Advanced Therapeutic Interventions Targeting *Mycobacterium Tuberculosis*

Abstract: Tuberculosis infection (TBI), caused by *Mycobacterium tuberculosis* (*M.tb*), presents with or without clinical signs of active TB and is a persistent global threat despite efforts to eradicate it. The emergence of HIV/AIDS is one of the problems to complete eradication. Recent research has focused on vaccines, diagnostics, and treatment. This review examines vulnerable populations, high-risk groups, and socio-economic factors influencing TBI prevalence. It also explores the intersection of TBI and the COVID-19 pandemic, including healthcare disruptions and transmission dynamics. Advances in TBI diagnosis, biomarkers, prophylactic therapies, and combination treatments are discussed, along with the integration of artificial intelligence (AI) in TBI therapy to optimize treatment and personalize care. Vulnerability to TBI varies based on age, socio-economic status, and immune status. High-risk groups include those with compromised immune systems, the elderly, and those in crowded or poorly ventilated settings. Socioeconomic factors such as poverty and limited healthcare access also contribute to TBI prevalence. The COVID-19 pandemic has disrupted TBI diagnosis and treatment, with limited healthcare access impacting outcomes. Changes in healthcare delivery, like telemedicine, may have long-term impacts on TBI care. Improved biomarkers, like interferon-gamma release assays (IGRAs), offer faster TBI diagnosis. Prophylactic therapies, such as isoniazid preventive therapy (IPT), reduce active TB risk in high-risk groups. Combination treatments are being evaluated for drug-resistant strains. AI integration in TBI therapy could lead to better outcomes by analyzing patient data for personalized treatment plans. In conclusion, TBI remains a global health threat requiring ongoing research and innovative approaches for diagnosis and treatment. Advances in diagnosis, prophylactic therapies, and combination treatments, along with AI integration, offer hope for improved outcomes and better patient care.

Keywords: Tuberculosis infection (TBI), *Mycobacterium tuberculosis (M.tb)*, Vulnerable populations, High-risk groups, Socio-economic factors.

1. Context

Tuberculosis infection (TBI) manifests as a sustained immunological response triggered by *M.tb* antigens, either with or without evident clinical symptoms of active TBI disease. Many individuals with this infection remain asymptomatic and do not exhibit visible signs, posing an absence of risk of spreading the disease. These individuals are susceptible to active tuberculosis, potentially contributing to disease transmission. TBI is now recognized as a dynamic continuum of reactions to M.tb infection, influenced by the immunological response interactions between TBI bacteria and the host's immune system. A recent study suggests that persons diagnosed with TBI who showed no symptoms may have different infection levels. The infection might be eliminated, while on the other end, active tuberculosis may be present in a subclinical state (1). An estimated 10% of the world's population is infected with *M.tb*. However, there is minimal evidence on the frequency of resistance to basic anti-TBI therapies (isoniazid and rifampicin) owing to the difficulties in isolating and testing infecting strains for resistance evaluation (2). Globally, the prevalence of infections caused by strains resistant to isoniazid is increasing, which demands the need to understand the dynamics of tuberculosis infection and the potential difficulties presented by drug-resistant variants (3). The global efforts to eliminate TBI have been hindered by the appearance of human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), perpetuating it as a continuous worldwide menace. The World Health Organization has designated tuberculosis as a "global emergency" due to its significant severity. In 2010, Gandhi and colleagues emphasized these concerns (4) such as drug-resistant strains of tuberculosis emerged due to the improper use of antibiotics during tuberculosis chemotherapy, lack of adherence to anti-TBI drugs, and insufficient monitoring of drug resistance. According to the World Health Organization (WHO), almost 9.6 million people were impacted by TBI, leading to 1.5 million fatalities and TBI-related deaths (5) There is a critical need for a method for effective TBI control that can rapidly and accurately diagnose *M.tb* and facilitate effective treatment on a global scale. However, existing point-of-care (POC) detection methods lack the speed and effectiveness required to reduce infection rates and TBI-related mortality significantly. These methods primarily focus on detecting active TBI infections. The diagnosis of active TBI involves detecting the presence of the causative *M.tb*, within the patient. While traditional microbiological culture methods are available, they suffer from slow growth rates and a higher risk of contamination, making them vulnerable to spreading the MDR strain. Sputum smear microscopy has traditionally been the main method for diagnosing TBI despite challenges in rapidly identifying TBI in a clinical setting (6). Patients suspected of having TBI may test negative in sputum smears, prompting the use of additional diagnostic procedures. Examples of diagnostic tests for tuberculosis include the TBI skin test (TST), interferon-gamma release assay (IGRA), culture examination, chest radiography, or amplification techniques to confirm the diagnosis. The cultural approach is the most precise and sensitive diagnostic technique, outperforming sputum smears' accuracy. Employed in clinical and research settings, the culture of *M.tb* proves to be a highly reliable method for diagnosing active tuberculosis. The growth in nanoscience and nanotechnology has greatly contributed to advancements in diagnostic procedures, especially in sample preparation and detection. The emphasis is on creating technologies that provide a direct, economical, sensitive, precise, and quick diagnosis of TBI at the POC levels. A conventional method entails using magnetic nanoparticles and antibodies in immune magnetic separation fields to diagnose viral and inflammatory diseases (7) which developed a diagnostic system that integrates microfluidics, magnetic nanoparticles tagged with anti-BCG antibodies, and nuclear magnetic resonance (NMR) equipment to analyze unprocessed biological materials. In addition, nanotechnology methods have been extensively used to create a highly responsive bio-sensing platform at both the micro and nanoscale using nanoparticles. This approach offers enhanced sensitivity, specificity, simplicity of assembly, and the ability to produce large volumes at a reasonable price for POC testing. Functionalized quantum dots combined with immune magnetic separation have shown exceptional sensitivity and specificity when used with clinical samples (8).

