INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 7.581]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 9, Issue - 7, July - 2023 Publication Date: 31/07/2023

DOIs:10.2015/IJIRMF/202307031 --:-- Research Paper / Article / Review

An Overview on the Sensitivity and Specificity of the Routine Diagnostic Tests of Tuberculosis

[1] Abdul Kareem, [2] Paramita Dey
[1]Student, Department of Paramedical and Allied Health Sciences, Galgotias University, UP, India
[2]Assistant Professor, Department of Paramedical and Allied Health Sciences, Galgotias University, UP, India
Email – [1]abdul_kareem.smasbsc@galgotiasuniversity.edu.in, [2]paramita.dey@galgotiasuniversity.edu.in

Abstract: *The best way to diagnose tuberculosis is to show that mycobacteria are present in various bodily fluids. Detecting tuberculosis in children can be challenging due to the limited presence of mycobacteria in bodily fluids. However, recent advancements in our understanding of the molecular biology of Mycobacterium tuberculosis have led to the development of new diagnostic methods. Polymerase chain reaction (PCR) is a promising technique for diagnosing tuberculosis in children, particularly those with substantial lung disease or HIV infection. It should be noted that a negative PCR result does not rule out a tuberculosis diagnosis, and a positive result does not necessarily confirm it. Another approach that has gained attention is serodiagnosis, which involves detecting children's antibodies to specific M. tuberculosis antigens using ELISA. Although serology is quick and does not require a sample from the affected area, it has not been widely adopted as a routine diagnostic method for pediatric tuberculosis. The sensitivity and specificity of serological tests can be influenced by factors such as the type of antigen used, the gold standard for tuberculosis diagnosis, and the type of tubercular infection. Moreover, variables like age, prior BCG vaccination, and exposure to environmental mycobacteria can affect the accuracy of these tests. It is worth noting that serodiagnosis currently does not play a significant role in diagnosing pediatric pulmonary tuberculosis, as it may not effectively distinguish between disease and infection.For diagnosing latent tubercular infection, a novel test called QuantiFERON-TB (QFT) has emerged. This test analyzes the release of interferon-gamma in whole blood when stimulated by pure protein derivative. It has shown comparable results to tuberculin skin testing and offers a potential alternative for diagnosing latent tuberculosis infection. In summary, tuberculosis diagnosis in children has seen advancements through molecular biology insights. PCR and serodiagnosis hold promise but still require further clarification and standardization for routine clinical practice. QFT provides a potential avenue for diagnosing latent tuberculosis infection in children.*

Key Words: *Immunohistochemistry, multidrug-resistant TB, phytohemagglutinin, Cartridge Based Nucleic Amplification, lymphadenopathy.*

1. INTRODUCTION:

Tuberculosis remains a severe infectious disease, causing approximately two million deaths each year, predominantly in developing nations. The escalation of drug-resistant strains of Mycobacterium tuberculosis, which are unresponsive to first-line anti-TB medications, has contributed significantly to the current TB epidemic. Insufficient or incorrect treatment of TB has been a major catalyst for the development of drug-resistant strains.[5,6] These strains acquire resistance to anti-TB drugs through chromosomal mutations in the genes responsible for drug targets. Accumulation of successive mutations in the target genes leads to the emergence of multidrug-resistant (MDR) strains, which are resistant to rifampin and isoniazid.[8] The spread of MDR-TB strains poses a significant challenge to TB control efforts, jeopardizing the World Health Organization's goal of eradicating tuberculosis by 2050.Efficient and timely identification of patients with MDR-TB is crucial for effective treatment. Several phenotypic and molecular diagnostic techniques have been developed for rapid detection of drug-resistant strains, particularly in resource-limited settings.[16] However, once MDR-TB is diagnosed, successful treatment necessitates a combination of multiple potent but potentially toxic and expensive medications. The extensive treatment duration of 18 to 24 months poses challenges in ensuring patient adherence. Successful treatment of MDR-TB involves various strategies, including antibiotic susceptibility testing, supervised therapy with appropriate drugs at facilities equipped with culture facilities, regular patient monitoring for adverse effects, and tracking bacteriological and clinical improvement. Despite significant global

