for use in settings with a high HIV burden.

Performance of various immune based tests to detect antibodies to M. tuberculosis antigens has been reviewed extensively. Conventional tests like tuberculin skin test are still in common practice,though their role in diagnosis of tuberculosis is very limited. The WHO recently made a negative recommendation against the use of serological tests{Interferon-γ release assay (IGRA)} for TB, based on data suggesting that these tests could neither replace sputum microscopy nor be used as an add-on test to rule out TB. Latest diagnostic tools like

Sensing volatile organic compounds and electronic nose devices are under research and yet to be included in mass screening programs.

About detecting HIV among individuals with active TB, provider initiated HIV testing is recommended for all TB patients, as standard of care. The rapid expansion of HIV testing for TB patients has been particularly encouraging in Africa, where only 4% of TB patients were tested for HIV in 2004, but by 2008 that number had increased to 45% t4. In a pilot study of implementation of provider initiated HIV testing and counselling in India, HIV status was successfully ascertained for 70% of TB patients and this was found to be feasible and acceptable. The policy has been rapidly scaled up with over 60% of TB patients being aware of their HIV status in 2011.

The WHO currently recommends that all HIV-infected persons be screened for TB, and HIV-infected persons without active TB disease be evaluated for treatment of latent TB infection 69. The National AIDS Control Organization (NACO) intends to test the effectiveness and feasibility of the WHO IPT guidelines in ART clinics as a precursor for adopting this recommendation [9].

The basic principles of treatment for HIV-associated TB are the same as for HIV uninfected individuals. Certain areas of uncertainty remain, including the regimen duration, dosage and frequency of administration of anti-TB drugs, optimal timing of initiation of ART and optimal anti-TB drug combination for patients on second line treatment [9].

Currently, standard therapy consists of four drugs in the intensive phase for two months namely isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) followed by H and R in the continuation phase of four months. In India, under RNTCP, a fully intermittent thrice-weekly regimen Category I ($2EHRZ_3/4HR_3$) is recommended for newly diagnosed TB. This regimen is reinforced with streptomycin (Sm) in the intensive phase and the total duration increased to eight months for retreatment cases - Category II [9] ($2EHRZS_3/1EHRZ_3/5EHR_3$)78. Rifampicin plays a key role in the treatment of HIV-associated TB because of its ability to destroy both intracellular and intermittently and slowly growing TB bacilli. Non-rifampicin containing regimens are associated with inferior cure rates and prolong the period of treatment 79. A meta-analysis on the duration of rifampicin showed that recurrences were 2-3 times higher if rifampicin use was restricted to 2 months [9].

The WHO guidelines for management of HIV-infected TB patients in resource- limited settings recommend a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) along with one non-nucleoside reverse transcriptase inhibitor (NNRTI) for first line therapy 89. In India, the NACO recommends a regimen containing zidovudine or stavudine along with lamivudine and efavirenz 90. Rifamycin's induce the cytochrome CYP-450 enzyme system in the liver and intestinal wall, there by increasing the metabolism of protease inhibitors (PIs) and

NNRTIs 91. The effect is weaker with rifabutin than with rifampin [9].

Evidence from randomized controlled trials shows that early initiation of ART during TB treatment is associated with reduced mortality rates, especially in patients with profound immunosuppression (CD4<50 cells/microlitre) [9].

Transient worsening of symptoms and signs of tuberculosis or radiological deterioration after the initiation of ART, despite a reduction in HIV load (>1 log10 copies/µl) and immunological recovery, is known as IRIS. Drug resistance and other opportunistic infections need to be ruled out before a diagnosis of IRIS is made. Hypercalcemia is a unique feature of tuberculosis IRIS. There are two types of IRIS presentation: unmasking of undiagnosed tuberculosis and a paradoxical deterioration of existing tuberculosis lesions or appearance of new lesions after initial improvement.

In a study conducted among HIV/TB patients in Tamil Nadu, the prevalence of drug resistance among patients with no history of previous treatment was 13.2% to INH, 2.4% to EMB, 7.8% to SM and 4.2% to RMP, either alone or in combination with other anti-tuberculosis drugs [9].

At least four effective drugs - including a fluoroquinolone, an injectable agent (capreomycin, kanamycin, or amikacin) and at least two agents from the remaining second-line antituberculosis drug classes (cycloserine, thioamides like ethionamide or prothionamide, and p-aminosalicyclic acid)- along with pyrazinamide and EMB, if still sensitive, should be used. Therapy may be individualized on the basis of drug susceptibility test results; however, many countries use standardized regimens that are based on surveillance of antituberculosis drug resistance in the community. DOTS plus regimen is currently followed in India comprising of kanamycin, levofloxacin, ethionamide, cycloserine, ethambutol, and pyrazinamide given for a period of 6-9 months daily in the intensive phase followed by all drugs except kanamycin and pyrazinamide during the continuation phase of 18 months, with dosages prescribed for 3 weight bands [9].

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Diagnosis and Management of Tuberculosis

Chapter 2

New Insights to Resistance of a Novel Drug Bedaquiline using *in-vitro* Mutants of ATP Synthase in Mycobacterium Tuberculosis

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Abstract

Bedaquiline (BDQ) is the new first-in-class anti-tuberculosis (TB) compound belonging to the class of diarylquinolone with activity against drug-sensitive and drug-resistant *Mycobacterium tuberculosis* (M. *tuberculosis*). This novel drug has the immense potential to shorten TB treatment duration and has been advocated for multi-drug resistant (MDR)-TB treatment. Therefore, BDQ resistance can be considered as a major public health problem and molecular investigation of the same is the utmost need of the hour. The target based concept of resistance to BDQ is caused by mutation in c-ring of adenosine tri phosphate (ATP) synthase, a critical enzyme in the synthesis of ATP in M. *tuberculosis* coded by *atpE* gene. BDQ inhibits the proton pump of mycobacterial ATP synthase. To understand the molecular basis of BDQ resistance using mutants (MTs) as the emergence of strains resistant to BDQ may pose a potential threat to the TB control program, we undertook a initiative to study seven *in vitro* mutants of *AtpE* viz., Asp28Gly, Asp28Ala, Asp28Pro, Asp28Val, Glu61-Asp, Ala63Pro and Ile66Met (D28G, D28A, D28P D28V, E61D, A63P, and I66M) which involves in resistance against BDQ. Molecular modeling and docking was performed to understand the interacting behaviour of mutant (MT) enzymes with BDQ in comparison to wild-type (WT). These results indicate that the substitutions in AtpE except D28G showed high affinity towards the drug in comparison to the WT. This could be due to the favourable interactions in mutants compared to WT.

It can be inferred from this concise analysis that the mutants (D28A, D28P D28V, E61D, A63P, and I66M) due to high affinity binds with the BDQ tightly leading to slow or no release of the BDQ which eventually results in high level BDQ resistance, compared with D28G that could lead to low-level resistance.

key words: Mycobacterium tuberculosis; BDQ resistance; ATP synthase; mutants

Unissa AN

1. Introduction

Despite being a controllable, preventable and curable disease, tuberculosis (TB) still remain as a major public health problem in many parts of the world. The increase in multi-drug resistant (MDR-TB) is defined as resistance to the two most effective first line TB drugs: rifampicin (RIF) and isoniazid (INH) has intensified the magnitude of the situation. Extensively drug-resistant TB (XDR-TB) is emerging as an even more ominous threat, that is resistant to any fluoroquinolone, and at least one of three injectable second-line drugs (capreomycin, kanamycin, and amikacin), in addition to INH and RIF. Drug resistance in TB is essentially a potential threat to the TB control programs. The most recent drug-resistance surveillance data issued by the World health organization (WHO) estimates that an average of roughly 9 % of MDR-TB cases are XDR-TB [1].

1.2. Properties of Bedaquiline (BDQ)

Bedaquiline (BDQ) is also known as TMC207 or R207910, is a drug belonging to the class of diarylquinoline and found to be effective against both drug susceptible and drug resistance TB [2]. It is a very promising and relatively new candidate drug for treatment of TB and mycobacterial infections [2]. The activity spectrum of TMC207 includes the mycobacterial species that are pathogenic to humans, such as *Mycobacterium tuberculosis* (*M. tuberculosis*), but also atypical pathogenic species, such as *M. avium complex, M kansasii,* and the fast growers *M. fortuitum* and *M. abscessus* [2]. It is found to be active within macrophages, and an important agent in shortening the duration of anti- TB treatment [3].

1.3. BDQ in MDR treatment

Preclinical studies have shown the efficacy of BDQ in terms of reduction in bacterial load and treatment duration. In 2012, food and drug administration (FDA) approved BDQ for treatment of MDR-TB and XDR-TB [3]. It is currently in phase IIIb clinical trials for patients with MDR-TB. Phase II clinical studies have established the safety, tolerability and earlier sputum conversion time in patients with MDR-TB [3]. A study from France showed that BDQ-containing regimens achieved favourable outcomes in a large proportion of patients and prolonged BDQ treatment was overall well tolerated in this cohort [14].

1.4. Mechanism of action of BDQ

BDQ exhibits a novel mechanism of action which efficiently inhibits the adenosine

5-triphosphate (ATP) synthase of mycobacteria such as *M. tuberculosis* [2]. It targets subunit c of the ATP synthase of *M. tuberculosis,* leading to inhibition of the activity of proton pump in the enzyme [5]. The structure of ATP synthase was described in terms of two sectors, a membranous F_0 ($ab_2c_{10–15}$) and a membrane-extrinsic F_1 ($\alpha_3\beta_3\gamma\delta\varepsilon$) [6]. Binding of BDQ at the level of the proton-binding site to the oligomeric and proteolipic subunit c in F0 domain of ATP synthase blocks rotation of discs of ATP synthase, which culminates in the inhibition of ATP synthesis and eventually to the death of mycobacteria [7,8].

1.5. Mechanism of resistance of BDQ

Target based resistance was found to occur in resistant isolates with mutations in subunit c at positions 28 (Asp→Gly/Ala/Val/Pro), 61 (Glu→Asp), 63 (Ala→Pro), and 66 (Ile→Met) based on the *M. tuberculosis* amino acid numbering system [9,10]. The assumption was supported by the fact that the mycobacterial species naturally resistant to BDQ, i.e., *M. xenopi, M. novacastrense,* and *M. shimoidei*, display a Met at position 63 in subunit c in place of a conserved Ala in the species susceptible to the drug [9,10].

Recent elucidation of crystal structure of ATP synthase from *M. Phlei* (4VIF, 4VIG) [11], enables the mechanism of action and resistance of BDQ in *M. tuberculosis* in a more precise manner compared to earlier studies [9,12]. In the light of the above in this study, to understand the molecular basis of BDQ resistance, seven mutants (MT) proteins of ATP synthase with important resistant mutations at codon position 28 such as Asp→Gly/Ala/ /Pro/Val (D28G, D28A, D28P and D28V) and the remaining three mutants at codon 61, 63 and 66 with Asp, Pro, Met in place of Glu, Ala and Ile (E61D, A63P, and I66M) were modeled and docked with BDQ using *in silico* approaches.

2. Materials and Methods

2.1. Proteins: Template selection and model building

In the present study, ATP synthase was modeled using MODELLER 9.14 [13], because its crystal structure is so far not deduced. ATP synthase is coded by atpE gene; the target AtpE protein (Rv1305) sequence of *M. tuberculosis* obtained from the Tuberculist database was submitted to BLASTp [14] program and searched against protein data bank (PDB). The WT protein from *M. phlei* (PDB code-4V1F) [11] was considered as template protein displaying maximum identity with the WT protein. In the PDB file of template 4V1F, heteroatoms such as water and other chains were removed; retaining chain A and command line options were provided for model building in MODELLER9v14. The software aligns the FASTA sequence of template and target, following which it takes up the PDB template file and generates the model based on the concept of sequence predict structure. Sequence alignment between WT and template protein was performed with the command line options, and a series of commands

were provided for model building using the software MODELLER9v14. Residues at positions 28, 61, 63, and 66 of WT AtpE protein were substituted for generating seven different MT proteins, following the above procedure. The same set of protocol was followed for chain B generation for the WT and seven mutants.

2.2. Chain combination

In this study, two (A and B) chains were modeled separately following the above procedure; chains were then combined using Amber software (Version- 12) [15] to enable docking efficiently, since, ATP synthase is a complex oligomeric protein comprises of 11 AtpE subunits, for proper binding of BDQ at least two chains are required.

2.3. Model evaluation

Validation of the models was done by ramachandran plot [16]. Further the deviation between the WT and the template 4V1F upon structural superimposition was determined using PDBeFOLD [17].

2.4. Ligand

The ligand (BDQ) chosen in this study was obtained from PDB structure of 4V1F [11].

2.5. Molecular docking

The GOLD protocol is based on the principle of genetic algorithm, with the rigid receptor and the flexible ligand during the refinement process, details of which have been described elsewhere [18]. Docking was performed between BDQ and seven MT proteins of AtpE (D28G, D28A, D28P, D28V, E61D, A63P, and I66M) in comparison to WT with the help of software GOLD (Version- 4.0.1). The input atom files for both the proteins and the ligand were created. The ligand and the models were added with hydrogen atoms before docking. The cavity atom file containing the atom number of binding residues (Gly58, Ala62, Phe65, Ile66 and Ala69) was prepared for BDQ. The binding residues were selected on comparison between binding regions of BDQ with crystal structures of 4VIF. Dockings were performed under ‘Standard default settings’ mode. - number of islands was 5, population size of 100, number of operations was 100,000, a niche size of 2, and a selection pressure of 1.1. Ten docking poses were obtained for each ligand. Poses with highest GOLD score were used for further analysis. Ideally, the score should correspond directly to the binding affinity of the ligand for the protein, so that the best scoring ligand pose are the best binders.

2.6. BIOVIA software

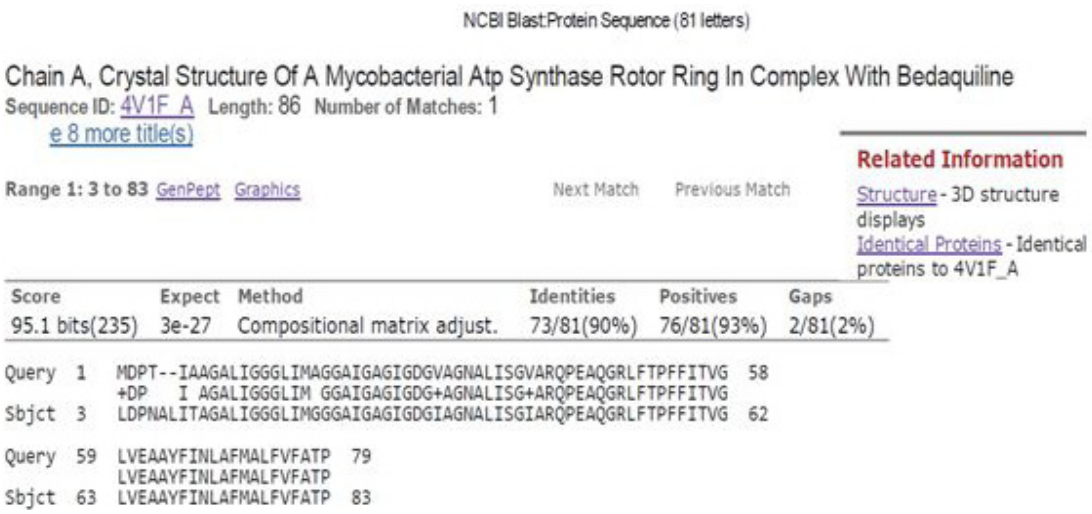
Biovia -2015 was used was used for visualization purpose of modelled proteins, dock-

ing data and to determine the interactions between the ligand and proteins [19].

3. Results and Discussion

BDQ or TMC207 is a new anti-TB drug belonging to the class of diarylquinoline, which selectively inhibits the mycobacterial energy metabolism *i.e.* ATP synthesis. BDQ is found to be effective against all states of *M. tuberculosis* like active, dormant, replicating, non-replicating, intracellular and extracellular. Although the contribution of BDQ against the treatment of drug resistant TB seems significant to the TB control program, however, some *in vitro* studies [9,10] have shown the emergence of resistant strains to BDQ. In a report [9], *in vitro* resistant mutants of BDQ from *M. tuberculosis* and diverse atypical mycobacteria were isolated. Six distinct mutations, Asp28→Gly, Asp28→Ala, Leu59→Val, Glu61→Asp, Ala63→Pro, and Ile66→Met have been identified in the subunit c forming a C ring in the ATP synthase, in order to map the amino acid residues involved in the binding of BDQ [9,10].

The catalytic core of the membrane-embedded rotor ring of the sodium ion–translocating ATP synthase contains α3β3γ subunits arranged in a hexagon of alternating α and β subunits with helices of γ in the center. ATP synthesis and hydrolysis reactions occur at three catalytic sites [6]. In the present study, ATP synthase was modeled because its crystal structure of *M. tuberculosis* is not so far deduced due to the complexity involved in crystallizing the protein. The template chosen was the crystal structure of 4V1F from *M. Phlei* at 1.7 Angstrom (Å) resolution that showed 90% identity with the target *M. tuberculosis* protein (**Figure 1**). Moreover, 4V1F was in complexed with BDQ, which enabled the docking process easier [11].


NCBI Blast:Protein Sequence (81 letters)

Chain A, Crystal Structure Of A Mycobacterial Atp Synthase Rotor Ring In Complex With Bedaquiline

Sequence ID: 4V1F_A Length: 86 Number of Matches: 1

e 8 more title(s)

Range 1: 3 to 83 GenPept Graphics Next Match Previous Match

Related Information

Structure - 3D structure displays

Identical Proteins - Identical proteins to 4V1F_A

Score	Expect	Method	Identities	Positives	Gaps
95.1 bits(235)	3e-27	Compositional matrix adjust.	73/81(90%)	76/81(93%)	2/81(2%)

```
Query  1   MDPT--IAAGALIGGGLIMAGGAIGAGIGDGVAGNALISGVARQPEAQGRLFTPFFITVG  58
           +DP   I AGALIGGGLIM GGAIGAGIGDG+AGNALISG+ARQPEAQGRLFTPFFITVG
Sbjct  3   LDPNALITAGALIGGGLIMGGGAIGAGIGDGIAGNALISGIARQPEAQGRLFTPFFITVG  62

Query  59  LVEAAYFINLAFMALFVFATP  79
           LVEAAYFINLAFMALFVFATP
Sbjct  63  LVEAAYFINLAFMALFVFATP  83
```

Figure 1: pBLAST results showing maximum identity (90%) between the template 4V1F of ATP synthase from *M. Phlei* and the target WT protein sequence - Rv1305 of ATP synthase of *M. tuberculosis.*

In this study, seven MT models of AtpE were built based on the WT sequence of AtpE protein (Rv1305) through substitution at position 28 with four mutants: Gly, Ala, Pro, Val in place of Asp, the remaining three mutants at codon 61, 63 and 66 with Asp, Pro, Met in place of Glu, Ala and Ile, respectively. (**Figure 2**). They were validated by ramachandran plot (RM) which showed 100% and above 97% of residues in the favoured regions for WT and seven

mutants, respectively (**Figure 3 and Table 1**). In addition to evaluation by RM plot, they were also validated by structural superimposition (**Figure 4**) which showed a root mean square deviation (RMSD) of 1.2 Å between 4V1F and WT suggesting a reliable model.