efforts, high rates of TB-related morbidity and mortality persist.[13,17] Factors contributing to the ongoing TB pandemic include the expansion of HIV infection, which increases the risk of active TB, and the rise of M. tuberculosis strains resistant to first-line drugs. Other variables such as population growth, low case detection and treatment rates in underdeveloped countries, active transmission in overcrowded environments, immigration from high-prevalence nations, drug abuse, and homelessness also contribute to the problem. The primary source of community infections is individuals with confirmed pulmonary TB and positive sputum tests. Although an efficient immune response typically halts M. tuberculosis multiplication after infection, only 10% of individuals completely eliminate the bacteria, while the remaining 90% manage to contain the infection and remain latently infected. According to estimates from the World Health Organization, 5-10% of individuals infected with M. tuberculosis will develop active TB illness over their lifetime. However, for those co-infected with HIV, the annual probability of active illness is 5-15%, reaching 50% over their lifetime.Drug resistance in tuberculosis is a consequence of genetic mutations that render medications ineffective against the bacteria.[7,8] Patients with a high bacterial load are more likely to develop drug-resistant strains due to a greater likelihood of spontaneous mutations. Inadequate treatment plans contribute to the selection and dominance of drug-resistant strains in TB patients. Multidrug-resistant tuberculosis (MDR-TB) refers to strains resistant to at least rifampin and isoniazid, the primary drugs used in TB treatment. MDR-TB poses significant public health challenges and is associated with higher rates of treatment failure and death, particularly in individuals co-infected with HIV.[6] Patients with MDR-TB experience slower sputum culture conversion, which is a predictor of treatment outcome. The use of second-line medications for MDR-TB is more challenging due to their adverse effects and high cost. Recurrent TB occurs when active tuberculosis reoccurs after successful treatment. It can be classified as reinfection with a different strain of M. tuberculosis or as a relapse of the initial infecting strain. The risk factors for recurrent tuberculosis, including distinguishing between relapse and reinfection, can be assessed using genotyping techniques.[5,6]

2. BACKGROUND:

Disease is defined as the absence of well-being due to physical or mental discomfort, encompassing any condition that disrupts normal bodily functions. This concept is often used when discussing infectious ailments, and one such affliction that has plagued humanity throughout history is tuberculosis (TB). Going back to the Neolithic era, tuberculosis has been caused by two distinct organisms, namely Mycobacterium tuberculosis and Mycobacterium bovis. In ancient Greece, physicians referred to this malady as “phthisis,” highlighting its destructive nature. In 1882, a breakthrough came when Robert Koch identified the tubercle bacillus, leading to the recognition of tuberculosis as a highly contagious disease. There are three main types of tuberculosis: pulmonary tuberculosis, extra pulmonary tuberculosis, and Millary tuberculosis. Pulmonary tuberculosis manifests as a long-lasting infection affecting the lungs. People with compromised immune systems are particularly susceptible to extra pulmonary tuberculosis. Millary tuberculosis often presents with pulmonary vein infection erosion as a characteristic symptom. [7,10] The symptoms of the disease vary depending on the specific location where tuberculosis bacteria proliferate within the body. Pulmonary tuberculosis often arises when the lungs become a breeding ground for the tuberculosis bacterium. Symptoms of this condition may include chest discomfort, a persistent and severe cough that lasts more than two weeks, and the expulsion of blood or mucus during coughing. Aiming to understand the distribution of different types of tuberculosis in Dindigul, an investigation will be conducted to explore the relationship between disease patterns and factors such as medical history and demographics. The analysis will be based on existing data sources, and an assessment of the primary aspects of tuberculosis treatment will be carried out using factor analysis. Furthermore, the utilization of GIS techniques will allow for the mapping and characterization of various types of tuberculosis in the study area.[7]

Active tuberculosis and latent tuberculosis are distinct conditions with notable differences. In most cases, individuals with active TB display noticeable symptoms, which commonly consist of unexplained weight loss, decreased appetite, night sweats, fever, fatigue, and chills. On the other hand, those with latent tuberculosis do not experience any sickness or display symptoms. Depending on the location of the infection, individuals may manifest various symptoms, such as a persistent cough or the coughing up of blood in pulmonary tuberculosis cases, or experiencing back pain in instances of spinal tuberculosis. Individuals with latent TB infection are non-contagious and do not pose a risk of transmitting TB to others. However, only infections affecting the larynx and lungs have the potential to be contagious and spread to other individuals when TB becomes active.[14,17] In the case of an asymptomatic individual, the presence of an infection is often detected through a skin test or blood test, indicating a latent TB infection. Typically, sputum tests for TB yield negative results, and chest X-rays do not reveal any signs of infection. Essentially, there are no observable symptoms or indicators of an active disease, apart from a positive blood or skin test. Active TB is diagnosed when symptoms and other indications of an active disease coincide with a positive test result, such as bacterial growth in a sputum sample or a positive nucleic acid amplification test.[17] Tuberculosis (TB), caused by the bacterium Mycobacterium tuberculosis (MT), has been a long-standing issue in human history, carrying significant social

consequences. The Mycobacterium genus is estimated to have emerged over 150 million years ago. During the Middle Ages, a novel manifestation of TB affecting the cervical lymph nodes was identified as scrofula. It acquired the intriguing moniker "king's evil" in France and England, with a widespread belief that the touch of royalty could heal the afflicted.[13,14]

In 1720, an English physician named Benjamin Marten proposed the infectious nature of tuberculosis, marking a seminal moment in its understanding. The invention of the sanatorium cure represented the initial breakthrough in effectively treating the disease. On March 24, 1882, the distinguished scientist Robert Koch accomplished a momentous feat by isolating the tubercle bacillus, making this astounding discovery known to the Academy of Physiology in Berlin. Subsequent to Koch's pioneering work, remarkable advancements in tuberculosis treatment emerged. The BCG vaccine, developed by Albert Calmette and Camille Guérin, along with Selman Waksman's streptomycin and other anti-tuberculous medications, were formulated in the decades that followed this pivotal discovery. [13] The development of tuberculosis in children follows a continuous process, although it can be beneficial to consider three primary stages: exposure, infection, and illness. Exposure occurs when a child has recently and extensively interacted with an adult or adolescent who is suspected or confirmed to have infectious pulmonary tuberculosis (known as a source case). Public health experts often identify exposed children through follow-up examinations of individuals with suspected pulmonary TB. During this stage, the child's tuberculin skin test (TST) shows no reaction, the chest radiograph appears normal, and there are no apparent physical symptoms or signs of TB. However, it should be noted that some children who have been exposed to the disease may carry Mycobacterium tuberculosis. It is important to recognize that the emergence of delayed-type tuberculin hypersensitivity can take up to three months, making it difficult for clinicians to Immediately determine if exposed children are infected. Sadly, in children under the age of five, meningeal and widespread TB are particularly prevalent.[10]

EPIDEMIOLOGY:

The significant challenge In establishing a coordinated regional response to tuberculosis (TB) stems from the considerable variations in TB epidemiology and contextual factors across different nations. Notably, in countries with low TB burdens like Australia and New Zealand, over 80% of reported cases occur in individuals born outside of the country, highlighting TB as essentially an imported disease. In countries with aging populations such as Japan, the Republic of Korea, and the Hong Kong Special Administrative Region SAR (China), people over 65 account for a substantial proportion of TB case notifications, with percentages reaching 66.7%, 45.4%, and 43.7% respectively in 2018.[3] Low-income, high-burden nations often observe undernutrition as a significant risk factor for TB, while high rates of cigarette smoking contribute to a higher prevalence of TB in men. In the Pacific island nations, diabetes is a common condition believed to be a significant contributor to the TB epidemic. To develop tailored strategies, it is crucial to comprehend population-level factors associated with TB by analyzing regular surveillance data, survey results, and facility records.[3,6] Given the diverse epidemiological and contextual settings, including small Pacific islands with unique geographic challenges and high TB incidence rates per capita (such as Kiribati, the Marshall Islands, and Tuvalu), the Western Pacific Region requires a specially designed regional strategy to guide the response effectively.[6] To assess national TB programs, it is essential to conduct in-depth investigations and interpretation of the various factors impacting TB case notifications. While a decline in case notifications may indicate a genuine reduction in TB incidence, it is crucial to consider other possibilities such as a decrease in case detection or a reduction in the funding and operational capacity of the TB program. Notably, the Global Fund to Fight AIDS, Tuberculosis, and Malaria plays a significant role in providing substantial financial support for TB initiatives in the region.[3] The treatment of multidrug-resistant tuberculosis (MDR-TB) presents formidable challenges and often results in relapse or treatment failure, thereby posing a grave threat to public health worldwide. Furthermore, the emergence of extensively drug-resistant tuberculosis (XDR-TB) becomes a worrisome possibility. [6]

ETIOLOGY:

In adults, the distinction between TB infection and disease is generally evident as most cases are triggered by reactivation of dormant organisms years after the initial infection. Adult patients commonly exhibit symptoms and are often contagious. On the other hand, children rarely pose a contagious risk, and the time gap between infection and disease, especially in those primarily affected, can vary from several months to several years. Furthermore, radiographic abnormalities in children frequently occur without accompanying symptoms. Distinguishing between infection and disease is crucial in children since the perception of the condition can influence the treatment approach. Typically, infection is managed with a single anti-TB medication, whereas active disease requires a combination of two or more drugs. This difference in treatment strategy is justified by the higher likelihood of medication resistance emerging as the bacillary population grows. However, in the case of children, the line between infection and primary disease is