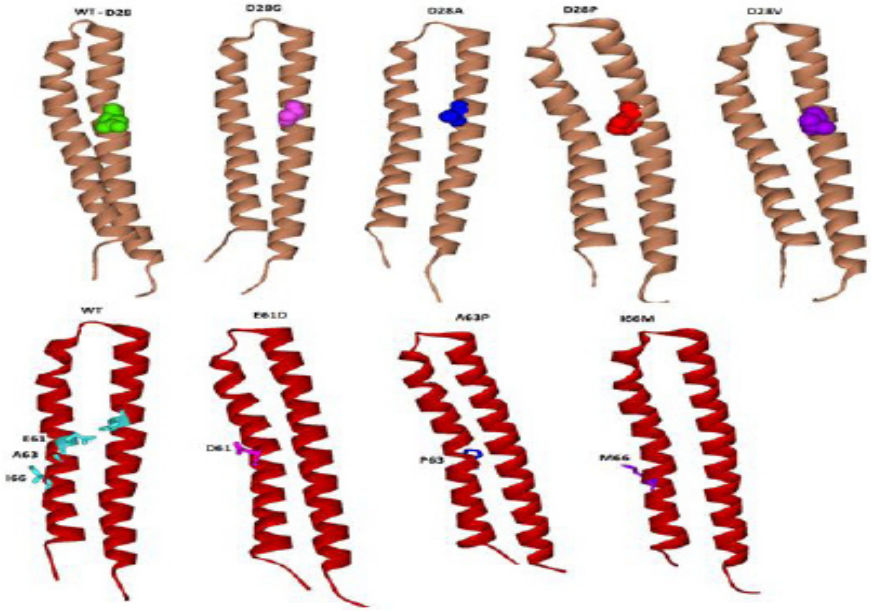


Figure 2: Three-dimensional models of AtpE showing WT and mutated residues at respective codon positions in *M. tuberculosis*.

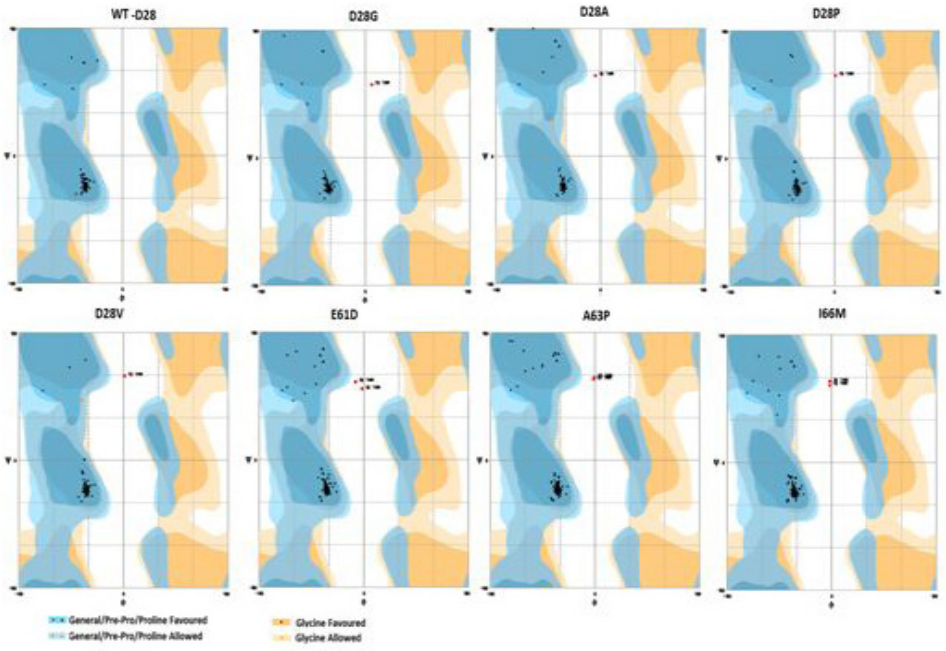


Figure 3: Ramachandran Plot for WT and MT models of AtpE from *M. tuberculosis*

Table 1: RM plot analysis of WT and MT models of AtpE from *M. tuberculosis*

Evaluation of residues	WT	D28A	D28G	D28P	D28V	E61D	A63P	I66M
Number of residues in favoured region (~98.0% expected)	79 (100)	77 (97.5)	78 (98.7)	77 (97.5)	77 (97.5)	78 (98.7)	78 (98.7)	78 (98.7)
Number of residues in allowed region (~2.0% expected)	0	1	0	1	1(1.3)	0	0	0
Number of residues in outlier region	0	1	1	1	1	1(1.3)	1(1.3)	1(1.3)

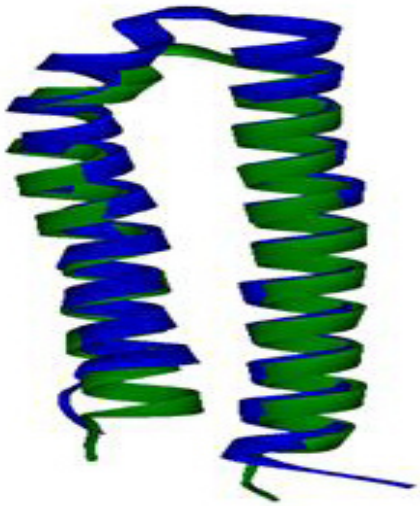


Figure 4: Validation of modeling through superimposition of template 4v1F (green) with WT protein of AtpE from *M. tuberculosis* (dark blue).

The mutant models such as D28G, D28A, D28P D28V, E61D, A63P, and I66M were created and docked with BDQ along with WT, the docked BDQ and AtpEs complex are illustrated in **Figure 5**.

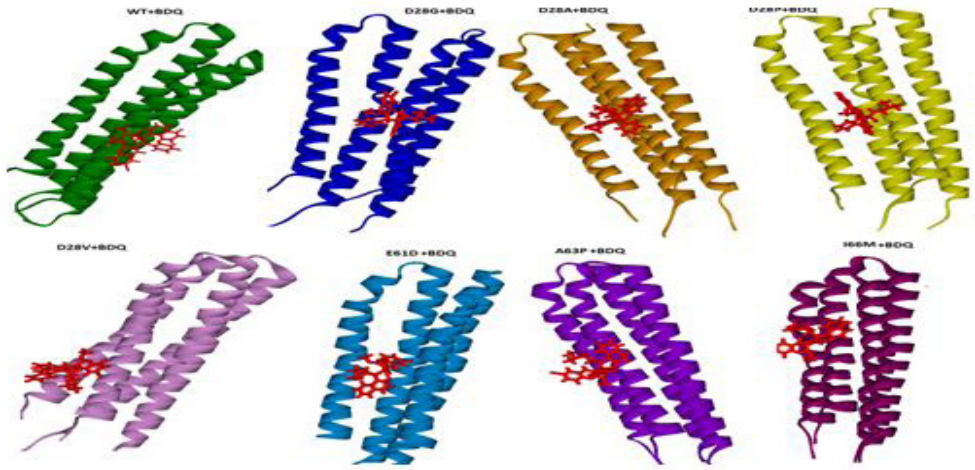


Figure 5: Docking of BDQ (red) with WT and mutants of AtpE.

The docking of BDQ with AtpEs resulted in ten poses. Of the ten poses produced, the best ligand pose was selected based on top GOLD score. Among the seven mutants, the high score of - 76.04 kcal/mol was obtained for the MT-D28P, followed by D28A, I66M, A63P, E61D and D28V compared to the WT and the D28G-MT showed relatively low score of -22.86 kcal/mol compared to the WT. The binding energy between WT and BDQ was found to be -32.1 kcal/mol (**Figure 6**). Thus, the docking results suggests that in comparison to the WT, binding affinity of D28P with BDQ was shown to be more, followed by others. In contrast, D28G, displayed low binding affinity compared to the WT protein.

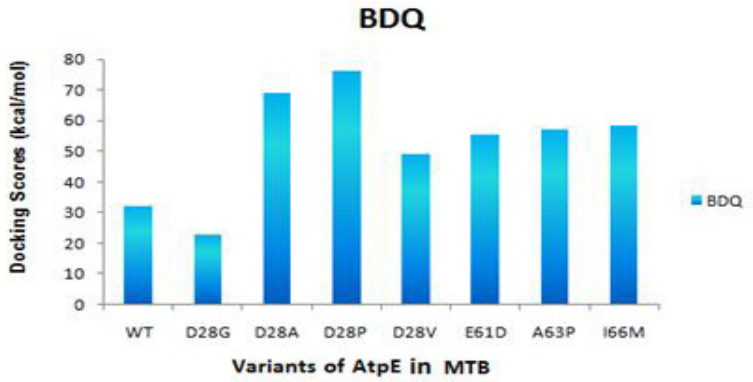


Figure 6: Docking score of BDQ with WT and mutants of AtpE

The interaction profile of WT and MT-AtpEs with BDQ at its binding site are illustrated in **Figure 7**. In general, the inhibitor and enzyme make a pattern of complementary hydrogen (H) bonds between their respective backbone atoms. In case of WT-AtpE complexed with BDQ, single carbon H bond was seen followed by two Pi-pi stacked interactions with Phe65, and other residues in van der Waals contact distance as shown in **Figure 7**. In case of MT-D28G, three carbon H bonds were observed between BDQ and residues Glu61, Phe62 and Phe65, respectively, followed by weak Pi-alkyl interactions. In contrast to WT in MT-D28A, several interactions principally of alkyl, Pi-alkyl, Pi-Pi stacked were found and Pi-sigma between the drug and Phe66 was also found. In MT-D28P complexed with the drug molecule, two carbon H bonds were formed with residues Phe54 and Gly58, followed by alkyl, Pi-alkyl Pi-pi stacked interactions. Surprisingly, in MT-D28V complexed with BDQ no H bonds were found. Notably, bromine atom of the BDQ was involved in alkyl interaction with the residue Leu68. In addition, alkyl, Pi-alkyl and two amide Pi-stacked types of interactions were observed. Interestingly, bromine atom of the BDQ was involved in alkyl interaction with the residue Leu68 (**Figure 7**). In case of MT- E61D, a carbon H bond with Asp142 was formed and Pi-alkyl, Pi-pi stacked interactions were found as shown in **Figure 7**. In MT- A63P complexed with BDQ, an amide-Pi stacked and Pi alkyl interactions were found with Glu61 and Ala62 residues. In MT- I66M with BDQ, interestingly, sulphur based interactions with Met66 itself was found. Then, a pi-Sigma with Leu59 and Pi-Pi T-shaped with Phe146 were also found (**Figure 7**).

The reason for the high score in all mutants (D28A, D28P D28V, E61D, A63P, and I66M except D28G) with BDQ could be attributed to the presence of favourable interactions that lacks in WT. This could in turn be due to the substitution of Ala/Pro/Val in the MT proteins in place of Asp in WT at position 28. In these mutants (D28A, D28P D28V), Ala contains one extra methyl group, Pro contains a hetero cyclic group and Val contains two methyl groups instead of Asp which is an acidic amino acid, might have induced more structural changes in the protein's side chain, which was obvious in changes in the pattern of interactions (many alkyl, Pi-alkyl, Pi-Pi stacked and Pi-sigma in D28A; two carbon H bonds, alkyl, Pi-alkyl, Pi-pi stacked interactions in D28P and alkyl, Pi-alkyl and two amide Pi-stacked in D28V) and consequently reflected in high score. In case of E61D, A63P, and I66M, Asp contains an

acidic group, Pro contains a hetero cyclic group and Met contains functional side chain of methyl group and sulphur atom in place of Glu61, Ala63 and Ile66, might have induced more structural changes in the protein's side chain, which was obvious in changes in the pattern of interaction.

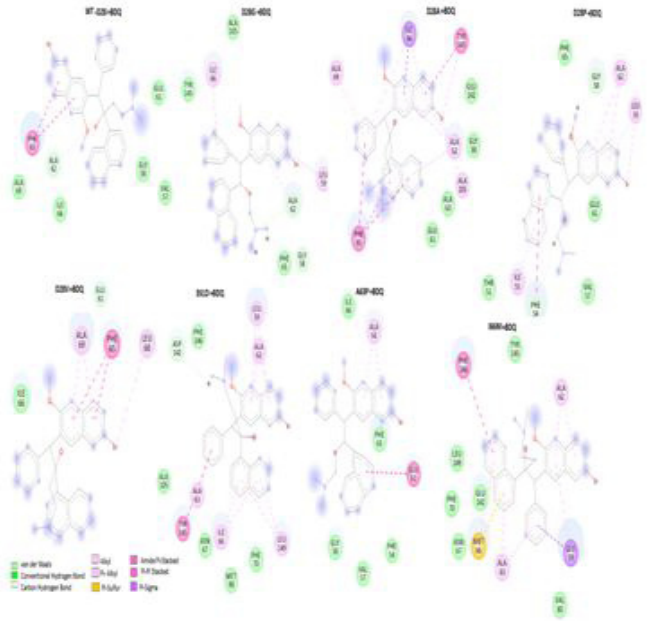


Figure 7: Differences in network of interactions between WT and mutants of AtpE from *M. tuberculosis* with BDQ

A carbon H bond, Pi-alkyl, Pi-pi stacked interactions in E61D and amide-Pi stacked and Pi alkyl interactions in A63P; interactions such as sulphur based with Met66 itself was found. A pi-Sigma with Leu59 and Pi-Pi T-shaped with Phe146 were found in MT- I66M. These types of interactions in all mutants consequently reflected in high score than the WT. In contrast in case MT-D28G, as Gly does not contains a functional group or side chain, resulted in lower score (only Pi-alkyl) than the WT (two Pi-pi stacked interactions). Thus, based on these findings it can be assumed that the mutants -D28A, D28P, D28V, I66M, A63P, and E61D could lead to high level resistance compared to MT-D28G that may lead to low level resistance. Therefore, in this pilot study, a primary effort was taken to understand the effect of binding affinity of WT and MT proteins of AtpE with BDQ, which showed more affinity towards the mutants compared to the WT. Therefore, it can be suggested that the mutants displayed more affinity with BDQ, because of the substitution that induces structural changes and BDQ binds very tightly leading to the slow or no release of the drug to mediate its inhibitory activity, thereby leads to BDQ resistance.

4. Conclusions

The findings in this concise report have provided some useful insights towards understanding the basis of *in vitro*-BDQ resistance in *M. tuberculosis*. Although, the effect of docking could be better explained after performing molecular dynamics for understanding their function precisely, yet, the information provided over here can be useful to understand the impact of such substitution and consequent changes in binding ability. However, further struc-

tural studies are needed to get a deeper understanding of the mechanism of ATP resistance to BDQ that will aid in development of inhibitors that are selective against ATP synthase which can circumvent the problem of BDQ resistance. In addition, to understand more about the binding aspects of BDQ with other non-target based resistance *i.e.,* efflux-based resistance to BDQ which was identified in paired isolates from patients treated with BDQ, as well as in mice, showing cross-resistance to clofazimine [20]. Thus, structural studies related to efflux-based resistance mutants leading to cause of BDQ resistance are also needed.

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Diagnosis and Management of Tuberculosis

Chapter 3

Tuberculosis Treatment and Management

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Abbreviations

AFB: Acid-fast Bacilli; ART: Anti -Retroviral Therapy; CTC: Care and Treatment Centre; DOT: Direct Observed Treatment; DST: Drug Susceptibility Test; EPTB: Extra- Pulmonary Tuberculosis; HIV: Human Immunodeficiency Virus; M. TB: Mycobacterium Tuberculosis; MDR-TB: Multi-drug Resistance Tuberculosis; NTPs: National TB Programs; PAS: Para-Aminosalicyclic Acid; PCT: Patient Centred TB Treatment; PTB : Pulmonary Tuberculosis; TB: Tuberculosis; WHO: World Health Organization; XDR-TB: Extreme Drug Resistance Tuberculosis

1. Introduction

1.1 Chapter objectives

This chapter has four main sections; introduction, TB treatment, management and challenges: The introduction section defines what is tuberculosis (TB) and how it is spread, risk factors, highlights on the historical perspective of TB including; its discovery and treatment (discovery of drugs). The section also explains on the types of TB and burden of TB globally. The treatment section describes the importance of TB treatment, treatment duration and phases, categories of patients, current treatment regimen for adult and for children. The management section gives details on different approaches used in the management of TB patients with main focus on involvement of communities. The section on challenges highlights challenges in TB treatment, management, TB/HIV co-infection, programmatic challenges and in special situations such as MDR-TB and prisons.

1.2 What is tuberculosis?

Tuberculosis is a chronic infectious disease mainly caused by *Mycobacterium tuberculosis (M. TB)* and occasionally by *Mycobacterium bovis* or *Mycobacterium africanum*. The disease is spread through air (an air born disease).Transmission from one person to another oc-

curs through inhalation of droplets during coughing, laughing, talking, sneezing and singing. However, the most common means of transmission is through coughing. The potential sources of infection are untreated smear-positive patients who are in the community.

1.3 Risk factors contributing to infection and development of disease

An individual's risk of infection depends on several factors, including;

- Concentration and size of infectious droplets,
- Extent or duration of exposure,
- Prevalence of TB in the community; (the higher the prevalence of TB in the community, the higher the risk of exposure and infection).
- Overcrowding and prolonged stay with an infectious person in a poorly ventilated and lighted environment, and
- Individual's immune status and susceptibility to infection.
- Malnutrition and other conditions such smoking, diabetes (Daniel, et al., 2011).

The risk of progression from infection to active disease depends on the status of the individual's immune system. It is well documented that 90% of people without human immunodeficiency virus (HIV) infection who are infected with *M.tuberculosis* do not develop TB disease. Tuberculosis and HIV co-infected people have annual risk of 5-10%,and20-30 times higher risk of developing TB disease during their life time than people without HIV infection (NTLP Manual 2013).

1.4 Historical perspective of Tuberculosis

Tuberculosis has been known to mankind since ancient times. Historically, earlier TB disease has been called by numerous names including; Consumption (because of the severe weight loss and to "consume" the patient); Phthisis pulmonaris and the White plague (because of the extreme pallor seen among those infected). Consumption, phthisis, scrofula, Pott's disease, and the White Plague are all terms used to refer to tuberculosis throughout history. It is generally accepted that the microorganism originated from other, more primitive organisms of the same genus Mycobacterium. Even today after the development of advanced screening, diagnostic and treatment methods for the disease, a third of the world's population has been exposed and is infected with the organism. In the 19th century, TB was known as **"the captain of all men of death".** It is still true to a large extent today.

Tuberculosis in Ancient times: The organism causing tuberculosis – M. TB existed since 15,000 to 20,000 years ago. It has been found in relics from ancient Egypt, India, and China. Among Egyptian mummies spinal TB, known as Pott's disease has been detected by archaeologists.

Tuberculosis in the middle ages: Evidence of TB of the cervical lymph nodes or lymph nodes of the neck termed **scrofula** was found in the middle ages. It was termed as the **"king's evil"** and was widely believed that the kings of England and France could cure scrofula simply by touching those affected.

Tuberculosis in the 18th century: In the 18th century in Western Europe, TB reached its peak with a prevalence as high as 900 deaths per 100,000 population. Poorly ventilated and overcrowded housing, primitive sanitation, malnutrition and other risk factors led to the rise. In deed the term White plague emerged around this time. During this period, famous men and women over ages suffered from this disease. Notable among these were poets John Keats and Percy Bysshe Shelley, the authors Robert Louis Stevenson, Emily Bronte, and Edgar Allen Poe, the musicians Nicolo Paganini and Frederic Chopin to name a few.

Discoveries pertinent to tuberculosis: The tubercle bacilli or the causative organism of tuberculosis was demonstrated by Robert Koch in 1882. Robert showed that the organism's unique protein coat made it difficult to visualize earlier until a specific stain called the ZeihlNeelson stain was discovered. After this discovery, the bacteria was called Koch's bacillus and since it took up the red acidic dye, it was called acid fast bacilli (AFB). Koch was awarded the Nobel Prize in 1905. In 1895 Wilhelm Roentgen developed X-rays which further advanced diagnostics of tuberculosis. Discovery of X-ray allowed early diagnosis and isolation of infected individuals.

Isolation: In the 19th century the concept of keeping TB patients isolated in a sanatorium started. It was initially started in Silelsia in 1859 by Hermann Brehmer.In 1884, Edward Livingston Trudeau started the first sanatorium in the United States. From that time, infectious persons were isolated from society and treated with rest and improved nutrition.

Development of vaccine for TB: In the 1880s Louis Pasteur began the concept of development of vaccines against anthrax, chicken cholera, and, later, rabies. In 1908, the French scientists **Albert Calmette and Camille Guerin** grew Koch's bacillus in several mediums to decrease their virulence and increase the capacity to produce immunity. This led to the now famous vaccine called BCG named after the two founders. BCG was introduced in 1921.