somewhat arbitrary as they both exist on a continuum. Consequently, the prevailing approach leans towards overtreatment rather than undertreatment in children, considering their good tolerance to anti-TB medications and the relative affordability of these drugs in industrialized nations. Consequently, mild alterations in lung parenchyma and asymptomatic lymphadenopathy are diagnosed and treated as diseases.[10] The onset of tuberculosis (TB) disease can be identified when signs, symptoms, or radiographic indications related to M. tuberculosis become apparent. In the case of infected children, their clinical symptoms and radiographic abnormalities are predominantly influenced by the host's inflammatory response rather than the actual quantity of organisms present. Studies indicate that within 1 to 2 years, untreated TB infection leads to the development of illness in approximately 40% to 50% of newborns. However, the risk decreases to around 15% for older children. Additionally, extrapulmonary TB occurs in about 25% to 35% of cases among children, posing a challenge for confirming the bacterial presence. [10] When evaluating new diagnostic tests for tuberculosis (TB), it is crucial to consider the fundamental differences in the pathophysiology of the disease between adults and children. In children who have recently contracted TB, active multiplication of mycobacteria can occur regardless of the presence or absence of radiographic abnormalities or clinical signs. Even infants with normal chest radiographs may have a small percentage that produces M. tuberculosis in gastric aspirate cultures. Consequently, some children who are typically classified as having TB infection may test positive for M. tuberculosis on most diagnostic tests designed for identifying the disease in adults with TB. To determine the implications of these novel test results on defining and managing TB infection and disease in children, careful consideration and thorough investigation are necessary. [10]

DIAGNOSIS:

Tuberculosis (TB) remains a significant health challenge in low and middle income countries, posing a considerable burden on public health. Unfortunately, the lack of reliable diagnostic tests has severely impeded our efforts to combat this disease effectively. Meeting the requirements for a diagnostic test that is rapid, affordable, and easy to use remains a challenge. Extensive research has been conducted to improve the diagnosis and treatment of pulmonary TB. Diagnosing Extra Pulmonary TB (EPTB) presents additional difficulties due to the often low presence of Mycobacterium Tuberculosis Bacilli (MTB) at the affected site. Obtaining clinical specimens from deep-seated organs can be challenging, further complicating the diagnosis process. Histology, while providing valuable insights, is time-consuming and labor-intensive, making it challenging to achieve precise TB diagnoses. Fine needle aspiration cytology (FNAC) has proven to be a valuable technique for diagnosing early-stage tubercular peripheral lymphadenopathy and monitoring post-therapy progress due to its high accuracy. However, FNAC does not require lymph node excisional biopsy, minimizing invasiveness, pain, and morbidity associated with the diagnostic procedure. In cases of superficial TB lymphadenopathy, excisional biopsy offers the highest sensitivity compared to FNAC. Despite being used as a first-line diagnostic method for nearly two decades, excisional biopsy still exhibits sensitivity and specificity ranging from 79% to 94%. However, reliance solely on FNAC for diagnosis is not without limitations. Challenges such as contamination, subjective interpretation, inability to distinguish between different sources of granuloma, lack of effectiveness in drug-resistant TB, and limited diagnostic utility in immunocompromised patients underscore the need for a new diagnostic tool. [1] Molecular diagnostic techniques are poised to shape the future of accurate and reliable diagnosis for Extra Pulmonary TB (EPTB). Globally, the Nucleic Acid Amplification Test (NAAT) is increasingly being employed as a faster diagnostic tool for TB. Genotypic methods offer numerous advantages, including the potential for programmatic management and surveillance of multidrug-resistant tuberculosis (MDR-TB), rapid diagnosis, standardized testing, high throughput capabilities, and reduced laboratory biosafety requirements. Several Amplification of Nucleic Acids Technology (NAAT) assays have been developed to efficiently detect M. tuberculosis DNA in specimens and identify DNA alterations associated with drug resistance. One such example is the Cartridge Based Nucleic Amplification Test (CB-NAAT), an automated DNA test that can detect M. tuberculosis and rifampicin resistance (a marker for MDR-TB) within two hours. These advancements hold promise in improving the study and diagnosis of patients suspected to have TB. [1] The global underdiagnosis of multidrug-resistant tuberculosis (MDR-TB) remains a pressing concern. To address this, the Genotype MTBDRplus® fast drug susceptibility tests (DST) assay has shown promising diagnostic accuracy in detecting resistance to isoniazid and rifampicin, thereby aiding in the identification of MDR-TB. However, limited evidence exists regarding its impact on patient outcomes in routine clinical practice. [4,6] In the city of Kimberley, South Africa, the National Health Laboratory Facilities (NHLS) TB reference laboratory conducted laboratory tests as part of their regular program. Sputum samples were collected by daily couriers, following the criteria set by the TB program. The samples were treated with N-acetyl L-cysteine-NaOH after centrifugation and then reconstituted in phosphate buffer (pH 6.8). Fixed smears were subjected to Auramine staining and fluorescence microscopy examination, and the slides were graded using the International Union Against Tuberculosis and Lung Disease scale. For inoculation, 0.5 mL of the digested and resuspended sputum was added to