Surgery for TB: Before antibiotics were found effective against TB, surgical treatment of TB was common and often lifesaving. Dr. James Carson, a Scottish physician (1821), began treatment by draining pleural effusion from around the lungs and found surgery helped prolong life. Various techniques evolved but due to lack of efficacy faded away after advent of anti-

tubercular drugs.

Anti-tubercular drugs: In 1944 -antibiotics were used against TB for the 1st time after the discovery of streptomycin. Use of this agent alone led to antibiotic resistance that is still a major problem.Better results followed the development of para-aminosalicyclic acid (PAS). Unlike streptomycin, PAS was an oral agent. In 1950's - More effective drugs like isoniazid (INH) came in and treatment with rifampicin followed. Currently, there are about 20 agents with activity against mycobacterium.

1.5 Types of tuberculosis disease

There are two main types of TB: pulmonary and extra-pulmonary. The most common form is pulmonary tuberculosis (PTB), which accounts for 80% of all cases of TB. Pulmonary TB is the infectious form and affects the lungs. Extra-pulmonary tuberculosis (EPTB) affects organs other than the lungs (e.g., pleura, lymphnodes, abdomen, genitourinary tract, skin, joints and bones, meninges); it is usually non-infectious and accounts for 20% of all cases of TB (NTLP Manual 2013).

1.6 Burden of Tuberculosis

Tuberculosis is a major public health problem throughout the world. About a third of the world's population is estimated to be infected with the tubercle bacilli and is at risk of developing active disease. According toWHO, in 2016 there were 6.3 million new cases of TB globally, equivalent to 61% of the estimated incidence of 10.4 million. TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people and an additional of 374 000 deaths among HIV-positive people. An estimated 10.4 million people fell ill with TB in 2016: 90% were adults, 65% were male, 10% were people living with HIV (74% in Africa) and 56% were in five countries; India, Indonesia, China, the Philippines and Pakistan. Furthermore, drug-resistant TB is continuing to be a threat, for instance in 2016, there were 600 000 new cases with resistance to rifampicin (RRTB), the most effective first-line drug, of which 490 000 had multidrug-resistant TB (MDR-TB). Almost half (47%) of these cases were in India, China and the Russian Federation (WHO report 2017).

1.7 HIV infection and multi-drug resistance TB

The most important recent changes in the history of TB have been the impact of the HIV epidemic and the emergence of resistance to anti-TB drugs. HIV infection exacerbates the TB epidemic through its impact on susceptibilityto *M. tuberculosis* infection and progression from infection to active disease. HIV infection increases the rate at which *M. tuberculosis* infections are acquired and increases the likelihood that people who are already infected will

develop active TB disease (WHO 2008). The impact of HIV has been greatest in countries of southern and eastern Africa, where up to 40% of adults may be infected with HIV and where the incidence of TB has increased 4–5-fold within 10 years. Infection with both *M. tuberculosis* and HIV is prevalent in some population groups in certain countries of South-East Asia, including Cambodia, China, India, Thailand and Viet Nam.The development and increasing importance of anti-TB drug resistance are of concern to TB control programs because drug-resistant TB is much more difficult to manage and more expensive to treat than fully drug-susceptible TB.

Section Two: Treatment

2. Tuberculosis Treatment

Treatment for TB is usually initiated once the diagnosis is confirmed. An effective TB treatment is of the paramount importance in TB care and control, since it terminates the transmission chain and eventually leads to a reduction of the disease burden in the community. The effectiveness of TB treatment depends on the use of the right anti-TB drug combinations both in the intensive and the continuation phases, prescribed and taken in the correct doses according to the schedule and for the required length of time.

2.1 Treatment phases and duration

There two phases of TB treatment; initial or intensive and continuation. Treatment of TB usually takes 6-8 months depending on weather the patient is newly diagnosed or previously treated case. Newly diagnosed are treated for six months while previously are treated for eight months (**Table 1**).

Table 1: Treatment phases and duration by patient category

Patient category	**Treatment phases and Duration**		**Total treatment duration**
	Intensive	Continuation	
Newly diagnosed	2 months	4 months	6- months
Previously treated (Re-treatment)	3 months	5 months	8- months

2.2 Aim of TB treatment

Specifically, treatment of TB aims to:

1. Cure the patient and restore quality of life and productivity.
2. Prevent relapse of TB.
3. Reduce transmission of TB to others.
4. Prevent the development and transmission of drug-resistant tubercle bacilli.

5. Prevent death from active TB or its late effects.

2.3 Definition of TB case classifications

Tuberculosis cases are classified according to anatomical sites and other conditions to facilitate recording and reporting and to identify the most infectious patients for infection control. The classifications include the following:

a) Anatomical site of disease

b) History of previous treatment

c) Drug resistance

d) HIV status

a) Classification based on anatomical site of disease

This classification is based on the site of the infection; pulmonary or extra pulmonary.

Pulmonary tuberculosis (PTB): Refers to a case of TB involving the lung parenchyma. Miliary TB is classified as PTB because there are lesions in the lungs. Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of EPTB. A patient with both pulmonary and extra-pulmonary tuberculosis is classified as a case of PTB.

Extra-pulmonary tuberculosis (EPTB): Refers to a case of TB involving organs other than the lungs (e.g., pleura, lymphnodes, abdomen, genitourinary tract, skin, joints and bones, meninges). EPTB cases can be either bacteriologically confirmed or clinically diagnosed. Identification of *M. tuberculosis* (as opposed to histology) should be the basis of bacteriological confirmation of EPTB. The case definition of an EPTB case with several sites affected depends on the site representing the most severe form of the disease.

b) Classification based on history of previous TB treatment (patient registration group)

This classification focuses on history of previous treatment only and is independent of bacteriological confirmation or site of disease (**Table 2**).

Table 2: Classification of patients based on history of previous TB treatment

	Patient categories	Description of the category
1	New patient	Has never had treatment for TB, or has taken anti-TB drugs for less than one month.
2	Previously treated patient	Has received one month or more of anti-TB drugs in the past.

	Patient categories	Description of the category
3	Relapse patient	Has been previously treated for TB, was declared cured or treatment completed at the end of the most recent treatment episode, and is now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by re-infection).
4	Treatment after failure patient	A patient who was previously treated for TB and whose treatment failed at the end of the most recent treatment episode.
5	Treatment after loss to follow-up patient	A patient previously treated for TB who was declared lost tofollow-up at the end of the most recent treatment episode (previously known as “return after default”).
6	Other previously treated patient	A patient who was previously treated for TB but with an unknown or undocumented outcome for the most recent treatment episode (previously known as “others”). A patient with an unknown previous TB treatment history does not fit into any of the categories listed above.

c) Classification based on drug – resistance

Drug-resistant patients are defined as those with tubercle bacilli that are resistant to both rifampicin and isoniazid (MDR-TB). Patients with MDR-TB whose tubercle bacilli have additional resistance to a second-line injectable (capreomycin, kanamycin, or amikacin) and a fluoroquinolone (ofloxacin, moxifloxacin, levofloxacin, or gatifloxacin) are classified as extreme drug resistance TB patients (XDR-TB).

There are about five categories of drug-resistance classification based on drug susceptibility test (DST) results:

- **Monoresistance:** Resistance to one first-line anti-TB drug only.
- **Polydrug resistance:** Resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin.
- **Multidrug resistance (MDR TB):** Resistance to at least both isoniazid and rifampicin.
- **Extensive drug resistance (extensively drug-resistant tuberculosis, or XDR TB):** Resistance to any fluoroquinolone and at least one of three second-line injectable drugs (capreomycin, kanamycin, and amikacin), in addition to multidrug resistance.
- **Rifampicin resistance:** Resistance to rifampicin detected using culture or molecular tests (phenotypic or genotypic methods), with or without resistance to other anti-TB drugs. This includes any resistance to rifampicin, in the form of monoresistance, multidrug resistance, polydrug resistance, or extensive drug resistance.

d) Classification based on HIV status

HIV-positive TB patient: A patient with TB (bacteriologically confirmed or clinically diagnosed) who has a documented HIV-positive result, such as a CTC1 card, or is registered in an HIV Care and Treatment Centre (CTC), or has a positive HIV result from testing conducted at the time of TB diagnosis.

HIV-negative TB patient: A patient with TB (bacteriologically confirmed or clinically diagnosed) who has a documented negative HIV result from a test conducted at the time of TB diagnosis. HIV-negative TB patients subsequently found to be HIV positive should be reclassified as HIV-positive TB patients.

2.4 HIV and multi-drug resistance TB

The most important recent changes in the natural history of TB have been the impact of the HIV epidemic and the emergence of resistance to anti-TB drugs.HIV infection exacerbates the TB epidemic through its impact on susceptibility to *M. tuberculosis* infection and progression from infection to active disease.

HIV infection increases the rate at which *M. tuberculosis* infections are acquired and increases the likelihood that people who are already infected will develop active TB disease. The impact of HIV has been greatest in countries of southern and eastern Africa, where up to 40% of adults may be infected with HIV and where the incidence of TB has increased 4–5-fold within 10 years. Infection with both *M. tuberculosis* and HIV is prevalent in some population groups in certain countries of South-East Asia, including Cambodia, China, India, Thailand and Viet Nam (WHO 2008).

The development and increase of MDR-TB pose a great challenge to the National TB control programs (NTPs) because drug-resistant TB is much more difficult and costly to treat than fully drug-susceptible TB. It is estimated at 450,000 cases of multidrug-resistant TB (MDR -TB) occur each year among new and previously treatedTB cases, and extensively drug-resistant TB (XDR -TB) has been reported frommany countries including South Africa. Drug resistance is more common in settings where cure rates are low, for examplewhere anti-TB drugs are available without medical prescription. For this reason the NTPs have been advised over the years to focus on achievinghigh cure rates, optimizing the quality of and access to anti-TB drugs, increasing case detection rates, ensuring good treatment outcomes for patients with MDR or XDR -TB and, in settings where HIV is prevalent, on ensuring that TB patients are tested for HIV and that people with HIV are examined for TB.

2.5 Direct Observed Treatment (DOT)

Direct observation of treatment is an important element of the first component of the Stop TB Strategy. DOT means that a trained health care provider or other designated individual, including family members, observe the patient when swallowing tablets. The importance of DOT is to ensure that a TB patient takes the right drugs, in the right doses, at the right intervals and this ensures that the patient finishes treatment within the required duration, which helps to prevent transmission of the disease to others and hence reduce the chance of treatment failure and relapse.

2.6 Standard TB treatment regimens

Treatment of TB use standardized regimen, this means that all patients in a defined group receive the same treatment regimen. Standard regimens have several advantages over the individualized prescription of drugs, these include:

- Reduces likelihood of prescription errors, thus reduce risk of drug resistance.
- Facilitates estimation of drug needs, purchasing, distribution and monitoring.
- Facilitates uniform staff training.
- Reduced costs.
- Ease of maintaining a regular drug supply when patients move from one area to another.
- Make it easier for outcome evaluation and comparable results.

2.5.1 Available first-line anti-TB drugs

Based on WHO recommendations, currently, there are five essential first-line anti-TB drugs for adults and children. The drugs are formulated in fixed dose combinations (FDCs).

Individual dosages are shown belowin **Table 3.**

Table 3: Recommended daily doses of first-line anti-TB drugs for adults

Drugs	Recommended daily dose	
	Dosage and Range(mg/Kg body weight)	Maximum (mg)
Isoniazid (H)	5 (4-6)	300
Rifampicin (R)	10 (8-12)	600
Pyrazinamide (Z)	25 (20-30)	1,600
Ethambutol (E)	15 (15- 20)	1,100
Streptomycin (S)	15 (12-18)	1,000

2.5.3 Fixed-dose combinations

First-line anti-TB drugs are usually supplied in a form of blister packs. Each blister pack contains 28 tablets and each box of blister packs contains 24 blisters of four-drug FDCs (RHZE), which is enough for an average of four adult patients during the intensive phase. FDCs are colour coded. The FDC dosage is given based on individual's body weight (**Table 4**).

Note: Newly diagnosed adult TB patients should receive a six-month regimen containing rifampicin (**2RHZE/4RH)**: The drugs should daily be taken under observation by a health care worker or treatment supporter throughout the six months.

Table 4: Daily dosage of TB drugs in relation to body weight for FDCs in new adult TB patients

Body weight	Number of tables in the initial phase: 2 months (R 150/H75/ E 400/E275)mg	Number of tables in the continuation phase: 4 months (R150/H75) mg
21 – 30 kg	2	2
31 – 50 kg	3	3
51 – 74 kg	4	4
≥75 kg	5	5

R= Rifampicin; H = Isoniazid; Z = Pyrazinamide; E = Ethambutol

Note: All patients who are rifampicin resistant should receive MDR -TB treatment in a designated health facility or clinic.

2.7 Treatment of paediatric TB

For treatment of children diagnosed with TB, treatment will depend on the availability of paediatric formulation, when not available adult formulation is used based on the Doctor's descriptions and child's body weight. The recommended type of drugs and duration for treatment of children is shown below on **Table 5.**

Table 5: Recommended treatment regimens for children

TB disease group	Intensive phase	Continuation phase
All forms of PTB and EPTB except TB meningitis and TB of thespine/bone/joints	2 RHZE	4 RH
TB meningitis, miliary TB, TB of the	2 RHZE	10 RH
spine/bone/joints		
Previously treated smear-positive PTB (relapse, return after default, treatmentfailure)	2 RHZE	5 RHE

R= Rifampicin; H = isoniazid; Z = Pyrazinamide; E = Ethambutol

The two tables below (**Table 6 and Table 7**) show the recommended regimen and dosage for treatment of children.

Note: If the paediatric formulation is not available, the health care provider will need to give clear instruction to the parent or caregiver on how to cut and crush adult tablets to achieve the prescribed dosage.

Table 6: Weight-based dosing of anti-TB drugs for children (2-20 kg body weight)

	Intensive phase (2 months)		Continuation phase (4 months)
Weight in kg	RHZ 60/30/150 mg	Ethambutol 100mg	RH 60/30 mg
2 - 2.9	½ tablet	½ table	½ tablet
3 – 3.9	1 tablet	½ tablet	1 tablet
4 – 5.9	1 tablet	1 tablet	1 tablet
6 – 7.9	1.5 tablets	1.5 tablets	1.5 tablets
8 – 10 .9	2 tablets	2 tablets	2 tablets
11 - 13.9	3 tablets	2 tablets	3 tablets
14 – 19.9	4 tablets	3 tablets	4 tablets

RHZ = Rifampicin Isoniazid and Pyrazinamide; RH = Rifampicin and Isoniazid

Table 7: Weight-based dosage for children using adult anti-TB drug formulations

Weight in kg	**Intensive phase (2 months)**	**Continuation phase (4 months)**
	RZHE 1500/75/400/275 mg	**RH 150/75 mg**
5 – 5.9	½ tablet	½ tablet adult
10 – 14.9	1 tablet	1 tablet adult
15 – 19.9	$1^{1/2}$ tablets	$1^{1/2}$ tablets adult
20 -24.9	2 tablets	2 tablets
25 – 29.9	$2^{1/2}$ tablets	$2^{1/2}$ tablets
30 - 40	3 tablets	3 tablets
>40	4 tablets	4 tablets

Section Three: Management

3. Management of Tuberculosis (TB)

3.1 Introduction

Management of TB patients encompasses identification, diagnosis; initiation of treatment, assurance of available drug supplies, adherence to treatment, monitoring of drug intake as well as making sure that the patient is cured or complete treatment. Proper management of TB patients within the existing health system has become very difficult due to several factors, such as: increased number of TB patients, inadequate health personnel and limited coverage of public health services. The later has continued to impede accelerated access to TB control services (WHO 2008). Inadequate health services infrastructures, insufficient decentralization

of diagnostic and treatment services and inadequate human, material and financial resources have also contributed to poor management of patients. In additional, the direct impact of the HIV epidemic has led to an exponential increase of TB incidence, increasing pressure on hospital and public health services already stretched by the need to ensure effective delivery of essential packages of health care services (WHO 2008).

In order to address some of these challenges, several efforts have been made by different organisations including; WHO, Ministry of Health, National TB control programs (NTPs), civil society organizations (CSOs) and partners through involving community in the delivery of TB control services all aiming at better TB management, treatment adherence and better treatment outcome. For the effective engagement of communities in TB control activities, (TB care and management), guidelines and manuals have been developed by WHO including; "ENGAGE –TB"operational manual (WHO 2012) as well as training manuals on how to engage the community, NTPs (PCT manual 2005) and by other non-government organisations and civil society organisation in the delivery of TB care and management.

3.2 Involvement of community in TB treatment and management

A community consists of people living together in some form of social organization and cohesion. Although it may vary significantly in size and socioeconomic profile, its members usually share social, cultural, economic characteristics as well as common interests, including health.

The challenges posed by major epidemics such as HIV/AIDS, TB and malaria,and the role civil society has played in helping individuals and families to cope with them, have certainly contributed to make people and health policy-makers more aware of the essential and complementary role that communities can play in ensuring high-quality patient care.Community participation in TB control highlighted that a sphere of close friends and neighbours plays an important role in every person's daily life and acts as an immediate point of reference for help and advice.

Advantages of community involvement in TB care and management: Involvement of communities in the management of TB control activities has shown a significant contribution in terms of; case finding and treatment adherence, cure rate, reduction in defaulters (Egwaga, 2009), death as well as reduction of workload to the health care workers (HCWs). Community involvement facilitate access by bringing services to people's homes, and reducing the cost of care-seeking for patients and health services (WHO TB Strategy 2008, WHO 2012). Involvement of community initiatives facilitates patient and community empowerment. Through the involvementof local communities, education on relevant health issues and stimulation of change in health-related behaviour, communities become increasingly knowledgeable and self-reliant (WHO 2008).

Community engagement is critical to improve the reach and sustainability of TB interventions, helping save lives from this top infectious killer. Engagement of communities and civil society organizations in TB care is one of the core components of the End TB Strategy. The ENGAGE-TB approach aims to better identify and treat people with TB by integrating services into community-based work of previously unengaged nongovernmental and other civil society organizations. ENGAGE-TB approach seeks to shift the global perspective of TB from only a medical illness to a more comprehensive socioeconomic and community problem. The approach emphasizes the value of collaboration and partnership between NGOs and other CSOs and the NTPs. The ENGAGE-TB emphasizes among other things in TB monitoring and reporting, in order to ensure that national data is adequately captured the contribution of community-based TB activities is evidently.

Section 4: Challenges

4. Challenges in Treatment and Management of Tuberculosis

Despite that there have been great achievements in the treatment and management of TB both in terms of high treatment success rates and integration of TB-HIV care and management there are still several challenges. Below are some of the challenges in terms of treatment, management among TB, TB/HIV co-infected and in special situations (MDR-TB and prisons).

4.1 TB Treatment challenges

- At times the NTPs experience drug interruption due to inadequate or limited supply of 1st line anti-TB drugs.
- Long TB treatment duration of (6-8 months).
- Irregular treatment;i.e. treatment failure, drug resistance and relapse.
- Coping strategies for difficulties of “real” DOT: since health care workers cannot coup to daily observe the TB patients taking drugs at the health facility, communities are engaged to observe patients taking drugs at their homes. Involvement of lay persons in the communityor in the family is a challenge; In **community:** Issues of accountability, confidentiality, remuneration, and sustainability, In **family**: DOT is rather a disguised self-administered treatment.

4.2 TB/HIV co-infection challenges

About 40–65% of HIV-infected African patients with respiratory disease have TB.

- Weakened health system due to inadequate number of health care workers and increased

number of patients to be managed (TB-HIV co-infected).

- Health care delivery is weakened due to absenteeism of staff because of illness.
- TB control programmatic delivery and patient management are adversely affected by HIV epidemic.
- Increased risk of drug toxicities
- Higher pill burden
- Drug interactions when anti-TB and anti-HIV treatments are taken together
- Occurrence of immune-reconstitution syndrome

4.3 Programmatic delivery challenges

- Health services struggle to cope with increasing numbers of TB suspects and patients
- Stock-outs of sputum containers as the demand fails to keep pace with supply frequent
- More smears have to be prepared and examined with risk of increase false-negative results.
- Paradoxically, with increase in TB burden due to HIV, case detection rates for smear positive PTB decline.