MGIT tubes, which were incubated and monitored daily according to the manufacturer's guidelines. A culture period of 35 days without growth was considered negative. Positive MGIT cultures were further examined for acid-fast bacilli using Ziehl-Neelsen staining.[4] The diagnosis of active tuberculosis (TB) involves a combination of clinical suspicion, chest radiographs, acid-fast bacilli (AFB) smears, solid and liquid cultures, as well as newer techniques such as hybridization- and PCR-based amplification to detect the nucleic acid of M. tuberculosis in clinical samples. This serves as a basic overview of the diagnostic process, but for more detailed information, additional review articles are available. In cases where it is challenging to cultivate the organism in culture or differentiate between Mycobacterium tuberculosis and non-tuberculous mycobacteria (NTM), molecular approaches have revolutionized the diagnosis of TB, particularly in smear-negative samples. The FDA has approved two molecular assays specifically designed for respiratory specimens. It is important to note that the choice of amplification target, detection methods, as well as the sensitivity and accuracy of in-house PCR tests may vary. By utilizing two reverse hybridization-based techniques, both M. tuberculosis and various NTM species can be identified in clinical samples and cultured isolates.[4,6] Molecular assays play a crucial role in confirming the presence of M. tuberculosis in positive sputum and non-sputum smears, replacing the traditional methods of microscopy and culture. However, it is worth noting that while molecular assays have demonstrated their value, they have not entirely replaced microscopy and culture, as these methods continue to provide essential diagnostic information. [6]

3. DIAGNOSTIC METHODS:

In order to assess the diagnostic accuracy and validate transcript classifiers, it will be necessary to conduct testing in multiple independent cohorts comprising individuals with various respiratory and systemic illnesses. This approach aims to enhance the current diagnostic methods and establish proposed classifiers as reliable tools. To achieve the highest levels of specificity and sensitivity in diagnosis and analysis, a dominant algorithm consisting of multiple sets of approximately 12-100 genes associated with active tuberculosis could prove to be the most effective strategy. Considering the current cost constraints, this approach is essential for practical implementation in clinical settings.[8] However, as technology continues to advance in terms of processing, gene expression analysis, and affordability, it is conceivable that a comprehensive set of genes capable of differentiating tuberculosis from other diseases could form the basis of diagnosis, along with selected classifier genes. Integrating blood transcriptomics into the diagnostic process for tuberculosis, in conjunction with existing methods such as clinical symptoms, sputum mycobacterium smear positivity, and confirmatory tests like culture or nucleic acid amplification technology, would be highly valuable. This approach would particularly benefit individuals who are smear-negative, culture-negative, or children for whom these tests may not always be feasible. Leveraging blood transcriptomics could expedite diagnosis, enable timely treatment, and ultimately reduce transmission rates. [8]

4. TUBERCULINS SKIN TEST:

After administering the tuberculin skin test (TST) using 5 Tuberculin Units (TU) based on the Monteux method, the size of skin induration was measured in millimeters within 48-72 hours. The interpretation of TST results took into consideration the level of risk, following the guidelines currently in place. It's important to note that all patients underwent testing due to their heightened susceptibility to tuberculosis (TB) infection. [8]

5. QUANTIFERON-TB GOLD:

The test procedure followed the instructions provided by the manufacturer. The test setup consisted of two sample wells containing whole blood stimulated with either Early Secretory Antigen Target 6 (ESAT-6) or Culture Filtrate Protein 10 (CFP-10), along with a positive control well (mitogen well) stimulated with the mitogen phytohemagglutinin (PHA), and a negative control well (nil well) with no antigens or mitogen. The whole blood samples were then incubated at 37°C in a humid environment for 20 hours. The results of the mitogen well and the antigen-stimulated wells were adjusted based on the background IFN-level found in the nil well. To determine the test outcome, regardless of the positive control result, the concentration of IFN- in the sample well following stimulation with ESAT-6 and/or CFP-10 was compared to a threshold of 0.35 IU/ml (after subtracting the value of the nil well) in the mitogen well. If the IFN-level in response to the specific antigens (after subtracting the value of the nil well) was below 0.35 IU/ml and the IFN-level of the positive control (after subtracting the value of the nil well) was equal to or greater than 0.5 IU/ml, the test result was considered negative. If both antigen-stimulated sample wells yielded values below 0.35 IU/ml (after subtracting the value of the nil well) and the positive control well value was below 0.5 IU/ml (after subtracting the value of the nil well), the test result was classified as undetermined.[15]