4.4 Patient management challenges

The following are some of the challenges that adversely affect the image of the TB program

- Diagnosis of TB more difficult in HIV infected persons:
- Clinical picture and radiological findings are atypical
- More patients likely to die during the course of treatment
- High early case fatality or more deaths during the first two months of anti-TB treatment and higher rate of recurrent TB among HIV+ compares to HIV- patients.

4.5 Other challenges are those in special populations including; Patients with MDR-TB and prisoners with TB

For MDR-TB challenges include;

- Management of MDR-and XDR-TB is complex and expensive
- 2nd line medications not routinely available
- Treatment of MDR-TB is long, expensive with many side-effects and requires special dispositions for follow-up and care

• For prisoners with TB – challenges include;

- Overcrowding
- Poor ventilation of cells
- Malnutrition
- Higher rates of HIV than in general population
- Hygiene and health services sub-standard or inexistent
- TB in prisons affects the general population through transmission when prisoners are released and via prison staff and visitors.

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Diagnosis and Management of Tuberculosis

Chapter 4

Early Diagnosis of *Mycobacterium tuberculosis* using Next Generation Sequencing

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1. Introduction

Tuberculosis is a chronic granulomatous infectious disease caused by tubercle bacteria known as *Mycobacterium tuberculosis* (*Mtb*). It is a facultative intracellular organism that is readily phagocytosed but is resistant to intracellular killing by the macrophage. It can remain dormant for years till hosts' immunity persistence and then reactivates itself. *Mycobacterium tuberculosis* was the first bacterial human pathogen described, which dates back to 1882 by Robert Koch but even today continues to produce devastating illness. Since then, a large number of Mycobacterial species responsible for causing pulmonary and extra-pulmonary infections have been identified in humans as well as in animals [1]. *Mycobacterium tuberculosis* creates global epidemic disease, which occurs in many parts of the body, but most commonly found (80%) in the lungs and is rampant throughout the world (Center for disease control and prevention, Morbidity Mortality weekly report, 1993a,b; [2-4]. Every third person on this earth is believed to be infected with *Mtb*, leading to eight million cases of active tuberculosis per year and approximately three million deaths annually. This situation has been more aggravated by the emergence of epidemic multi-drug resistant strains of *Mtb* in conjunction with HIV infection have rendered this problem all the more serious [5]. Furthermore, the accelerating and amplifying influence of HIV infection is contributing to the increasing incidence of disease caused by multi-drug resistant strains of *M. tuberculosis* [6]. Thus, Tuberculosis has become

a major concern worldwide with significant mortality and morbidity reported from various parts of the world [7-12]. We have suggested the theoretical basis of high TB burden among the healthcare workers of developing countries which can be used for further improvement in strategies for the prevention of TB infections in hospital settings [13,14]. Apart from that we have also suggested the potential diagnostics and therapeutics based strategies to combat the drug resistance TB [13,15,16]. Without increased of active commitment at National and International levels, tuberculosis will claim about 30 million more lives in the next decade and there will be about 90 million new cases of active tuberculosis. Such a vast epidemic creates challenges as it raises the demand for public health solutions. Recent advances in diagnosis done by deep sequencing namely Next-generation sequencing this technology decreases the time in diagnosis delay and improve treatment of MDR, XDR etc. outcome.

2. Next-Generation Sequencing

Like other medical fields, the role of next generation sequencing (NGS) techniques is about to revolutionize diagnostics of infectious diseases. Initially, the diagnosis of the microbial origin of diseases was based on the laboratory test by culture assay for bacteria, the lacunae were cultivability could only be detected rather than its hypotheses. In order to seek specific pathogens, specialized media rich or selective and culture conditions defined oxygen tension or temperature can be used but was laborious and their intrinsic inefficiency in the propagation of fastidious bacteria like *Mycobacterium leprae*. These difficulties have been progressively replaced by nucleic acid-based tests like PCR or NASBA. The advantages of PCR are numerous: speed, low cost, automation, sensitivity, and specificity main drawback of targeted, pathogen-specific PCR is that it is only able to identify predefined targets, which supposes that the physician has elaborated an etiological hypothesis. To broaden the range of detection methods like the direct hybridization of non-amplified or random amplified nucleic acids (NA) from samples on DNA arrays was used but this has not been proven satisfactory, due to relative lack of sensitivity for medical diagnosis. Bacterial typing achieved by sequencing the 16S gene or other regions of the genome that is sufficiently conserved to allows the definition of consensus primers yet sufficiently variable to allow for typing. Use of NGS has increased the depth of sequencing by several orders of magnitude and thereby the capacity to detect rare species. But, with 16S PCR, the taxonomic assignation remains often at the level of the genus, an intrinsic limit due to the conservation of the locus between species of the same genus. Multiplexed PCR assays for multiple loci have been developed to provide simultaneous detection of several agents. A meta-analysis study for 6012 patients with sepsis results in an overall sensitivity and specificity of 0.75 (95% CI: 0.65–0.83) and 0.92 (95%CI: 0.90–0.95), respectively, to detect bacteremia [17]. Multiplex PCR range can be improved by designing primers targeting numerous pathogens and varied loci within pathogens and resolving these amplicons using electrospray ionization-mass spectrometry [18] or NGS([19].

To this all setbacks an alternative strategy takes advantage of the increasing availability and speed and decreasing cost per base of NGS offered by deep sequencing machines which allows metagenomics, which is the study of the microbial genetic sequences recovered directly from a given human, animal, or environmental sample here the sequence of all the NA species of the sample are determined and compared with those in databases. At first, this technology was used to describe the dynamics of microbiomes from different origins, from the gut, other mucosal sites and the skin, as well as from various human-made (e.g., sewage) and natural (e.g., sea) environments. It has also been used to discover new infectious agents. At present such metagenomic study cover known but unexpected viruses, phages, bacteria, parasites or fungi [20], which paves the way to the application in the field of diagnosis of infectious diseases. In principle, a whole genome NGS (WG-NGS) would be advantageous in clinical diagnostics, as there is no need to design specific primers to pre-amplify target sequences. In the field of bacteriology, most studies have dealt with sequencing of clinical isolates cultured in vitro, but good results have been obtained by direct sequencing from clinical samples, for example for the diagnosis of tuberculosis lesions [17], fecal samples from diarrheic patients [21], or urinary samples from patients with suspected urinary tract infection [22]. Another advantage of the technique is its capacity to identify co-infections, which is of great help to adapt therapeutics. Old methods to identify strains of *M. tuberculosis* rely on the analysis of small level of the genome, and assumed that the DNA sequences in these level are of mostly variable so not enough to allow researchers to separate strains of *M. tuberculosis* that are evolutionarily close or distant and also the true complexity of disease dynamics cannot be resolved by tracking strains using a small section of the genome. The availability of next-generation sequencing platforms has allowed viewing the complete genetic information of the bacteria, which should improve the accuracy of efforts to monitor strains of *M. tuberculosis* over space and time [23]. It also has been proven where NGS platform are used to expose the genetic heterogeneity of Mycobacterium tuberculosis in extra pulmonary TB patients [24].

3. Epidemiological Sound

For epidemiological investigations, diagnosis, and for testing whether strains of bacteria are susceptible to particular drugs rapid whole genome sequencing would be a promising tool. It has been reported that long-term large-scale whole genome sequencing strategy has been used to decipher the tuberculosis epidemic in a high prevalence setting with multiple sources of infection [25]. The whole genome sequences of 1687 *M. tuberculosis* samples (isolates) from patients in the Karonga District of Malawi for 15 years, which represented 72% of the total number of confirmed tuberculosis cases during that time. Guerra-Assunc et al. found that the epidemic was largely driven by members of one lineage, which implies that either this lineage arrived in the area earlier than the others, or that the members of this lineage were more successful. The genome of *M. tuberculosis* consists of ~4.4 million bases and is generally

believed to be relatively stable [26]. Guerra-Assunc¸ et al. developed a clustering formula to group together directly related isolates. Using this formula in combination with network-analysis they found that strains from certain lineages were more likely to be transmitted between patients than others. This suggests that there are differences in the abilities of bacteria in the different lineages to cause disease. In this high-incidence setting, 66% of identified cases clustered together, of which 38% of the patients had evidence of recent infection, implying ongoing transmission of the bacteria. This indicates that reactivation of the previous infection was the primary driving force behind this epidemic. Glynn, also showed that the proportion of tuberculosis cases due to reactivation increased over the duration of the 15-year study, as demonstrated by a marked decrease in transmission between 1999–2001 (45%) and 2008–2010 (30%). Guerra-Assunc¸ et al. reported due to the implementation of the antiretroviral therapy and isoniazid preventative therapy in Karonga. Significantly, this study reported that the tuberculosis control program in Karonga has reduced transmission of the bacteria and also demonstrated that whole-genome sequencing can provide new insights into tuberculosis epidemics, which could be used to advice and fine tune control programs. Despite the advantages of whole genome sequencing, it is important to acknowledge the complexity of the technology and data analysis. This questions how useful it could be in high incidence settings where tens of thousands of cases are diagnosed annually. Furthermore, the current technology is restricted to clinical isolates that need to undergo a lengthy culturing and DNA extraction process, which prevents its use as a real-time monitoring tool. Additionally, whole genome sequencing is labor intensive and financially demanding, although costs have decreased significantly over the last decade. Regardless of these challenges, this technology has the potential to immediately revolutionize drug susceptibility testing by identifying the complete repertoire of mutations in target genes that confer drug resistance [27].

4. **Key Advantage of Next Generation Sequencing (NGS) for *Mycobacterium Tuberculosis:***

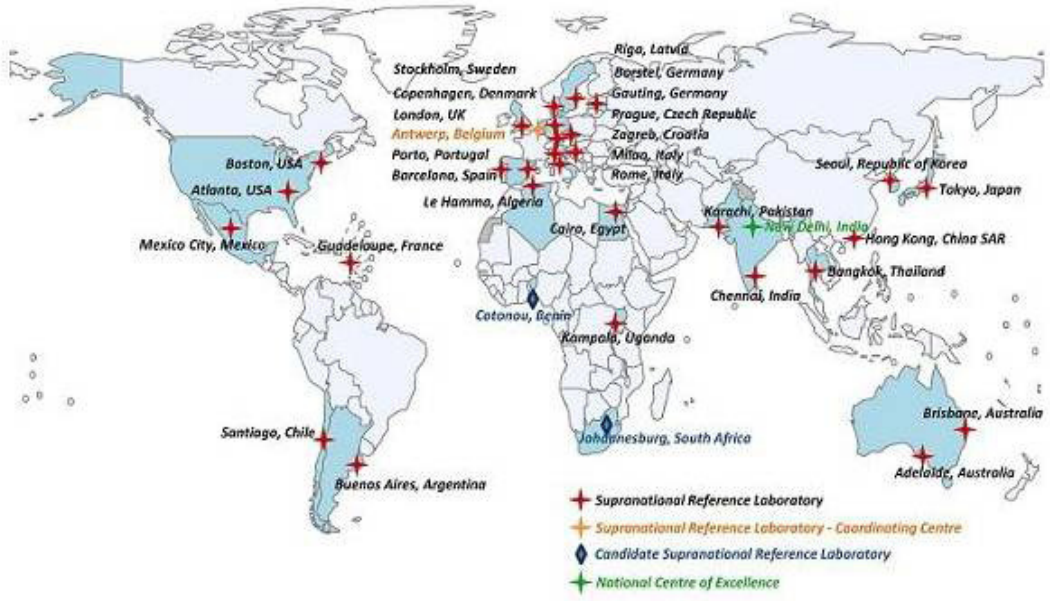
Next generation sequencing (NGS) approaches, where the complete genome is sequenced that not only assistances in pointing out minute variances between the various sequences but also saves time, cost, good Strengths – simpler, faster, Less expensive than WGS, Up to 200 gene targets and easy to handle.

Following key advantages

(i)-Sequence DNA direct from sputum, (ii)- Direct from sputum sample , no need BSL laboratory (iii) Detect primary 1st and 2nd line drug resistance mutations (iv) Rapidly sequence whole genomes (v)- 3.Utilize RNA sequencing (RNA-Seq) to discover novel RNA variants and splice sites, or quantify mRNAs for gene expression analysis (vi) Analyze epigenetic factors such as genome-wide DNA methylation and DNA-protein interactions (vii) These techniques enable

the identification of mycobacterial strains

Next Generation sequencing (NGS) is also found to be useful in identifying single nucleotide polymorphisms (SNPs), comparative genomics and also various aspects about transmission dynamics and also facilitate the study of their phylogenetic and evolutionary traits.



Place of destination: Culture-Free, NGS for Rapid DST in TB Reference Laboratories by 2020.

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Diagnosis and Management of Tuberculosis

Chapter 5

CNS Tuberculosis: An Overview

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1. Introduction

Tuberculosis (TB) is foremost amongst 'the captains of death'of men. It has afflicted humanity from time immemorial. Predictions of its eradication a few decades ago seemed premature and misjudged as the resurgence of TB on the back of the AIDS pandemic derailed benefit paradigms and the emergence of a spectrum of drug resistance brought in new challenges. WHO declared TB a global emergency in April 1995.The historical names for the disease reflect the wasting that is associated with uncontrolled disease. The English called it "consumption" and the Vedas "Yakshma". The word Tuberculosis is derived from the Latin term 'Tubercula' which means small lump.

The day on which Robert Koch declared his discovery of the Tubercle bacillus, on the 24th of March 1882, is commemorated as the 'World Tuberculosis Day. As the discovery of X-rays by William Conrad Rontgen offered an accessible window into the pathological ravages of pulmonary disease, the discovery of Streptomycin, PAS and INH in the 1940's sparked the era of effective antitubercular therapy. Today, 3.7% of new cases of Tuberculosis are caused by Multidrug resistant organisms. Amongst patients previously treated for TB, the incidence is 20%. Finally9% of recurrent TB is XDR (extremely drug resistant).

Tuberculosis (TB) is a global disease, 9.2 million new TB cases occur globally every

year. Of these patients around 13% are HIV positive. Around 1.7 million deaths each year are attributable to Tuberculosis. WHO aims to reduce deaths due to Tuberculosis by 95% by the year 2035.HIV 1 infection is the strongest risk factor for tuberculosis and in India TB is the most common opportunistic infection in HIV positive patients. CD4 counts, the mark of immune competence in HIV,is of strong predictive value in prognosis. The commonest cause of HIV associated deaths in Africa is Tuberculosis. At a global level one third of all TB mortality is associated has an HIV- TB association.

Around 20% of tuberculosis patients have extrapulmonary tuberculosis. Tuberculosis can affect a variety of organ systems including the CNS. Neuromeningeal tuberculosis is rarely seen in immunocompetent individuals in the west. In developing countries TB meningitis is the most common and devastating form of CNS tuberculosis resulting in Vasculitis, Infarctions and Hydrocephalus with consequent Cognitive, Intellectual and Endocrine sequelae.

The clinical manifestations of TB reflect the myriad interplays between Mycobacterium Tuberculosis (MTB) and the human host. DNA evidence suggests that man and the tubercle bacillus have coevolved from the Neolithic ages [1]. With prosperity and progress the incidence of this once global scourge now expresses profound geographic variations. Today the disease predominantly afflicts denizens of the third world [2].India and China together account for 40% of the world wide incidence. The incidence of HIV with TB is highest in Africa. Around two million people die of tuberculosis yearly across the world. The immunocompromised population, whether afflicted by AIDS or with immune deficiency induced by cancer therapy or post transplant regimes remains vulnerable to Tuberculosis. An additional confounding factor has been the emergence of drug resistant variants which pose additional and sometimes unsurmountable impediments to effective treatment. Indeed the professed WHO target of eradicating tuberculosis by 2050 has been stymied by evolution of multidrug resistant (MDR), extensively drug resistant (XDR) and functionally untreatable strains of MTB.

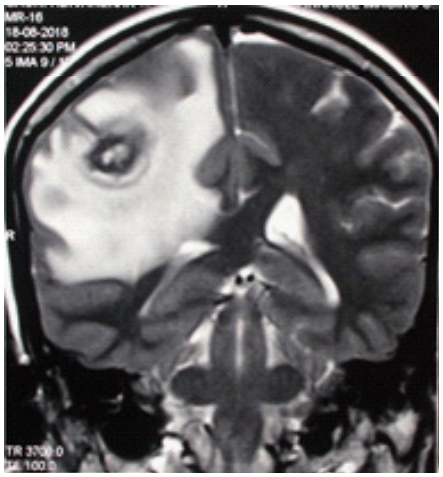
Tuberculosis is almost inevitably transmitted through the respiratory tract. The initial inoculum is often eliminated or kept in check by host defence mechanisms. Both host and pathogen factor variables influence the efficacy and outcome of this encounter. Vitamin D is one amongst the nutritional supplements which contributes to an effective immune restraint [3]. In about 15% of patients the initial infection progresses to clinical disease syndromes. In around 12% of cases the organism escapes the initial immune confined to reactivate and result in clinical disease [4]. Genetic susceptibility to TB is due to multiple genomic traits [5].

Hematogenous dissemination of the tubercle bacillus from the primary focus culminates in the occurrence of extrapulmonary tuberculosis. Some of the more sinister syndromes of the disease are consequent to CNS invasion. The blood brain barrier (BBB), constituted by tight endothelial junctions supported and nourished by supporting astrocytic foot processes

prevents passive intracranial egress of infections and toxins [6]. Endothelial cells may be the initial residence of MTB in the CNS. Tubercle bacilli cross the BBB via infected monocytes/ neutrophils [7]. The initial focus in the brain is called the 'Rich' focus after Arnold Rich, who along with Howard McCordick first demonstrated cascading foci in the meninges and brain parenchyma of patients with TB meningitis.MTB enter the CSF consequent to rupture of the 'Rich' focus. A T-cell response occurs. Cytokines like TNF alpha and Interferon gamma are activated.Inflammation causes obstruction of CSF pathways resulting in hydrocephalus and vasculitis which leads on to infarctions. Suppressing the inflammatory response therefore is integral to therapeutic regimens in CNS Tuberculosis [8].

The brain has limited regenerative capacity. This necessitates a certain degree of immunological privilege with a restricted supply of dendritic cells and limited expression of MHC class 2 molecules [9].The tailored immune capability has been used to explain many unique features of CNS tuberculosis. Selective privilege is responsible for the delayed onset of inflammatory meningitis in disseminated tuberculosis. The phenomenon of delayed enlargement of Tuberculomas, the so called 'paradoxical' growth which occurs well after the initiation of antitubercular treatment is also explained by the unique immunological milieu of the brain. Both host and pathogen factors may play a part in the unique clinicopathological manifestations of an index case of CNS tuberculosis [10].

Tuberculomas which are tubercular granulomas occur in the cerebral parenchyma because of haematological dissemination. Tuberculomas in the subarachnoid space are more often the consequence of tubercular meningitis. Around 39% of patients with TB meningitis develop tuberculomas which may appear paradoxically after initiation of antitubercular treatment. Factors in tubercular meningitis that predispose to tuberculoma formation include high levels of CSF protein (>3g) and the presence of meningeal enhancement on contrast imaging [11, 12].



2. Manifestations of CNS Tuberculosis

CNS Tuberculosis occurs in 1% of Tuberculosis patients but has the most dire morbidity and mortality.