6. ELISPOT ASSAY:

The T-SPOT test employed specific ELISPOT assays using test plates. To obtain PE mononuclear cells (PEMCs), Ficoll-Hypaque gradient centrifugation was utilized to separate Pes. In brief, 250,000 PBMCs or 250,000 PEMCs were plated overnight in 96-well plates coated with a mouse anti-human IFN- antibody, with each well containing 100 L of culture medium. The wells were divided into three groups: unstimulated (negative control), stimulated with 50 L phytohaemagglutinin (positive control), or treated with 50 L each of the peptides ESAT-6 and CFP-10 separately. Following the provided instructions, the plates were cultured, washed, counterstained, visualized, and assessed for spots.[12] A positive response was determined in the stimulated cultures when the test well exhibited at least six additional spots and twice the number of spots compared to the control well. The negative control wells typically displayed around 10 background spots per well. [12]

MTB-specific nucleic acid technique amplification:

To achieve amplification of MTB-specific nucleic acid technology (MTB-NAT), either the BDProbeTec ET test or the Amplified MTD assay was employed. Various methods were utilized for histopathology and identification of Mycobacterium tuberculosis (MTB). For mycobacterial culture, half of the fresh biopsy specimens were utilized for growth on Lowenstein-Jensen egg medium. The remaining half of the tissue was preserved in 4% phosphate-buffered formaldehyde for conventional paraffin embedding. Subsequently, standard haematoxylin and eosin staining and Ziehl-Neelsen (ZN) staining were performed to detect acid-fast bacilli (AFB). ZN staining involved the application of heat-carbol-fuchsin technique. [11]

Immunohistochemistry:

Immunohistochemistry (IHC) has emerged as an alternative to conventional acid-fast staining for detecting mycobacterial antigens, utilizing a combination of polyclonal and monoclonal antibodies. Various mycobacterial antigens, including BCG and lipoarabinomannan, have been identified in tissues using IHC, albeit with varying success rates. However, the widespread presence of these antigens poses a challenge in distinguishing M. tuberculosis from other mycobacteria. Nevertheless, our recent pilot investigation yielded promising results. In this study, we reported the exceptional sensitivity of an in-house rabbit polyclonal antibody in identifying a secretory mycobacterial antigen, MPT64, exclusively present in the M. tuberculosis complex. Biopsies from patients with tuberculous lymphadenitis showed high sensitivity and specificity (89-93% and 95-98%, respectively) when IHC was employed, demonstrating its diagnostic potential on diverse tissue types. The strength of this technology lies in its accessibility, robustness, and the ability to detect fragmented tubercle bacilli in standard surgical pathology laboratories. Compared to the limited sensitivity (10-45%) of Ziehl-Neelsen (ZN) staining, which requires intact cell walls, IHC offers a significant improvement in diagnostic accuracy. It is particularly suitable for diagnosing pauci-bacillary extrapulmonary tuberculosis (EPTB).[11] Despite these advantages, the adoption of IHC for tuberculosis diagnosis in histopathology labs has been relatively slow. This hesitation may be attributed to the uncertainty surrounding the precise diagnostic role of IHC for mycobacteria. To establish its utility in endemic areas, comprehensive evaluations including adequate control groups and appropriate antisera are essential. Our extensive investigation is the first to demonstrate the reliability of IHC utilizing an antibody against MPT64 in establishing an etiological diagnosis of M. tuberculosis complex infections across multiple tissues in EPTB cases. [11]

Treatment:

Many countries in the region continue to face challenges in achieving favorable treatment outcomes, especially for individuals with drug-resistant tuberculosis (DR-TB) and TB/HIV co-infection. To address this issue, the World Health Organization (WHO) has provided a comprehensive set of recommendations. These include screening people living with HIV (PLHIV) for tuberculosis, initiating antiretroviral therapy (ART) early, improving infection control measures, providing tuberculosis preventive treatment (TPT), implementing more effective interventions for multidrug-resistant tuberculosis (MDR-TB), closely monitoring drug safety, and promoting patient-centered care models.[2] Digital solutions are increasingly being utilized to enhance adherence to tuberculosis medication. By identifying and bridging gaps in such interventions and promoting the use of innovative technologies, treatment outcomes can be improved. It is crucial to identify at-risk groups and regions with reported poor treatment outcomes to tailor targeted interventions effectively. Treatment outcomes typically vary based on geographic location, as observed in tuberculosis epidemiological reviews for the Philippines and the Lao People's Democratic Republic (both 2019, unpublished).[2,3] Japan's low overall treatment success rate can be attributed to the high mortality rate among the elderly population, who are disproportionately affected by tuberculosis. As other nations face aging populations and increasing rates of tuberculosis cases among the elderly, they may encounter similar challenges in the future. It is imperative for global and