The wide spectrum of CNS TB includes the following:

2.1. Brain

1. TB meningitis
2. Tubercular encephalopathy
3. Vasculopathy- End arteritis
4. Hydrocephalus
5. Tuberculoma
6. Brain Abscess

2.2. Spinal Cord

1. Intramedullary tuberculoma
2. Spinal meningitis
3. Secondary injury to spinal cord in Potts spine

3. Clinical Syndromes associated with CNS tuberculosis

1. The meningitis syndrome
2. Hydrocephalus syndrome
3. Intracranial space occupying lesion syndrome including

a. Seizure phenomena

b. Focal Neurological deficits which can be due to infarcts or mass lesions

c. Focal neurological defects

4. The vasculitis syndrome resulting in infarcts.
5. Spinal Cord Syndromes

a. Intramedullary syndromes

b. Potts spine manifestations

c. Radicular syndromes associated witharachnoiditis

6. Visual impairment which can occur

   a. Secondary to increased icp

   b. Due to basal arachnoiditis

   c. Associated with optic nerve granulomas

   d. Adverse effect of ATT especially Ethambutol

4. Tubercular Meningitis

TB meningitis is the commonest form of CNS Tuberculosis. It accounts for 5% of extrapulmonary tuberculosis. TB meningitis affects children often and the age group less than one year is most vulnerable. In India, the estimated mortality attributed to CNS TB is 1.5/100,000.

4.1. Pathogenesis

Human infection is transmitted by the respiratory route. MTB is essentially and dominantly a lung residing pathogen. In the lungs the bacillus is phagocytosed by alveolar macrophages. Intracellular signalling pathways result in the formation of a phagocytic cup. In successful antibacterial defence the phagosome fuses with lysosomes to neutralise the invasive agent.

Macrophagic strategies to lyse bacteria include:

1. The induction of nitric oxide and reactive oxygen species. These kill bacteria by oxidising membrane lipids and fragmenting DNA.

2. Preventing intracellular phagocytosed bacteria from having access to nutrients like amino acids, fatty acids and iron.

3. Induction of antimicrobial peptides and cytokines.

4. Macrophages use autophagy for intrinsic cleansing of senescent organelles as well as of phagocytosed bacteria [12].

Approximately one third of the world population harbours a dormant tubercular infection. Around 10% of these may progress to active disease. This rate may be higher in children, at around 50% reflecting a higher vulnerability to disease progression.Tubercle bacilli which escape autophagic destruction disseminate by the hematogenous route. They seed end arterioles of the Brain or Meninges and mature into tubercular micro-abscesses. These micro-abscesses may burst and release contents into the subarachnoid space culminating in TB meningitis.In

the brain parenchyma they may evolve into a brain abscess or into a tuberculoma depending upon the immunologic response or the host and on the virulence of the pathogen (MTB). In the subarachnoid space TBM produces adhesions and exudates. The adhesions may result in radiculopathy syndromes or in blockage of CSF pathways and consequent hydrocephalus. The basal meninges and cisterns are commonly affected. Cranial nerve involvement often occurs especially affecting the 6th and 7th nerves. Hydrocephalus is relatively common and is attributable to basal/ cisternal adhesion as well as to the high protein content of the CSF. Obstructive hydrocephalus is less common and occurs due to tuberculomas blocking CSF pathways or due to adhesive ependymitis. Exudates may coalesce into cisternal tuberculomas in a process which may be augmented by antitubercular therapy. This enlargement or appearance of fresh mass lesions is described as a paradoxical reaction. Paradoxically enlarging tuberculomas may merit surgical excision if they are in critical locations or producing clinical syndromes by mass effect. In this scenario ATT is continued and corticosteroids are added to the regime. A meta-analysis of 7 randomised controlled trials concluded that corticosteroids are beneficial in HIV-ve patients with TB meningitis. There is no clear consensus on the administration of steroids to TBM patients who are HIV positive. [13]

4.2. Clinical features

The clinical features of Tb meningitis in adults includes headache, vomits and altered sensorium with or withoutfever. Children with TBM have headache less frequently, but have seizures along with nausea and vomits. HIV positive individuals with tuberculosis are more likely to develop TB meningitis, with an incidence of 10% versus the 2% incidence in non HIV individuals. CD 4 counts of <100 are associated with a higher incidence of CNS TB. The risk of CNS Tuberculosis however can flare up even in the early phase of HIV when the CD 4 counts are still normal as well as in the phase after initiation of ART when the counts are recovering.HIV patients with TBM have a distinct syndrome wherein the classical triad of fever, headache and meningeal signs is replaced by early alteration in sensorium along with other manifestations of HIV like lymph adenopathy and of other extrapulmonary tuberculosis manifestations (13). Other immune compromised states influencing the course of Tuberculosis include malnutrition, extremes of age, alcohol dependence, diabetes mellitus, therapeutic immunosuppression and Vit D deficiency.

The most serious complications od TB meningitis include hydrocephalus, stroke and tuberculoma formation. The incidence of TB meningitis and stroke is higher in children than in adults. Children may develop hemiplegia as a consequence of TB meningitis induced vasculitis and stroke [14].

4.3. CSF picture in TB meningitis

1. Lymphocyte predominance (60-400/mm3)

2. Increased protein level (0.8 to 4 gms/L)

3. Decreased glucose level (<50% of serum levels but not as low as in pyogenic meningitis)

4. Demonstration of MTB in CSF smear or culture

5. Antibody titres

6. Interferon gamma release assays include TB Gold and T- Spot TB and are based on the detection of interferon gamma in response to antigens in MTB.Serum interferon gamma levels are not affected by BCG. 17-45% of patients with TB meningitis have a positive response to Purified Protein Derivative.

4.4. Radiology in TB Meningitis

40-60% of patients with TB meningitis have abnormal chest X-Rays. Every patient with TB meningitis should undergo a contrast enhanced imaging within 48 hrs of initiation of ATT. CT and MR images may show evidence of cerebral ischemia or infarction, meningeal enhancement and of hydrocephalus. Characteristically we see: [15]

1. Enhancing exudate in basal cisterns
2. Ventriculomegaly
3. Basal Ganglia infarcts
4. Gyral enhancement pattern

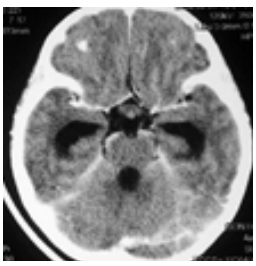


Figure 1: CECT showing enhancing basal exudates

4.5. Outcome

In about 50% of patients TB meningitis leads on to death or disability. Patients may develop a paradoxical worsening of their clinical state after initiation of ATT. It is recommended that steroids be used at initiation of ATT even in HIV positive patients. This ‘Paradoxical

reaction' is related to the hosts immune response. Steroids are commonly used. Other immunomodulatory drugs like Thalidomide also have a role in alleviation of this condition [16]. Biomarkers may be used as a marker of neurological injury in TBM. The markers which have been used include S 100 B, Neuron Specific Enolase and Glial Fibrillary Acidic Protein.

4.6. Grading severity of TB meningitis

TB meningitis is generally graded by the severity of neurological involvement. At the lower end of severity we have patients who are alert and oriented and without a neurological deficit. The next group is patients with a GCS ranging from 11 to 14 who may or may not have focal neurological deficits. The most serious patients have a GCS of 10 or less. The mortality rate in TB meningitis in patients who have an altered sensorium varies from 10.5 to 57.1% in various studies. In those who have a normal sensorium, the mortality figures quoted vary from 0 to 12.5%.

One of the validated grading systems of clinical severity in TB meningitis is the Vellore grading system described by Palur.

4.7. The Vellore/Palur Grading system

Grade 1- Patients with no deficits and a normal sensorium.

Grade 2- Patients with a normal sensorium who have focal neurological deficits.

Grade 3- Patients with an altered sensorium with or without focal deficits.

Grade 4- Moribund or deeply comatose patients.

Cranial nerve palsies occur in 20-30% of patients. The most commonly affected nerve is the 6th cranial nerve.

Visual loss in TB meningitis may be attributable to optochiasmatic arachnoiditis, by compression of the optic chiasm by third ventricular enlargement or by the occurrence and enlargement of tubercles in the optic nerves. Fundus examination may reveal papilloedema, single or multiple choroid tubercles may be present.

5. Intracisternal Tuberculomas

These form from Tubercular exudates in the cisterns and often occur paradoxically after initiation of treatment for TB meningitis.

6. Brain Tuberculomas

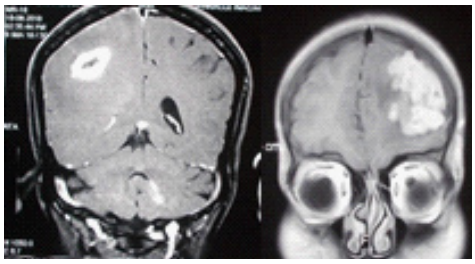


Figure 2: MRI scans showing supratentorial tuberculomas

CNS tuberculosis develops in approximately 1% of all patients with active Mycobacterium Tuberculosis infection.

6.1. Pathogenesis

Tuberculomas and TB meningitis both develop consequent to hematogenous dissemination of MTB in patients with Pulmonary Tuberculosis. Rarely there may be direct contamination from the paranasal sinuses. Hematogenous seeding results in the formation of a sub-pial or subependymal focus in the brain. These foci may rupture resulting in subarachnoid dissemination and TB meningitis. Tuberculomas generally form when the tubercular focus does not rupture, but is contained by the body's immune system. These tubercles however grow to produce mass lesions. Tuberculomas can also occur in patients with Tubercular meningitis. While intraparenchymal tuberculomas are the result of direct hematogenous seeding and establishment of a microfocus at a blocked arteriole level, basal and cisternal tuberculomas are secondary to TB meningitis. The incidence of Tuberculomas in TB meningitis is around 10%. Tuberculomas are multiple in about a third of patients [14]. Adult patients usually suffer supratentorial lesions while children have lesions in the posterior fossa.

In developed countries Tuberculomas are rare and constitute only 0.15% of ICSOL's. In the developing nations however 20 to 30% of ICSOL's may be tuberculomas. Tuberculomas can occur in any part of the brain. Brain tuberculomas which have a propensity to occur in the posterior fossa especially in children [14] are rarely a cause of obstructive hydrocephalus. In the supratentorial compartment there is a predilection for intra-axial tuberculomas to occur in the Frontal and Parietal lobes. Tuberculomas may present as intrinsic brain stem masses [15]. Isolated brain stem tuberculomas constitute only 5% of intracranial tuberculomas. Tubercular lesions may also occur in the subarachnoid, subdural or epidural spaces.

The spinal cord is another location where tuberculomas can occur [16]. They manifest like any other intramedullary spinal cord mass.Intramedullary tuberculomas constitute 2 in 1000 cases of CNS tuberculosis. The commonest site is the thoracic cord.

Sellar region tuberculomas comprise some of the rarer differential diagnoses of pituitary region tumours.

Intra-cisternal tuberculomas develop in patients with TB meningitis who have exudates in the basal cisterns. Surgical intervention may be required when there paradoxical growth after initiation of ATT.

6.2. Pathology

Pathologically a tuberculoma is a conglomerate mass of tissue made up of small tubercles. Tubercles are usually conglomerate. There is a central core of epitheloid cells (altered monocytes) surrounded by lymphocytes.

6.3. Radiology

6.1.1. CT scan features

Single or multiple low or high-density round or lobulated masses with irregular walls. Homogenous enhancement on contrast. Variable perilesional edema (depending upon age of lesion).'Target Sign' (17)- A spot of calcification surrounded by a ring of enhancement.

6.1.2. MRI features

Intracisternal tuberculomas appear as multiple coalescing contrast enhancing lesions. Intraparenchymal tuberculomas are usually hypo or hyperintense. They may have central hyperdense areas corresponding to caseous necrosis and may show ring enhancement.MR spectroscopy usually shows a lactate peak.

6.1.3. Clinical features

The clinical presentation of tuberculomas is with headache, seizures, focal neurological deficits or papilledema. The neurological deficits may be severe, with altered mental status and hemiplegia. Hydrocephalus may be present.

7. Hydrocephalus

Hydrocephalus is one of the commonest complications of TB meningitis.Hydrocephalus usually occurs in TBM after the initiation of ATT. The incidence of Hydrocephalus in children with TB meningitis is almost 90%. 12% of adults with TB meningitis develop hydrocephalus. The onset of symptoms is typically 4 to 6 weeks after the initiation of treatment. These patients benefit from early CSF diversion. Complications are however frequent.

7.1. Pathogenesis

Hydrocephalus in TBM may be purely obstructive,purely communicating or combined. The aetiology of hydrocephalus in TB meningitis is typically multifactorial. Thick gelatinous exudates develop, involving the cisternal spaces and the basal subarachnoid compartments.

This results in a communicating hydrocephalus. Blockage of the exit foramina of the fourth ventricle by exudatesor Leptomeningeal scarring produces a panventricular obstructive hydrocephalus. Occlusion of either Foramen of Monromay result in a monoventriculardilatation. The aqueduct of Sylvius may be occluded by tuberculomas or by adhesive ependymitisto produce triventricular obstructive hydrocephalus. Strangulation of the brainstem by basal exudates may also result in aqueductalblockage. Another possible contributary factor for hydrocephalus is the increase in CSF production consequent to inflammation of the ependyma and of the choroid plexus.

7.2. CT findings

These include periventricular lucency, basal exudates, infarcts and a rising Evan's ratio.

8. Calvarial Tuberculosis

Usually seen in children and may form collar stud abscesses. These respond to ATT.

9. Tubercular Brain Abscess

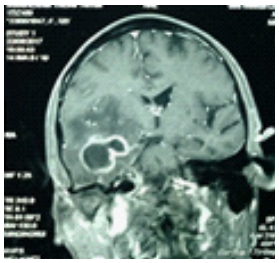


Figure 3: MRI scan showing right temporal TB abscess

Tubercular abscesses are often larger than tuberculomas and have a more acute presentation. Abscesses are more common in HIV afflicted individuals. While around 6% of HIV -ve patients with CNS tuberculosis have brain abscesses, nearly 20% of HIV positive patients suffering from CNS TB have brain abscesses.

Pathogenesis:

These abscesses are characterised by a thick wall with an encapsulated collection of pus. The abscess wall is generally thicker than in the case of pyogenic abscess. The pus can be demonstrated to contain viable tubercle bacilli. Histopathology reveals the absence of granuloma formation. The aetiopathological difference between Tuberculomas and Tubercular Brain abscess is the hosts immune response. HIV induced suppression of cell mediated immunity increases the chance of formation of a tubercularabscess.

10. Optochiasmatic Tuberculoma

This is a complication of TB meningitis [18].

11. Sellar Tuberculomas

Tuberculomas or tubercular abscesses may occur in the sellar region. The commoner causes of lesions in the sella include Pituitary Adenomas, Craniopharyngiomas and Rathkes cleft cysts. Colloid cysts, metastases and arachnoid cysts may also rarely occur.

12. Stroke in TB meningitis

Stroke in tuberculosis may occur due to vasculitis, vasospasm, endovascular thrombosis or by vascular entrapment in basal exudates. Early infarcts are believed to be due to spasm while later deficits represent vessel entrapment or thrombosis. Hemorrhagic infarctions are relatively rare but may occur.15-57% of patients with TB meningitis develop infarctions of which a significant majority are subclinical.

12.1. Pathogenesis

The commonest vessels involved are the Medial Striate, the Thalamotuberal and the Thalamostriate. The so called tubercular zone for infarcts includes the Caudate, the anterior Thalamus and the anterior limb and genu of the internal capsule. Major vessels may sometimes be involved to produce cortical stroke. The proximal MCA, ACA, PCA, Supraclinoidal ICA and Basilar may be involved in tubercular arteritis. Vessels may be embedded in exudates or stretched by hydrocephalus. Activation of cytokines including TNF alpha, VEGF and MMP's has been incriminated. Steroids (dexamethasone) and aspirin have not been conclusively shown to be of benefit in the alleviation of arteritis. However some beneficial effects have been described [18, 19]. Tubercular arachnoiditis and pachymeningitis is rare in children.

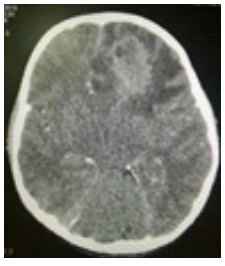


Figure 4: CECT showing left frontal tuberculoma with surrounding edema

In a setting of immune compromise the differential diagnosis of CNS mass lesions include other infections like Toxoplasmosis and malignancies like Lymphoma. The CSF picture also gets confounded. The HIV viral load and a declining CD4 T lymphocyte count correspond with a flare up or new onset CNS TB. Highly active antiretroviral therapy on the other hand decreases the risk of Tuberculosis exacerbations [20]. Patients who have undergone solid organ transplantation with the necessary post-transplant immunosuppression are also at a higher

risk of developing TB meningitis.

Drug interactions between Antitubercular drugs and HART (Highly active antiretroviral therapy) have to be kept in mind and the timing of initiation of HART and ATT need to be tailored to individual patients. The occurrence of IRIS (immune reconstitution inflammatory syndrome) will merit special treatment.

Therapeutic immunosuppression in solid organ transplant recipients and the use of immunomodulatory biologicals in rheumatoid arthritis may result in potentially catastrophic exacerbation of CNS TB.

14. Other sequelae

Although the disease is curable and complete eradication is possible with ATT the residual scaring of the CNS can produce various sequelae. Ventricular and ependymal involvement may cause obstructive hydrocephalus. Cortical scaring may lead to scar epilepsy.Rarely deep seated scaring in the thalamic region can lead to abnormal involuntary movements like dystonia.

15. Management of CNS Tuberculosis

15.1. Pharmacotherapy

Medical management of Tuberculomas include antiseizure medications, dexamethasone, other anti-oedema measures as appropriate and antitubercular drugs. INH, Rifampicin and Pyrazinamide have good CNS penetration and are bactericidal. Conventionally, SHRZ is administered. The total duration of therapy is 18 months. Short term intensive therapy regimes over six months have also been reported as successful. However intensive short course regimes have been described.

Drug resistance: The problem of emerging drug resistance, with Drug Resistant(DR or Resistant to one first line drug), Multidrug resistant (MDR or Resistant to INH and Rifampicin), Extensively drug resistant (XDR), Extremely drug resistant (XXDR), super XDR and Totally drug resistant (TDR) variants poses fresh and sinister challenges as does the entity of patient intolerance to ATT. Patient intolerance to first line ATT is often related to deranged liver functions.One of the established dictums is that a single new antitubercular drug should never be added to a failing regime of ATT. Drug resistance (MDR and XDR) may be considered in those with a prior history of tuberculosis, in those who have had exposure to drug resistant tuberculosis and in those with poor clinical response to ATT.

Paradoxical reaction: Patients with TB meningitis may manifest enlargement of existing lesions or the appearance of new ones while on ATT. This phenomenon, known as the ‘Paradoxical Reaction’ needs to be differentiated from treatment failure. Paradoxical reac-

tions occur in about a third of TB meningitis patients on ATT. Female gender, the existence of concomitant HIV and a relatively rapid onset of disease are all predictive of a higher risk of paradoxical progression. Paradoxical enlargement of tuberculomas responds to the addition of Dexamethasone to the ongoing antitubercular drug regime [21].Dexamethasone decreases mortality but does not affect neurological outcomes (21). Thalidomide may be considered in children with Tubercular abscesses and TB related optochiasmatic arachnoiditis.

15.2. Host Directed Therapies

Much of the damage in CNS tuberculosis is mediated by host responses. The relative immunologic impunity enjoyed by the bacillus is also a potential target for modulation. Tailored host genotype specific therapies have been designed to harness host responses effectively to control the bacillus and will be part of therapeutic strategies of the future. Drugs directed at the host may disrupt macrophage host signalling pathways, deprive the pathogen of nutrients, promote autophagy or activate antimicrobial killing mechanisms.

Drugs which have been proposed to modulate host responses include Vit D, Imantinib, Cyclic amp inhibitors like Cilostazol, Pentoxyphyline and Sildenafil, eicosanoids like Aspirin, Oxyphenbutazole and PGE2, Statins, Metformin and autophagy inducing drugs like Rapamycin. Drugs which augment host responses and those that mitigate the response of the host are both used. Amongst the advantages of host directed therapy is the action against resistant strain, synergy with antimicrobials and activity against non replicating MTB. Overall, the chances of disease recurrence are decreased.

Host directed therapies are important in the context of evolution of resistant strains.

There is some controversy on whether tubercle granuloma formation protects the host or the organism. The granulomas have macrophages and T and B lymphocytes with fibrous encapsulation. They may act as a mechanical and functional barrier to bacillary dissemination. On the other hand they may allow indolent bacilli to survive for prolonged periods. [20,21,22,23]

16. Role of Surgery

Tuberculosis therapy although mostly medical does require surgical intervention in some cases.