regional tuberculosis programs to be prepared in addressing tuberculosis among the elderly by incorporating evidence-based recommendations and successful interventions. [3] Bedaquiline (BDQ), delamanid (DLM), and pretomanid (PTM) are three newly developed drugs used in the treatment of tuberculosis (TB), specifically for patients with multidrug-resistant TB (MDR TB). These medications have demonstrated significant improvements in therapeutic success rates. Additionally, the effectiveness of alternative medications such as linezolid (LZD) and clofazimine (CFZ) has been reinforced, further expanding the options for MDR TB treatment.[6] In recent studies, a promising all-oral regimen, devoid of injectable aminoglycosides, has been evaluated and showcased encouraging outcomes. As a result, the World Health Organization (WHO) has modified its recommendations for MDR TB treatment, now advocating for a shorter all-oral therapy. However, the current guidelines for shorter regimens still endorse a 9-12 month course of treatment involving a combination of seven medications, including BDQ, levofloxacin/moxifloxacin, and high-dose isoniazid (INH). Although real-world data has demonstrated positive results, DLM and LZD have not yet been incorporated into the recommendations for shorter therapy due to limited data quality. [2,6] These modifications in MDR TB treatment guidelines have also brought about changes in the definitions of pre-extensively drug-resistant TB (pre-XDR TB) and extensively drug-resistant TB (XDR TB). Presently, TB caused by Mycobacterium tuberculosis strains that meet the criteria for MDR/RR-TB and exhibit resistance to any fluoroquinolone and at least one additional Group A medication is classified as XDR TB (BDQ and LZD). On the other hand, M. tuberculosis strains meeting the criteria for MDR/RR-TB and displaying additional resistance to any fluoroquinolone are categorized as having pre-XDR TB. [2] The successful treatment of tuberculosis (TB) relies on several crucial factors, Including the medication combination, treatment duration, cost, and potential adverse effects. A positive indicator of progress is the resolution of chest radiograph abnormalities within two months of initiating treatment, accompanied by a transition from culture-positive to culture-negative sputum. Conversely, if sputum cultures remain positive four months into multidrug therapy, it signifies treatment failure. Inadequate or inappropriate treatment plays a significant role in treatment failure, allowing the disease to persist and leading to the emergence of drug resistance (acquired resistance). When a newly infected host contracts the resulting Mycobacterium tuberculosis strain, it exhibits primary resistance as it is already resistant to the prescribed medications.[6]

7. CONCLUSION:

Early diagnosis and appropriate treatment are essential for reducing the impact of tuberculosis (TB) in terms of morbidity and mortality. However, the traditional method of direct smear testing with Ziehl-Nielsen staining (ZN) has limitations due to its low sensitivity. It can only identify a portion of "TB suspects" with positive cultures, ranging from 30 to 60%, depending on the number of samples analyzed. Moreover, the collection of sputum samples on consecutive days can be cumbersome for patients, slowing down the diagnostic process and hindering their cooperation. While chest X-rays are used to diagnose smear-negative TB, they have limited specificity and carry the risk of overdiagnosis. However, advancements in molecular techniques have shown promise in addressing these challenges by rapidly detecting mycobacterial DNA in sputum samples. In a study conducted in a high TB and HIV prevalence setting, the AMPLICOR PCR technique was used to diagnose pulmonary tuberculosis. Sputum samples from 1,396 suspected tuberculosis patients in Nairobi, Kenya, were analyzed using PCR to detect the presence of Mycobacterium tuberculosis, with culture on Löwenstein-Jensen medium serving as the "gold standard." The PCR technique demonstrated a sensitivity of 93% and specificity of 84%, unaffected by the HIV status of the patients. PCR successfully identified 82.1% of true smear-negative TB cases and 99.7% of true smear-positive TB cases. Notably, PCR detected M. tuberculosis in 11.7% of suspects with negative cultures, and 60% of these individuals had one or more positive sputum samples. Among the 490 positive cultures, M. tuberculosis was found in 486 cases. The high sensitivity of Amplicor PCR justifies its use in clinical settings with a significant burden of TB and HIV. Tuberculosis is a highly dangerous infectious disease with a high risk of transmission, morbidity, and mortality. The overall increase in Mycobacterium tuberculosis infection rates is attributed to factors such as the HIV epidemic and socioeconomic challenges, particularly in resource-constrained developing nations. Detecting pleural TB (plTB) is challenging, with various diagnostic methods offering varying sensitivities. Pleural biopsy, MTB culture, MTB nucleic acid amplification, and histological detection of caseating granulomas are considered the gold standard, with sensitivities ranging from 39% to 80%, 90%, and 50% to 97%, respectively. Pleural biopsies, while invasive, have higher sensitivity compared to pleural fluid in detecting active tuberculosis infection. Immediate diagnosis of plTB through pleural taps in a clinical setting would be desirable. However, MTB culture, MTB-DNA detection, and acid-fast bacilli detection from pleural fluid exhibit varying sensitivities ranging from 12% to 70%, 30% to 100% (in culture-negative cases, 30% to 60%), and 10%, respectively.

REFERENCES:

1. Espinosa-Pereiro J, Sánchez-Montalvá A, Aznar ML, Espiau M. MDR Tuberculosis Treatment. Medicina (Kaunas). 2022 Jan 26;58(2):188. Doi:10.3390/medicina58020188. PMID: 35208510; PMCID: PMC8878254.
2. Morishita F, Viney K, Lowbridge C, Elsayed H, Oh KH, Rahevar K, Marais BJ, Islam T. Epidemiology of tuberculosis in the Western Pacific Region: Progress towards the 2020 milestones of the End TB Strategy. Western Pac Surveill Response J. 2020 Dec 28;11(4):10-23. Doi: 10.5365/wpsar.2020.11.3.002. PMID: 34046237; PMCID: PMC8152824.
3. Hanrahan CF, Dorman SE, Erasmus L, Koornhof H, Coetzee G, Golub JE. The impact of expanded testing for multidrug resistant tuberculosis using genotype [correction of geontype] MTBDRplus in South Africa: an observational cohort study. PLoS One. 2012;7(11):e49898. Doi: 10.1371/journal.pone.0049898. Epub 2012 Nov 30. Erratum in: PLoS One. 2013;8(6). Doi: 10.1371/annotation/47420ec0-db00-4994-a0de-86b5e5d713f4. PMID: 23226229; PMCID: PMC3511489.
4. Bang D, Andersen ÅB, Thomsen VØ, Lillebaek T. Recurrent tuberculosis in Denmark: relapse vs. re-infection. Int J Tuberc Lung Dis. 2010;14:447-53.
5. Bang D, Lillebaek T, Thomsen VØ, Andersen ÅB. MulTidrug resistant tuberculosis: treatment outcome in Denmark, 1992-2007. Scand J Infect Dis. 2010;42:28893.
6. Bang D, Andersen PH, Andersen ÅB, Thomsen VØ. Isoniazid resistant tuberculosis in Denmark: mutations, Transmission and treatment outcome. J Infect. 2010;60:452-7.
7. Bang D, Andersen ÅB, Thomsen VØ. Rapid genotypic de-Tection of rifampin- and isoniazid-resistant Mycobacterium tuberculosis directly in clinical specimens. J Clin Microbiol. 2006;44:2605-8.
8. Vijdea R, Stegger M, Sosnovskaja A, Andersen ÅB, Thomsen VØ, Bang D. Multidrug-resistant tuberculosis:Rapid detection of resistance to rifampin and high or Low levels of isoniazid in clinical specimens and isolates. Eur J Clin Microbiol Infect Dis. 2008;27:1079-86.
9. Suhail Ahmad, Eiman Mokaddas; Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis, Open Archive Published: August 06,2009. DOI:https://doi.org/10.1016/j.rmed.2009.07.010
10. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. Emerg Infect Dis. 1995;1(4):115-123. Doi:10.3201/eid0104.950402.
11. Purohit, M.R., Mustafa, T., Wiker, H.G. et al. Immunohistochemical diagnosis of abdominal and lymph node tuberculosis by detecting Mycobacterium tuberculosis complex specific antigen MPT64. Diagn Pathol 2, 36 (2007). https://doi.org/10.1186/1746-1596-2-36
12. M. Losi, A. Bossink, L. Codecasa, C. Jafari, M. Ernst, S. Thijsen, D. Cirillo, M. Ferrarese, U. Greinert, L. M. Fabbri, L. Richeldi, C. Lange for the European Tuberculosis Network TBNET European Respiratory Journal 2007 30: 1173-1179; https://doi.org/10.1183/09031936.00067307
13. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg. 2017 Mar;58(1):E9-E12. PMID: 28515626; PMCID: PMC5432783.
14. Thomas M.Daniel; Center for Global Health and Diseases, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-7286, USA
https://doi.org/10.1016/j.rmed.2006.08.006
15. Bruchfeld J, Correia-Neves M, Källenius G. Tuberculosis and HIV Coinfection. Cold Spring Harb Perspect Med. 2015 Feb 26;5(7):a017871. Doi: 10.1101/cshperspect.a017871. PMID: 25722472; PMCID: PMC4484961.
16. https://www.ncid.sg/Health-Professionals/Articles/Pages/Latent-and-Active-Tuberculosis,-What-Is-the-Difference.aspx