The indications for surgery are:

1. to control raised ICP rapidly
2. to relieve hydrocephalus

3. to resect mass lesions
4. to provide tissue diagnosis
5. In lesions recalcitrant to ATT and
6. in the spine to decompress neural elements and stabilise the spine.

16.1. Hydrocephalus

Hydrocephalus is the commonest cause of increased ICP in CNS tuberculosis. Increased intracranial tension in CNS Tuberculosis may also occur with mass producing tuberculomas which act as SOLs, with brain oedema which is potentially multifactorial. Cerebral infarctions which swell and venous thrombosis are other rarer causes.

Medical management of hydrocephalus in TBM with Dexamethasone, acetazolamide and frusemide along with repeated ventricular taps can help in alleviation and avoidance of surgery in 70% of cases of TBM

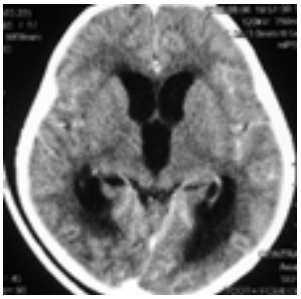


Figure 5: CT scan showing post TB meningitis hydrocephalus

16.2. CSF diversion procedures

Options for CSF diversion in hydrocephalus due to TBM include ventriculoperitoneal shunts, Thecoperitoneal shunts and Endoscopic Third Ventriculostomy.

VP shunts in TBM have a higher than expected incidence of complications [24,25,26,27]. Repeated lumbar punctures and External ventricular drain placement have been suggested as options to try and avoid a shunt [24,25,26,27]. In patients with poor grades, a trial of external ventricular drainage and assessment of benefit will help in the decision to place a permanent shunt.

16.3. Ventriculoperitoneal Shunts

Shunts in TB meningitis are prone to blockage by the high protein content and because of exudates encrusting the catheter tip. The fear of dissemination of Tuberculosis as a consequence of VP shunting is probably unfounded [28].

16.4. Endoscopic Third Ventriculostomy (ETV)

ETV is typically performed through a single burr hole in line with the Foramen of Monro and the Interpeduncular cistern.Fenestration is between the mamillary bodies and the infundibular recess at a point anterior to the basilar artery where the floor is thin and transparent. The stoma is usually enlarged with a Fogarty catheter. A Lillequist membrane, if present, should also be fenestrated.Endoscopic Third Ventriculostomy has been proposed as an option [28]. However, the Third Ventricular floor is often thick and opaque especially in the acute phase of TBM posing technical challenges in safe fenestration of the ventricular floor. An opaque ventricular floor markedly increases the risk of catastrophic vascular injury.The potential for vascular injury to the Basilar artery or its branches is only partially addressed by the use of intraoperative doppler or by neuro-navigation. The ideal patients for ETV are those in the chronic phase of TB meningitis who are well nourished and with a thin and transparent third ventricular floor. Malnourished patients in the acute phase, with cisternal exudatesand a thick opaque ventricular floor may be considered for ventriculoperitoneal shunting. It is often difficult to evaluate stoma patency after ventricular floor fenestration. Resolution of periventricular lucency, widening of subarachnoid spaces and a decrease in the ventricular size are all features of a patent diversion. However, a delayed absorption of CSF in these patients due to arachnoidal thickening may result in a relatively slow or tardy response. Demonstration of a flow void across the ventricular floor in MR ventriculography is conclusive evidence [28]. Ventriculography however is invasive. CISS MRI is also sensitive to CSF flow. Cine phase contrast MRI is the most commonly used modality for demonstrating fenestration patency [24,25,26,27,28,29]. In most of our patients we therefore prefer to use a the classical ventriculo-peritoneal shunts.

16.5. Theco-peritoneal shunt

Theco-peritoneal shunting is an option in treating communicating variety of hydrocephalus. A combination of ETV with a theco-peritoneal shunt is sometimes used in patients with obstructive hydrocephalus in TBM. In infants, choroid plexus coagulation may be used as an additional and complementary surgical modality [24,25,26,27].

16.6. Management of Tuberculomas and Tuberculous abscesses

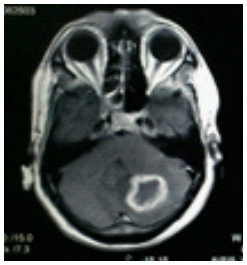


Figure 6: MRI showing tuberculoma of the left cerebellar hemisphere

Tuberculomas need surgery when they are large and cause mass effect on the underlying normal brain. The lesion with the usually extensive perilesional edema can cause significant midline shift leading to altered sensorium and impending herniation. Lesions in the cerebellar hemispheres should have a lower threshold for surgery if they cause mass effect on the fourth ventricle and are significant in size. Tubercular abscesses are managed by aspiration and excision when feasible along with antitubercular treatment. Complete excision is not a goal and indeed is impossible in eloquent areas. However partial or sub-total resection to relieve mass effect and adequate ATT usually give good results.

16.7. Management of Optochiasmatic arachnoiditis

This usually occurs in patients who have tubercular exudates in the basal cisterns. These patients may have paradoxical vision loss after initiation of antitubercular therapy regimes. Some of these patients benefit from surgical intervention [30,31,32].

16.8. Spinal Tuberculosis

Spinal Tuberculosis includes both the spectrum of spino-osseous Tuberculosis which produces clinical syndromes by spinal collapse and spinal cord or cauda equina compression and tuberculosis which affects the neural structures directly.

The clinical syndromes in spino-osseous TB are outside the purview of CNS tuberculosis.

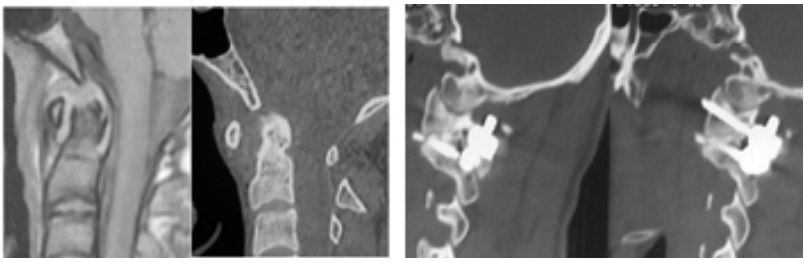


Figure 7: Atlanto axial TB and surgical treatment with C1 C2 fusion

The neurological syndromes consequent to vertebral involvement however are often devastating often producing mixed syndromes of myelo radicular compromise. Vertebral tuberculosis which affects the disc space and contiguous end plates initially often respond to rest and ATT. However structural instability with imminent collapse and consequent neurological devastation merits surgical stabilisation which has a high degree of success.

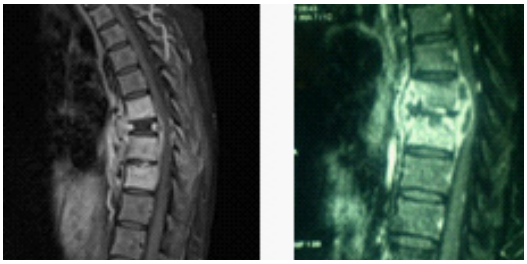


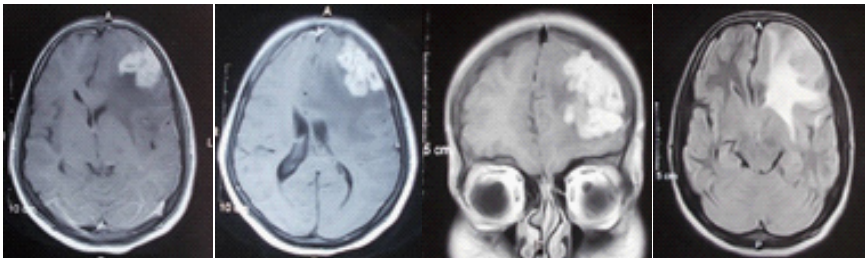
Figure 8: MRI showing TB of dorsal spine. Destruction of vertebra and kyphosis with para vertebral abscess

Spinal intradural tuberculomas including intramedullary and intradural extramedullary variants account for 2.5% of all CNS TB. Spinal intramedullary tuberculomas are usually solitary but may occasionally be multiple.Dorsal involvement is most common. However cservical lesions have been well described [33,34] The commonest presentations are spastic or flaccid paraplegia along with bladder and bowel involvement. Differential diagnoses include astrocytomas, ependymomas and hemangioblastomas of the spinal cord. The MRI picture in spinal intramedullary tuberculomas varies depending on the age of the lesion. They appear hypo to isointense on T1 weighted images and hyperintense on T2 weighted images. The hyperintensity on T2 weighted sequences becomes duller in mature tuberculomas as the cellularity increases. Liquefaction of the core adds brightness on T2 sequences. A target sign [35] with sharp borders is highly suggestive. Post gadolinium there is ring like or nodular enhancement. Around a quarter of healed tuberculomas show evidence of calcification.

Spinal intramedullary tuberculomas are well circumscribed and amenable to surgical excision [33]. Antitubercular treatment along with steroids is given to all patients whether or not they are subject to surgery.

Case discussion 1

This relates to a 10 year old girl with left frontal tuberculoma. The patient had progressively worsening headaches which progressed to deficits. She became hemiparetic on the right side and had features of raised ICP on examination as in presence of papilloedema. Her MRI of the brain showed a large conglomerate lesion in the left frontal region with edema and mass effect suggestive of TB. She underwent surgical excision of the lesion in view of progressively increased ICP and made excellent recovery with ATT.

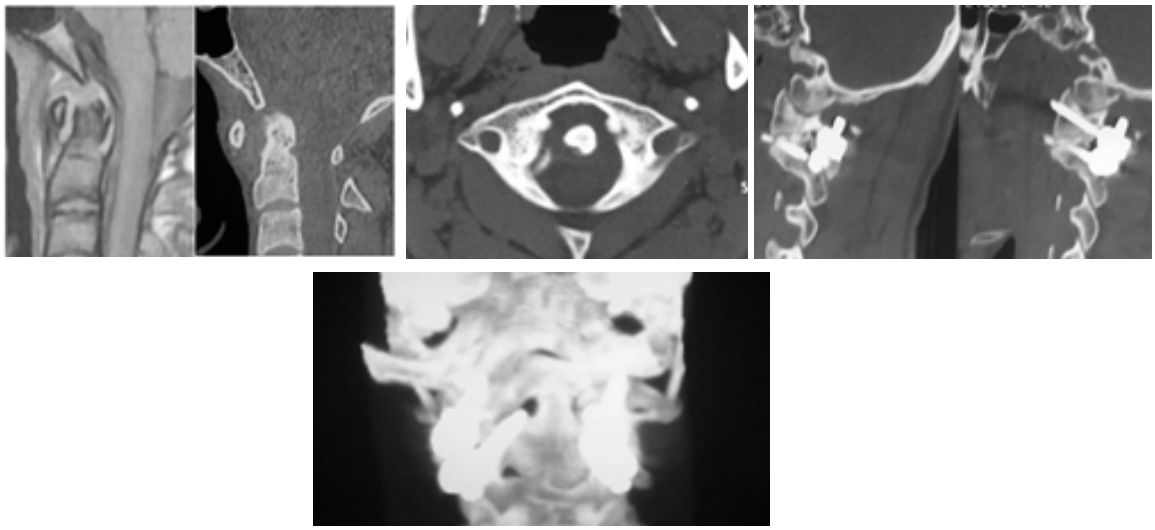


MRI scans showing the tuberculoma and adjoining extensive edema.

Post op image of the patient showing complete recovery with no neurological deficits (The Surgery was performed by the Fisrt Author)

Case discussion 2

This is a case of a young gentle man with neck pain who became increasingly weak leading to quadriparesis. Imaging revealed an atlanto axial dislocation with increased ADI. MRI revealed contrast enhancement suggestive of granulation along the odontoid and the C1C2 joints. There was cord compression on account of the AAD and inflammatory mass. The patient was operated and C1C2 lateral mass fixation done with decompression. With ATT subsequently, the patient made good recovery.



Imaging showing the AAD with MRI evidence of TB granulation around the odontoid. Post op scans showing the lateral mass screws and 3d recon. (The Surgery was performed by the Fisrt Author)

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Diagnosis and Management of Tuberculosis

Chapter 6

Diagnosis of Tuberculosis

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1. Introduction

Tuberculosis (TB) laboratories play a critical part in national TB programmes, providing clinicians with invaluable information that is used to diagnose and guide the care of patients. Because of the specialized nature of the different technical procedures needed to diagnose TB, and the need for quality assurance and effective laboratory management, TB control programmes require a tiered network of laboratories in which different tiers use complementary diagnostic tools and mechanisms for referring specimens [1].

Diagnostic capacity continues to be a major bottleneck in TB control, including scaling up management and control efforts to tackle drug resistant TB and TB associated with HIV. An unprecedented effort to improve and expand the capacity of TB laboratories is under way, coordinated by the World Health Organization's (WHO's) Global TB Programme and with the active involvement of the Global Laboratory Initiative (GLI), a working group of the Stop TB Partnership [1].

WHO's global strategy for TB prevention, care and control for 2015–2035 (known as the End TB Strategy) prioritizes the early diagnosis of TB, which should include the universal availability of DST, and systematic screening of contacts and high-risk groups [1,2]. Therefore, all national TB control programmes should prioritize the development of a robust network of TB laboratories that have adequate biosafety standards, use modern methods of diagnosis, use standard operating procedures (SOPs) and appropriate quality assurance processes, and that have qualified and sufficient human resources; these priorities should be comprehensively addressed in national strategic plans [1].

Overall, the development landscape for TB diagnostics is promising: many different organizations are developing products, and there is a robust pipeline of technologies. The

range of technologies that may replace sputum-smear microscopy continues to expand, and smaller, simpler and more robust products are expected to become available in the coming years. Several technologies aim to deliver results in less than 1 hour, including DST results; this should improve the time to treatment, enable point-of-care testing programmes and provide greater access to DST [1,3].

Until these new technologies become available, the use of existing WHO-recommended diagnostic techniques must be accelerated and strengthened. This will require ensuring that laboratories have adequate infrastructure and human-resources capacity. Additionally, there must be clear country-level policies on using these recommended tests in the most effective screening and diagnostic algorithms, depending on each country's specific epidemiology and resources [1].

2. Tuberculosis Diagnostic Tools/Methods

2.1. WHO's recommended techniques for diagnosing TB

2.1.1. Microscopy

Mycobacteria can be visually distinguished from other microorganisms by their thick lipid containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (known as 'acid fastness'). Given that the examination of two sputum specimens is adequate to identify the majority (95-98%) of smear-positive TB patients, WHO's current policy on case-finding using microscopy recommends that in settings with appropriate external quality assessment and documented good-quality microscopy two specimens should be examined. In settings with appropriate quality assurance procedures, a case is defined as someone with one positive smear – that is, at least 1 acid-fast bacillus in at least 100 microscopic fields [4].

In 2010, WHO confirmed the diagnostic accuracy of examining two consecutive smears on the same day to diagnose TB, so that treatment can be started during the patient's first visit to a health-care facility [5].

Advantages

- Microscopy of sputum smears is simple and inexpensive, and allows rapid detection of the most infectious cases of pulmonary TB. Sputum specimens from patients with pulmonary TB, especially those with cavitary disease, often contain sufficiently large numbers of AFB to be detected by microscopy.
- Microscopy is suitable for peripheral-level and higher-level laboratories.
- Microscopy can be done safely in a laboratory that has implemented only a low level of

precautions to mitigate the risk of laboratory acquired TB infection.

- It is a simple, rapid and inexpensive test and is necessary for treatment follow up of patients with susceptible TB.

Disadvantages

- Direct sputum-smear microscopy is relatively insensitive: at least 5000 bacilli per ml of sputum are required for a positive result. The sensitivity is further reduced in patients with extrapulmonary TB, children and in those who are co infected with HIV.

- A comprehensive quality assurance programme is necessary; although this may be challenging to implement, it is necessary to ensure high-quality test results.

Limitations

- Microscopy for AFB cannot distinguish Mycobacterium tuberculosis complex from non tuberculous mycobacteria; it cannot distinguish viable from nonviable organisms; and it cannot distinguish drug-susceptible strains from drug-resistant strains.

- Smears that have been stained with auramine will need to be stained again if they are to be rechecked as part of an external quality assessment programme.

1. ***Conventional light microscopy***

Direct Ziehl–Neelsen staining of sputum specimens and examination using light microscopy is suitable for use at all levels of laboratory, including peripheral laboratories at primary health-care centres or district hospitals. There is insufficient evidence that processed sputum specimens (for example, those that are concentrated or chemically treated) give better results than direct smear microscopy. Therefore, the use of such methods is not recommended [6].

The number of Ziehl–Neelsen smears examined by 1 microscopist each day should not exceed

20–25 because visual fatigue can occur and lead to a deterioration in quality.

In general, it is sufficient for there to be 1 centre using Ziehl–Neelsen staining and light microscopy per 100 000 population;[7] however, if services are expanded, then it is important to consider the location of the centre, the workload of the technicians, the accessibility of the centre to the population as well as the effectiveness of specimen transportation.

2. *Light-emitting diode fluorescence microscopy*

Light-emitting diodes (LEDs) provide a relatively inexpensive light source for fluorescence microscopy. LED microscopes or attachments require less power than conventional fluorescence microscopes and can run on batteries. Also, the bulbs have a long half-life and do not release potentially toxic products if they are broken.

Evidence shows that the diagnostic accuracy of LED microscopy is comparable to that of conventional fluorescence microscopy and it surpasses that of conventional Ziehl–Neelsen microscopy (by an average of 10%). Therefore, WHO recommends replacing conventional fluorescence microscopy with LED microscopy, and that LED microscopy should be phased in as an alternative to conventional Ziehl–Neelsen light microscopy in all settings, prioritizing high-volume laboratories [8].

2.1.2. Culture and species identification

Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually (that is, by identifying specific characteristics) or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC).

The *M. tuberculosis* complex comprises eight distinct closely related organisms, the most common and important agent of human disease is *M. tuberculosis*. The complex includes *M. bovis* (the bovine tubercle bacillus—characteristically resistant to pyrazinamide, once an important cause of TB transmitted by unpasteurized milk, and currently the cause of a small percentage of human cases worldwide), *M. caprae* (related to M. bovis), *M. africanum* (isolated from cases in West, Central, and East Africa), *M. microti* (the "vole" bacillus, a less virulent and rarely encountered organism), *M. pinnipedii* (a bacillus infecting seals and sea lions in the Southern Hemisphere and recently isolated from humans), *M. mungi* (isolated from banded mongooses in southern Africa), *M. orygis* (described recently in oryxes and other Bovidae in Africa and Asia and a potential cause of infection in humans), and *M. canetti* (a rare isolate from East African cases that produces unusual smooth colonies on solid media and is considered closely related to a supposed progenitor type) [9].

Differentiation of the members of the MTBC is necessary for the treatment of individual patients and for epidemiological purposes, especially in areas of the world where tuberculosis has reached epidemic proportions or wherever the transmission of *M. bovis* between animals or animal products and humans is a problem. In addition, it can be important to rapidly identify isolates of *M. bovis* bacillus Calmette-Guérin (BCG) recovered from immune compromised patients. Differentiation of species with the MTBC can be achieved using either phenotypic [10] and/ or genotypic methods [11].

The use of rapid immune chromatographic assays (or strip tests for speciation) to identify cultured isolates is recommended because they provide definitive identification of all members of the MTBC (including *M. bovis*) in 15 minutes [12].

Much remains to be understood about the pathogenesis of non-tuberculosis mycobacteria (NTM) infection and disease in humans. There is no evidence to suggest either animal-to-human or human-to-human transmission of NTM and it is assumed that most persons are infected by NTM from the environment. NTM may cause both asymptomatic infection and symptomatic disease in humans. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood.

Awareness of the context from which an NTM isolate is obtained can be critically important in determining the need for speciation of that isolate.

Hence, communication between the clinician and laboratorian is essential for determining the importance and extent of identification and for drug susceptibility testing of an NTM isolate. Differentiation of NTM species can be achieved busing a variety of phenotypic or genotypic Methods [13]. Conventional solid or liquid culture is required to monitor the treatment of patients with MDR-TB.

Advantages

- Culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB as well as significantly increasing in the number of cases identified when compared with microscopy: there is often an increase of 30–50%.

- Culture also provides the necessary isolates for conventional DST.

Disadvantages

- Culture is more complex and expensive than microscopy; it also takes longer, requiring facilities for preparing media, processing specimens and encouraging the growth of organisms.

- Culture also requires specific laboratory equipment, technicians with additional skills, and appropriate bio safety conditions.

Limitations

- Specimens must be decontaminated before culture to prevent overgrowth by other microorganisms. To some extent, all decontamination methods are also harmful to mycobacteria;

therefore, culture is not 100% sensitive.

- Good laboratory practices must maintain a delicate balance between the yield of mycobacteria and contamination by other microorganisms.

- Solid and liquid culture methods are suitable for central reference laboratories (regional laboratories in large countries) or intermediate level laboratories. Solid culture methods are less expensive than liquid, but the results are invariably delayed because of the slow growth of mycobacteria. Liquid culture increases the case yield by approximately 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks. However, liquid systems are more prone to contamination, and the manipulation of large volumes of infectious material mandates appropriate additional bio safety measures [14].

2.1.3. Drug-susceptibility testing

DST determines whether a strain is susceptible to particular anti-TB agents: a result indicating that the strain is sensitive to particular agents means that treatment with those agents will most likely be successful; a result indicating that a strain is resistant means that there is a high possibility that treatment with those agents will fail and, therefore, other agents should be used. Thus, using standardized and reliable DST for *M. tuberculosis* provides guidance on treating a patient.

Techniques of drug susceptibility testing

- **Phenotypic methods** involve culturing *M. tuberculosis* in the presence of anti-TB agents to detect growth (which indicates resistance) or inhibition of growth (which indicates susceptibility).

- **Genotypic methods** target specific molecular mutations associated with resistance against individual anti-TB agents. Phenotypic DST methods are performed as direct or indirect tests in solid or liquid media.

Direct testing involves inoculating drug-containing and drug-free media directly with a concentrated specimen.

Indirect testing involves inoculating drug-containing media with a pure culture grown from the original specimen.

Indirect phenotypic tests have been extensively validated. Three methods are commonly used: the proportion, absolute concentration and resistance ratio methods. For first-line anti-TB agents, the results obtained do not differ significantly among the three methods. Liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for conventional solid media. Because liquid culture systems have increased

sensitivity and reduce delays in diagnosis, they may contribute significantly to improving patient management [14].

WHO recommends that formal links be established between the TB Supranational Reference Laboratory (SRL) Network and national reference laboratories to ensure that DST is available for both first-line and second-line anti-TB agents. Countries wishing to offer DST should seek advice from the TB Supranational Reference Laboratory Network to ensure they have continual, adequate expert input into the requirements for laboratory design, the transportation of specimens, processes, bio safety standards, SOPs, schedules for maintaining equipment, and processes for external quality assessment. The absence of capacity to treat patients with MDR-TB should not deter countries to build capacity for DST, as it is ethically justified [15].

Advantages

DST provides a definitive diagnosis of drug resistant TB.

Disadvantages

- Non-molecular DST methods take longer to provide results.
- These methods are suitable for use only at the central reference laboratory level, given the need for appropriate laboratory infrastructure (particularly bio safety precautions) and the technical complexity of the techniques and methods.
- Liquid DST fails to detect some clinically relevant "borderline rifampicin resistant strains" with *rpo*B mutations [16].

Limitations

- The accuracy of phenotypic DST varies according to the anti-TB agent being tested.

1) Drug-susceptibility testing for first-line anti-TB agents

DST is most accurate in detecting susceptibility to rifampicin and isoniazid; results are less reliable and reproducible for streptomycin, ethambutol and pyrazinamide.

At a minimum, national TB-control programmes should establish sufficient laboratory capacity to detect rifampicin-resistant TB (RR-TB) or MDR-TB (MDR-TB is TB that is resistant to at least isoniazid and rifampicin). In many settings and for many groups of patients, rifampicin resistance is a valid indicator of or proxy for MDR-TB. Persons at risk for MDR-TB should be targeted as a priority for rapid DST. Phenotypic culture-based DST methods, using the critical concentrations recommended by WHO in the updated table, are the current

reference standards for rifampicin resistance [17].

However, a number of recent studies have raised concerns about using phenotypic DST to detect rifampicin resistance, in particular the automated liquid system [18]. If rifampicin resistance has been detected, DST for resistance to isoniazid and second-line anti-TB agents should be performed, following WHO's recommendations [19]. WHO will be updating policy recommendations on DST in 2016.

2) Drug-susceptibility testing for second line anti-TB agents

Commercial liquid methods and the proportion method used on solid media have been studied; methods for the absolute concentration or resistance ratio methods on solid media for second line anti-TB agents have not been validated.

The recommended gold standard for DST for second-line anti-TB agents is the automated liquid system [20]. Routine DST for second-line agents is not recommended unless laboratory infrastructure and capacity have been established, rigorous quality assurance is in place and sustained proficiency has been demonstrated [21].

Phenotypic DST for second-line injectable agents (kanamycin, amikacin, capreomycin) and fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin) is generally reliable and reproducible across various settings [21]. The susceptibility of *M. tuberculosis* to all fluoroquinolones used by a national TB programmes should be tested to guide the choice of the most appropriate agent for treatment.

Current molecular methods cannot replace phenotypic DST for second-line agents because there is incomplete cross-resistance among second-line injectable agents. Current molecular methods cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens [22].

Routine DST for other second-line agents (such as ethionamide, prothionamide, cycloserine, terizidone, *p*-aminosalicylic acid, clofazimine, amoxicillin/clavulanic acid, clarithromycin and linezolid) is not recommended because the reliability and reproducibility tests for these anti-TB agents cannot be guaranteed. The WHO SRL network is currently developing and validating DST methods for the new and re-purposed second-line agents (bedaquiline, delamanid, clofazimine, linezolid).

3) Non-commercial methods

Non-commercial methods of culture and DST are less expensive than commercial systems but are prone to errors due to a lack of standardization and to local variations in the methods.

The performance of these methods is highly operator-dependent; therefore, it is imperative that good laboratory practices are followed, good microbiological techniques are used, and there is adequate quality assurance, supported by adequate training. Similar to the conditions needed with commercial systems, noncommercial systems require the implementation and enforcement of stringent laboratory protocols, SOPs and internal quality controls.

The evidence base for selected non-commercial methods of culture and DST has been reviewed by WHO, and the performance of these methods has been found to be acceptable *in reference or national laboratories in selected settings only when stringent laboratory protocols are followed* [23].The methods evaluated include the microscopic observation drug-susceptibility (MODS) assay, colorimetric redox indicator (CRI) methods, and the nitrate reductase assay (NRA).

The recommendations for their use are listed below.

- **MODS** is a microcolony method that uses liquid culture. Drug-free and drug containing media are inoculated, and this is followed by microscopic examination of early growth. MODS is recommended as a direct or indirect test for rapid screening of patients suspected of having MDR-TB.

- **CRI** methods are indirect methods. A coloured indicator is added to liquid culture medium on microtitre plate after *M. tuberculosis* strains have been exposed to anti-TB agents in vitro. Resistance is detected by a change in the colour of the indicator, which is proportional to the number of viable mycobacteria in the medium. CRI methods are recommended for use as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDRTB; however, the method is slower in detecting MDR-TB than conventional DST methods using commercial liquid culture and molecular LPAs, but it is less expensive.

- **NRAs** can be used as direct or indirect methods on solid culture. NRAs are based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a colour reaction. NRAs are recommended for use as direct or indirect tests to screen patients suspected of having MDR-TB; however, indirect NRA is not faster in detecting MDR-TB than conventional DST using solid culture.

- Both commercial and non-commercial culture and DST systems and methods are suitable for use only by central or regional reference laboratories. Non-commercial methods are recommended for use only as an interim option while capacity is being developed for rapid genotypic DST. Furthermore, non-commercial methods have not been validated for use with second line agents.

2.1.4. Molecular testing

Genotypic methods have considerable advantages when the programmatic management of drug-resistant TB is being scaled up, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for biosafety. The ultimate aim should be to use molecular assays – including LPAs, Xpert MTB/RIF, and any other molecular platform that may be recommended by WHO in the future – for rapid first-step identification of RRTB and MDR-TB.

Line-probe assays

Performing an LPA involves extracting DNA from *M. tuberculosis* isolates or directly from clinical specimens and using polymerase chain reaction **(PCR)** to amplify the resistance-determining region of the *rpo*B gene using biotinylated primers. Subsequently, labelled PCR products are hybridized with specific oligonucleotide probes immobilized on a strip. Colorimetric development of the captured and labelled hybrids enables the presence of *M. tuberculosis* complex to be detected as well as the presence of wildtype *M. tuberculosis*. It also detects mutations associated with drug resistance. If a mutation is present in one of the target regions, the amplicon will not hybridize with the relevant probe.

Therefore, mutations are detected by a lack of binding to wild-type probes as well as by binding to specific probes for the most commonly occurring mutations. The post-hybridization reaction leads to the development of coloured bands on the strip at the site of probe binding, and it can be read by the laboratory technician [23]. In 2015, WHO plans to update the policy recommendations on LPA for the detection of rifampicin resistance conferring mutations as well as utility of LPA in detection resistance to fluoroquinolones (FQ) and second-line injectable anti-TB agents.

Advantages

- Molecular LPAs enable rapid detection (in less than 48 hours) of resistance to rifampicin (alone or in combination with resistance to isoniazid); they were endorsed by WHO in 2008, and WHO has provided detailed policy guidance on introducing them at the country level [23].

- LPAs are a high throughput technology, allowing up to 48 specimens to be processed simultaneously and enabling several batches of tests to be done each day.

Disadvantages

- LPAs do not eliminate the need for conventional culture and DST.

- Available LPAs are recommended for use only on smear-positive sputum specimens and

isolates of *M. tuberculosis*.

• Current LPAs cannot replace phenotypic DST for second-line anti-TB agents. There is incomplete cross-resistance among second line injectable agents. LPAs cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens [20].

Limitations

• LPAs are suitable for use at the central or national reference laboratory level; they have the potential to be used at the regional level if the appropriate infrastructure can be ensured (three separate rooms are required).

• The sensitivity of LPAs to detect resistance to isoniazid is lower (approximately 85%) than that of culture methods.

Xpert MTB/RIF assay

The Xpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multi disease Gene Xpert platform. The Xpert MTB/RIF assay is performed directly on sputum, processed sputum sediment and selected extrapulmonary specimens from adults and children. GeneXpert instruments are modular, and options include systems with the capacity to have 1, 2, 4, 16, 48 or 80 independently functioning modules. The technology was first recommended by WHO in 2010, and a policy update was issued in 2013 following the meeting of an expert group to assess its use for detecting pulmonary and extrapulmonary TB and rifampicin resistance in adults and children [18, 24]. The "how to" Xpert MTB/RIF implementation manual was updated in 2014; it describes the operational aspects of and practical considerations associated with introducing and using the system.

Advantages

• The Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and rifampicin resistance in less than 2 hours.

• The sensitivity of the Xpert MTB/RIF assay for detecting TB is similar to that of to liquid culture (sensitivity, 88% when compared with liquid culture as a reference standard); the specificity is also high (99%).

• For smear-negative culture-positive TB, the pooled sensitivity of Xpert MTB/RIF has been found to be 68% [24]. The superior performance of Xpert MTB/RIF in detecting TB over that of microscopy makes it a particularly useful tool for case-finding among people living with HIV. As a tool for detecting rifampicin resistance, Xpert MTB/ RIF has a sensitivity of 95% and specificity of 98% when compared with phenotypic reference standards.

- The biosafety precautions required for Xpert MTB/RIF are similar to those for smear microscopy, and the training is minimal, which allows the technology to be used at relatively low levels in a laboratory network.

Disadvantages

- A stable uninterruptable electrical supply is needed; in settings where extended power outages may occur, uninterrupted power devices (UPS) and/or additional batteries may be needed to provide up to 2 hours of power.

- The ambient operating temperature of the instrument cannot exceed 30 °C, and cartridges must be stored at less than 28 °C.

- The shelf-life of the cartridges must be monitored to prevent them from expiring before they are used; thus, careful planning and management of supplies are essential.

- Security measures must be put in place to prevent the theft of the accompanying laptop or desktop computer.

- Limitations

- The modules require annual calibration; if modules fail the calibration test, using a specific calibration cartridge, they must be exchanged, which entails the importation of additional modules and exportation of the faulty modules.

- The use of Xpert MTB/RIF does not eliminate the need for conventional microscopy, culture and DST, which are required to monitor the progress of treatment and to detect resistance to anti-TB agents other than rifampicin.

- In patients who are not at risk for drug resistance but who initially test positive for rifampicin resistance by Xpert MTB/RIF, a second Xpert MTB/RIF test should be performed to control for preanalytical and postanalytical errors, and to improve the clinician's confidence in the diagnosis [19, 24].

- An increasing amount of evidence has shown that the infrequent occurrence of false-positive results may be linked to the detection by Xpert MTB/RIF of strains that are truly resistant to rifampicin but for which resistance is not detected by phenotypic culture-based DST, which is the present reference standard. Such strains appear to have clinically relevant mutations in the region conferring resistance to rifampicin, causing disease for which first-line treatment is likely to fail. In cases where discordant results are obtained from Xpert MTB/RIF and phenotypic DST or LPA, the culture isolate should be referred to a reference laboratory for DNA sequencing; while awaiting the results, a clinical decision should be made whether to continue the MDR-TB regimen [24].

2.1.5. Testing for latent TB infection

Persons with latent TB infection (LTBI) do not have active TB disease but may develop it in the near or remote future, a process called TB reactivation [25].

The lifetime risk of TB reactivation for a person with documented LTBI is estimated to be 5–10%, with the majority developing TB disease within the first five years after initial infection [26, 27]. A direct measurement tool for *M. tuberculosis* infection in humans is currently unavailable; hence, there is no gold standard for the diagnosis of LTBI. The tuberculin skin test (TST) and Interferon gamma release assays (IGRAs) indirectly measure

TB infection by detecting memory T-cell response signifying the presence of host sensitization to *M. tuberculosis* antigens. WHO recommends that either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100 000 population. IGRA should not replace TST in low-income and other middle-income countries.

Advantages

- IGRAs require a single patient visit; results are available in 24-48 hours, and prior BCG vaccination does not cause false positive results.

- TST is widely used, not expensive, and does not require any special laboratory infrastructure or supplies.

Disadvantages

- TST requires two patient visits, results are available in 48-72 hours, and requires an injection into the skin, and adequately trained staff. Moreover, it has poor specificity in BCG-vaccinated populations, cross-reactivity with non-tuberculous mycobacteria and poor sensitivity in immune compromised persons.

- IGRAs are expensive, require blood to be drawn, special laboratory infrastructure and supplies, and adequately trained staff. Given comparable performance but increased cost, replacing TST by IGRAs as a public health intervention in resource constrained settings is not recommended.

Limitations

- IGRAs and the TST cannot accurately predict the risk of infected individuals developing active TB disease.

- Neither IGRAs nor the TST should be used for the diagnosis of active TB disease.

2.2. Techniques not recommended by WHO for the diagnosis of active TB

2.1.1. Commercial sero diagnostic tests for diagnosis of active TB disease

It is strongly recommended that commercial sero diagnostic tests not be used for the diagnosis of pulmonary and extra-pulmonary TB. Currently available commercial sero diagnostic tests (also referred to as serological tests) provide inconsistent and imprecise findings. There is no evidence that existing commercial serological assays improve patient outcomes, and high proportions of false positive and false-negative results may have an adverse impact on the health of patients [28].

2.1.2. IGRA for diagnosis of active TB disease

There is no consistent evidence that IGRAs are more sensitive than TST for diagnosis of active TB disease. Studies evaluating the incremental value of IGRAs to conventional microbiological tests show no meaningful contribution of IGRAs to the diagnosis of active TB. IGRAs are considered inadequate as rule-out or rule-in tests for active TB, especially in the context of HIV infection. IGRAs should not be used for the diagnosis of active TB disease.

2.3. CDC's tuberculosis testing and diagnosis

2.3.1. Testing

There are two kinds of tests that are used to detect TB bacteria in the body:

1. TB skin test (TST)

2. TB blood tests.

A positive TB skin test or TB blood test only tells that a person has been infected with TB bacteria. It does not tell whether the person has latent TB infection (LTBI) or has progressed to TB disease. Other tests, such as a chest x-ray and a sample of sputum, are needed to see whether the person has TB disease.

2.3.2. Diagnosis

If a person is found to be infected with TB bacteria, other tests are needed to see if the person has latent TB infection or TB disease.

Who should be tested

1. Certain people should be tested for TB infection because they are at higher risk for being infected with TB bacteria, including:

2. People who have spent time with someone who has TB disease

3. People from a country where TB disease is common (most countries in Latin America, the Caribbean, Africa, Asia, Eastern Europe, and Russia)

4. People who live or work in high-risk settings (for example: correctional facilities, long-term care facilities or nursing homes, and homeless shelters)

5. Health-care workers who care for patients at increased risk for TB disease

6. Infants, children and adolescents exposed to adults who are at increased risk for latent tuberculosis infection or TB disease

7. Many people who have latent TB infection never develop TB disease. But some people who have latent TB infection are more likely to develop TB disease than others. Those at high risk for developing TB disease include:

8. People with HIV infection

9. People who became infected with TB bacteria in the last 2 years

10. Babies and young children

11. People who inject illegal drugs

12. People who are sick with other diseases that weaken the immune system

13. Elderly people

14. People who were not treated correctly for TB in the past

15. TB tests are generally not needed for people with a low risk of infection with TB bacteria.

Testing for TB Infection

There are two types of tests for TB infection: the TB skin test and the TB blood test. A person's health care provider should choose which TB test to use. Factors in selecting which test to use include the reason for testing, test availability, and cost. Generally, it is not recommended to test a person with both a TB skin test and a TB blood test.

TB skin test

The TB skin test is also called the Mantoux tuberculin skin test (TST). A TB skin test requires two visits with a health care provider. On the first visit the test is placed; on the

second visit the health care provider reads the test. The TB skin test is performed by injecting a small amount of fluid (called tuberculin) into the skin on the lower part of the arm. A person given the tuberculin skin test must return within 48 to 72 hours to have a trained health care worker look for a reaction on the arm. The result depends on the size of the raised, hard area or swelling.

a) **Positive skin test:** This means the person's body was infected with TB bacteria. Additional tests are needed to determine if the person has latent TB infection or TB disease.

b) **Negative skin test:** This means the person's body did not react to the test, and that latent TB infection or TB disease is not likely.

There is no problem in repeating a TB skin test. If repeated, the additional test should be placed in a different location on the body (e.g., other arm). The TB skin test is the preferred TB test for children under the age of five.

TB blood tests

TB blood tests are also called interferon-gamma release assays or IGRAs. Two TB blood tests are approved by the U.S. Food and Drug Administration (FDA) and are available in the United States: the Quanti FERON®–TB Gold In-Tube test (QFT-GIT) and the T-SPOT®.TB test (T-Spot). **A health care provider will draw a patient's blood and send it to a laboratory for analysis and results.**

a) Positive TB blood test: This means that the person has been infected with TB bacteria. Additional tests are needed to determine if the person has latent TB infection or TB disease.

b) Negative TB blood test: This means that the person's blood did not react to the test and that latent TB infection or TB disease is not likely.

TB blood tests are the preferred TB test for:

1. People who have received the TB vaccine bacille Calmette–Guérin (BCG).

2. People who have a difficult time returning for a second appointment to look for a reaction to the TST.

Testing in BCG-Vaccinated Persons

Many people born outside of the United States have been given a vaccine called BCG. People who were previously vaccinated with BCG may receive a TB skin test to test for TB infection. Vaccination with BCG may cause a false positive reaction to a TB skin test. A positive reaction to a TB skin test may be due to the BCG vaccine itself or due to infection with

TB bacteria.

TB blood tests (IGRAs), unlike the TB skin test, are not affected by prior BCG vaccination and are not expected to give a false-positive result in people who have received BCG. TB blood tests are the preferred method of TB testing for people who have received the BCG vaccine.

Testing Health Care Workers

Tuberculosis (TB) transmission has been documented in health care settings where workers and patients come in contact with people who have TB disease. Periodic testing of health care workers is recommended as part of a TB Infection Control Plan and may be required by state regulations.

TB testing programs should include anyone working or volunteering in health-care settings. Persons (health care workers and non- health care workers) who have face to face contact or potential exposure to TB through shared air or space with infectious patient(s) should be part of a TB testing program.

There are two types of testing for TB in health care workers.

1. Initial baseline testing upon hire: Two-step testing with a TB skin test or a TB blood test

2. Annual or serial screening: determined by state regulations or risk assessment outcomes.

Frequency of TB testing

Health care facilities have different TB testing requirements. Facilities should conduct staff TB testing based on risk classification.

Risk classification	Frequency of testing
Low	Baseline; then test if TB exposure occurs
Medium	Baseline, then annually
Potential ongoing transmission	Baseline, then every 8–10 weeks until evidence of transmission has ceased

1. Baseline Testing

A baseline test should be given prior to employment. The result of this test can be compared with later tests (due to potential exposure or as part of annual testing) to help determine if recent TB transmission has occurred in the facility.

2. Annual or Serial Testing

You may need to test for TB on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests.

TB Skin Test: Two Step Testing

1. Baseline Testing: Two-Step Test

Two-step testing with the Mantoux tuberculin skin test (TST) should be used for baseline or initial testing. Some people with latent TB infection have a negative reaction when tested years after being infected. The first TST may stimulate or boost a reaction. Positive reactions to subsequent TSTs could be misinterpreted as a recent infection.

- Step 1

  Administer first TST following proper protocol

- Review result

  Positive — consider TB infected, no second TST needed; evaluate for TB disease.

  Negative — a second TST is needed. Retest in 1–3 weeks after first TST result is read.

- Document result

- Step 2

  Administer second TST 1-3 weeks after first test

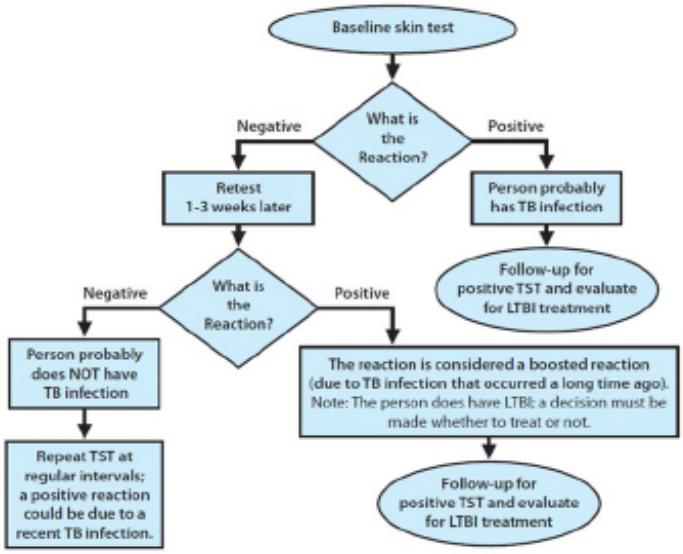
- Review results

  Positive — consider TB infected and evaluate for TB disease.

  Negative — consider person not infected.

- Document result

Two-Step TST Testing



2. Annual or Serial Testing

Once everyone in your facility has a baseline TB test, you may need to test on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests. There is no need for a two-step TST test process for annual TB testing. The process for annual testing with a TB skin test is as follows:

- Administer the TB skin test following proper protocol
- Review result — a change from a prior negative test result to a positive test result is evidence of recent TB infection
- Document result

TB blood Test

1. Baseline Testing

Using a TB blood test for initial or baseline testing does not require two-step testing and is not affected by BCG vaccination. The process for baseline testing using a TB blood test is as follows:

Administer TB blood test following usual protocol

Review result

Negative — consider not infected

Positive — consider TB infected and evaluate for TB disease

- Document result

2. Annual or Serial Testing

Once everyone your facility has an initial TB test result, you may need to test on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests. The process for annual testing for TB with a TB blood test is as follows:

- Administer the TB blood test following proper protocol
- Review result — a change from a prior negative test result to a positive test result is evidence of recent TB infection
- Document result

Testing During Pregnancy

There is a greater risk to a pregnant woman and her baby if TB disease is not diagnosed and treated. TB skin testing is considered both valid and safe throughout pregnancy. TB blood tests also are safe to use during pregnancy, but have not been evaluated for diagnosing TB infection in pregnant women. Other tests are needed to show if a person has TB disease.

Diagnosis of Latent TB Infection

A diagnosis of latent TB infection is made if a person has a positive TB test result and a medical evaluation does not indicate TB disease. The decision about treatment for latent TB infection will be based on a person's chances of developing TB disease by considering their risk factor [29].

3. Effectiveness of diagnosis of Tuberculosis

Diagnosis represents only one aspect of tuberculosis (TB) control but is perhaps one of the most challenging. The drawbacks of current tools highlight several unmet needs in TB diagnosis, that is, necessity for accuracy, rapidity of diagnosis, affordability, simplicity and the ability to generate same-day results at point-of-care (POC). When a return visit is required to access test results, time to treatment is prolonged, and default rates are significant. However, a good diagnostic tool is also critically dependent on obtaining an adequate biological sample [30].

3.1. Obtaining a biological sample and other considerations

Approximately 85% of the burden of TB is due to pulmonary TB. Diagnosis of pulmonary TB, particularly at primary care level, depends on obtaining an adequate expectorated sputum sample. However, in up to a third of TB cases, an adequate biological sample is not readily available or has a very low concentration of TB bacilli rendering the sample smear negative (cases of extrapulmonary TB requiring sampling at secondary care level, sputum scarce (unable to produce sputum), and smear-negative patients]. The latter is particularly relevant in children TB-HIV co-infection where up to 50% of persons are smear-negative [31]. Thus, alternative techniques such as sputum induction, gastric aspiration, bronchoscopy, and organ aspiration or biopsy may be required to obtain an adequate sample. However, the availability of these techniques is severely limited in high TB burden settings. Attention has therefore been focused on alternative biological samples, such as exhaled breath and urine, which are more readily available even in children.

Urine as a biological fluid for diagnostic testing is particularly attractive because it is sterile, less complex than other fluids such as sputum and serum, is readily available and TB-specific proteins and DNA may be found in the urine of patients with TB [32, 33]. Even

though a biological sample may be successfully obtained, other characteristics including sample volume (e.g. Xpert MTB/RIF requires _1 mL) and time-to-testing have the potential to impact results. It should be borne in mind that the reference standard for TB, that is, culture, is a suboptimal gold standard (prone to bacterial overgrowth, excessive decontamination, cross-contamination, etc.) and appropriate analytical strategies and methods may have to be employed to deal with this when evaluating new POC tests. Finally, more consideration should be given in combining tests and developing testing algorithms to rule-in TB [34], and to screening tests, including chest X-ray and computer-assisted diagnosis, to rule-out TB. These measures would help decrease the number of patients that require more expensive and complex tests, thus reducing burden on the patients as well as cost.

4. Effectiveness of Smear Microscopy

4.1. Direct Ziehl–Neelsen microscopy

Direct microscopy of Ziehl–Neelsen-stained sputum smears remains the mainstay of POC diagnosis in most TB endemic countries. The method is relatively rapid, inexpensive and has high specificity. However, direct Ziehl–Neelsen microscopy has low sensitivity (~50–60%) and is less sensitive in children, in HIV co-infected patients and in patients with extrapulmonary TB [35, 36]. Decontamination using chemicals, including bleach and NaOH and concentration of acid fast bacilli by centrifugation slightly improves the sensitivity [37, 38].

4.2. Fluorescence microscopy

An alternative to Ziehl–Neelsen -based direct microscopy is staining with a fluorescent molecule such as auramine O and visualization using a microscope with a mercury vapour bulb. This method is faster and improves sensitivity by ~10% without a compromise in specificity but its use has been limited by its higher cost, maintenance and darkroom requirements [39, 40].

4.3. LED microscopy

More recently, light-emitting diode (LED) microscopy was introduced. This low-cost method offers the benefit of fluorescence microscopy without the associated operational requirements, including a dark room and special microscope. LED has a lifespan of up to 50 000 h and may even be battery-operated. LED microscopy is endorsed by the WHO and also for use in resource-limited settings [41].

However, there are limited data about performance of LED microscopy in HIV-infected persons. A recent large study using samples from TB-HIV co-infected persons, LED microscopy was cheaper, faster and performed, as well as Ziehl–Neelsen and fluorescence microscopy independent of the staining and processing methods used [42].

4.4. Front-loaded microscopy

Another recent WHO-endorsed approach to smear based diagnostic work-up is front-loaded microscopy. Front-loaded microscopy addresses the problem of high dropout rates with focused collection of two or more sputum specimens during one clinic visit, and immediate referral and treatment of patients with positive smears [43, 44]. Front-loaded microscopy leads to a minor reduction in diagnostic sensitivity for the individual patient but is expected to improve case findings through enhanced quality of service and reduced dropout rates [45, 46].

5. Filtration techniques and magnetic beads

Other novel approaches to smear microscopy include filtration techniques and magnetic beads [47] to concentrate samples, and an automated slide-reading prototype that captures images and uses computerized algorithms to count acid fast bacilli [48].

6. Nucleic Acid Amplification Tests

A key advantage of near-patient rapid testing is that it may allow for the initiation of treatment within a very short time frame. New phenotypic methods such as commercial liquid culture drug susceptibility testing [49, 50] microscopic observation drug susceptibility [50, 51], colorimetric redox indicator methods [52] and the nitrate reductase assay [53], although approved by the WHO cannot provide results within a single clinic visit and also require extensive operator training, infrastructure needs and standardization before implementation [54]. By contrast, nucleic acid amplification tests (NAAT), which can rapidly detect small quantities of DNA through several different amplification methods, including the polymerase chain reaction, represent one of the most accurate known methods of detecting TB. With their improved simplification and automation in recent years, NAAT is becoming increasingly attractive candidate for use at the POC.

7. The Xpert MTB/RIF assay

Xpert MTB/RIF is a largely automated real-time polymerase chain reaction assay able to detect *M. tuberculosis* complex DNA and resistance to rifampicin [55]. It performs optimally on expectorated sputum specimens, using a disposable single-use cartridge and the test may be completed within 2 h, including a 15-min sample preparation step where sputum is homogenized using sterilizing sample buffer [56].

Several large-scale trials have assessed the accuracy of Xpert MTB/RIF [56, 57 58-61], where its sensitivity for TB detection in smear-positive and smear-negative patients was found to be ~98% and ~75%, respectively, [63] although some studies from high HIV prevalent settings have reported sensitivities in latter group to be as low as ~50% [57, 59, 62]. The

specificity of the assay for TB detection is ~98%. For the detection of rifampicin resistance in regions with high disease prevalence, the sensitivity and specificity are ~94% and ~97%, respectively [63]. Importantly, a recent large study [58] has shown that the improved accuracy of Xpert MTB/RIF over that of smear microscopy (the most widespread diagnostic test for TB, including at the POC) can translate into an improvement in the time-specific proportion of TB patients initiating TB treatment, where about 90% of TB patients could initiate treatment based on their Xpert MTB/RIF result on the same day they provided a sample. In contrast, only about 67% of TB patients were diagnosed by smear microscopy and able to initiate treatment rapidly, as this usually happened the day after a sample was provided for testing. However, whether this advantage is sustained and whether earlier diagnosis translates into reduction in morbidity and mortality remains unclear. It is critical that MDR-TB treatment capacity be scaled up in parallel to the rollout of the MTB/RIF assay.

8. Antigen Detection-Based Tests For Active Tb

The search for suitable TB-specific diagnostic antigens is ongoing and has been extensively studied in a variety of biological samples (e.g. sputum, blood, body cavity fluids and urine) [64]. A recent meta-analysis evaluated 47 studies using 12 single or combinations of TB antigens in different clinical specimens for pulmonary and extrapulmonary TB [64]. With the exception of LAM, TB antigen test sensitivity was as low as 2%, and specificity was suboptimal. However, antigen detection tests appear to offer a number of advantages over conventional diagnostics and have great potential for use as simple bedside tools. Antigen, as compared with whole *M. tuberculosis* organisms or TB-specific genetic material, is more likely to be detectable remote from the disease site in easily accessible biological fluids like urine. Antigen detection platforms, such as the lateral flow immune chromatographic assay (otherwise known as a strip test), require little or no sample processing to yield a rapid result. Unfortunately, despite the promise that antigen detection holds for POC diagnosis, available technologies have not yet delivered clinically useful results. Diagnostic accuracy measures vary widely, and with the exception of LAM, there are limited data about antigen-specific tests and none are currently in routine clinical use [64].

LAM, a 17.3-kDa immunogenic glycolipid component of the mycobacterial cell wall, has been the most extensively studied antigen and offers potential clinical utility in HIV-infected patients with advanced Immune suppression in both inpatient or outpatient (antiretroviral clinic) setting [65,66]. In HIV-infected patients, urinary LAM using an enzyme-linked immunosorbent assay kit had an overall sensitivity of ~50%, increasing to 67% and 85% in HIV-infected patients with CD4 count <50 cells/mL from outpatient and inpatient settings, respectively [65,66] and an overall specificity of 83–100% [65, 67, 68]. In addition, urine LAM correlated with bacterial burden [69] and may have prognostic utility by identifying TB HIV co-infected patients with the highest mortality [70]. Performance of the TB LAM enzyme-linked immunosorbent

assay using sputum or induced sputum samples has also been evaluated. Although sensitivity improved to over 80%, the specificity dropped to under 50% likely due to cross-reactivity with *Candida* spp and normal oral flora containing LAM-like molecules [65, 71]. The enzyme linked immunosorbent assay kit has now been superseded by the POC determine TB LAM Ag strip test (Alere), which is the first bedside TB test, provides a result within 25 min and will have a likely landing cost under USD 3.5 in the first quarter of 2013 [65]. Two initial evaluations in HIV-infected outpatients and inpatients showed similar diagnostic accuracy to the preceding TB LAM enzyme-linked immunosorbent assay and improved sensitivity (over the LAM strip test) when combined with sputum smear microscopy [72, 33]. However, specificity and inter reader agreement decreased when using the manufacturer's suggested grade-1 cut point. Thus, we recommend the grade 2 cut point at the expense of a lower sensitivity but with a higher specificity. In addition, the test either alone or combined with urine-based Xpert MTB/RIF testing was useful in sputum-scarce diagnostically challenging patients [32].

Further study is ongoing to clarify cut-point selection and the impact on patient-important outcomes, for example mortality, when LAM is used to guide the early initiation of treatment.

A number of antigens have also been evaluated in various compartments using non-sputum or urine samples, for example pleural fluid, cerebrospinal fluid, etc [64]. Urinary LAM had poor sensitivity and specificity in pleural and pericardial fluid, and in cerebrospinal fluid [64, 73, 74]. A limited number of studies with small numbers of patients have evaluated alternative diagnostic antigens using mainly 'in-house' assays with widely variable diagnostic accuracy [64]. These data reflect that antigen concentration in different body compartments are modulated by several factors including molecular weight, structure, and host degradation and processing. Thus, antigen performance may be highly variable between body compartments and sample specific. A possible solution may be to use combinations of antigens to improve overall diagnostic accuracy [64].

The availability, low cost, rapid format and modest performance of urine LAM in HIV-infected patients, although not ideal, gives us hope that antigen detection may still provide a broadly applicable and effective POC test. For this to be successful, candidate antigens or combinations of antigens will need to be specific for *M. tuberculosis*, be produced in abundance, be excreted into the extracellular environment and be resistant to rapid degradation associated with the host inflammatory response.

9. Antibody Detection and Microfluidic Technologies

Antibody detection tests based on lateral flow or other immune chromatic formats are attractive candidates. These tests monitor the humoral antibody immune responses to antigens and have proven to be rapid and accurate in the context of HIV diagnosis [75]. A number of

commercial antibody-based rapid TB tests are on sale [76] but significant clinical validation is absent and diagnostic accuracy is, at best, poor. In a recent updated meta-analysis including 67 studies of commercial serological tests, Steingart *et al*. showed that study quality was generally poor, and estimates of sensitivity and specificity were inconsistent and imprecise [40]. These findings lead the WHO to proclaim a negative recommendation (its first) against the use of TB serological tests [77].

The failure to develop antibody-based TB tests that meet clinical needs does not imply that such an approach should be abandoned. However, the heterogeneity in antibody responses from patient to patient suggests that a more complex multiplex approach is required [78-81]. Several novel promising antigenic targets have been identified [78, 82, 83]. A POC platform targeting several antigens, and co-developed by FIND (Geneva, Switzerland) and M Bio Diagnostics Inc. (Boulder, CO, USA), using multiplex serology on dot matrix readout will soon enter field evaluation studies. Microfluidics technology permits manipulation of fluids on a sub-millimetre scale enabling portability, affordability, easy disposal, user-friendliness, rapidity, multiplexing and feasibility with limited sample [84, 85]. A microfluidic platform seems well suited to a future multiplexed serological test.

10. POC Approaches for the Diagnosis of LTBI

In presumed LTBI, mycobacteria are not directly detectable, and therefore, diagnostic tests rely on measuring the presence of an adaptive immune response against *M. tuberculosis* [86].The major drawback of this approach is that a detectable response may represent exposure without infection or infection that has been cleared. The tuberculin skin test has been main stay of LTBI diagnosis for a century. This test is cheap and simple to apply, but there are several drawbacks including the need for a second test reading visit, subjective interpretation, cross reactivity in persons BCG vaccinated after birth and no assessment of immune energy. The discovery of regions of differentiation, that is, parts of the *M. tuberculosis* genome absent from most non-TB mycobacteria and BCG [87, 88], facilitated the development of specific immunodiagnostic tests—the interferon-g release assays (IGRA). IGRA are *in vitro* assays detecting interferon-g secretion from RD1 (ESAT6 and CFP10)-specific T cells. Two IGRA are commercially available—the Quanti FERON-TB Gold In-Tube assay (QFT, Qiagen, Hilden, Germany) and the T-SPOT.TB (Oxford Immuno tec, Oxford, UK). IGRA addresses several of the limitations of the tuberculin skin test; they have excellent specificity, but sensitivity (assessed in cases with active TB) is only 80%, and they are expensive and require specialized equipment and overnight incubation [89, 90]. Although data are variable [91, 92], a recent meta-analysis showed that ability to predict short-term progression to active TB was similar to the tuberculin skin test ~1–2% [89].

Although several biomarkers have been studied [93-97], the most promising interferon-g alternative is the chemokine inducible protein-10 (IP-10), which has comparable diagnostic accuracy and higher sensitivity in HIV-infected persons [97, 98, 99]. An IP-10 lateral flow platform can deliver quantitative results within minutes [100] and is stable in dried blood spots on filter paper allowing for letter based sample transport for centralized analysis [98].

Another interesting approach to LTBI diagnosis, similar to that of measuring interferon-g messenger RNA levels and transcriptional profiles [101], is IP-10 and MIG detection at messenger RNA level [102]. Advances in microfluidics and lab-on-a-chip technology could enable a novel generation IGRA-like test devices that combine incubation of small volumes blood (e.g. from a finger prick) and detection in a disposable device [103, 104]. A novel skin test using recombinant ESAT6 and CFP10 antigens, C-Tb (Statens Serum Institute, Copenhagen, Denmark) recently entered phase III clinical trials in South Africa and elsewhere, and performs comparably with the QFT in unexposed volunteers as well as in HIV-positive and -negative adults with confirmed TB. Diaskintest (Pharm standard, Ufa, Russia) is a similar product, but accuracy data are unavailable at the time of publication.

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