2. Evidence Acquisition

This review examines populations vulnerable to TBI, with a particular emphasis on demographics and socio-economic factors that contribute to disease prevalence. It also explores the relationship between TBI and the COVID-19 pandemic, analyzing how they mutually impact each other, including healthcare disruptions and patterns of TBI transmission. The review discusses advancements in TBI diagnosis, particularly the use of improved biomarkers for accurate identification, preventive therapies, and the effectiveness of combined treatments against drug-resistant strains. Additionally, it explores the integration of artificial intelligence in TBI therapy, highlighting its potential to enhance treatment approaches and personalized care strategies.

3. Results

3.1 Population Sensitive to TBI

Although not all persons infected with *M.tb* proceed to active tuberculosis infection the chance of disease progression varies among patients. Those with compromised immune systems exhibit a notably higher incidence of active TBI infection (9). This risk remains elevated even with effective antiretroviral treatment (ART). Household contacts (HHCs) of persons with bacteriologically confirmed cases proven for TBI are being targeted for intervention. Those who tested positive for TBI but did not undergo tuberculosis preventive therapy (TPT) showed a markedly increased prevalence of active tuberculosis during the initial two years of observation compared to their TBI-negative counterparts. Irrespective of age or TBI status, individuals with household contacts (HHCs) are at a higher risk of getting active tuberculosis compared to the general population (10). Demographic factors that influence active TBI infection are those persons who have migrated from countries with a high incidence of tuberculosis, those experiencing homelessness, incarcerated individuals, illicit drug users, and patients receiving immunosuppressive treatment, medication such as TNF inhibitors, and long-term corticosteroids. Host genes affect a person's vulnerability to TBI. However, the identities of the implicated genes have remained unknown so far. Two complementary approaches to identifying those genes are association-based candidate genes and a comprehensive linkage screen While candidate gene studies could identify the precise gene or genes responsible for the onset of TBI, the linkage will only pinpoint the genes' chromosomal position. A substantial study is needed to switch from mapping age networks to finding genes. Still, it becomes simpler as the human genome project generates increasingly high-resolution physical and genetic maps. The genome-wide map of translated sequence tags (EST), a short length of coding DNA for which a PCR test is currently being developed, would substantially aid in identifying genes associated with TBI susceptibility.

3.2 TBI association with the COVID epidemic

The regular TBI diagnostic and treatment services have been significantly affected by the COVID-19 pandemic. This is primarily attributed to the diminished capacity of healthcare systems, the reassignment of healthcare personnel to COVID-19-related duties, and the allocation of TBI diagnostic resources to address the similarity in symptoms between TBI and COVID-19. The enforcement and implementation of lockdowns and strict quarantine measures have caused delays in the diagnosis of TBI, interruptions in contact tracing attempts, and setbacks in the commencement of tuberculosis-preventative medication. These variables may have led to the increased spread of TBI within their homes (11). The number of reported TBI cases worldwide has significantly decreased, from 7.1 million in 2019 to 5.8 million in 2020, representing a remarkable historic fall. The southeast Asia and the western Pacific areas experienced the most significant reductions in TBI cases. Significant progress has been achieved in employing innovative strategies to address tuberculosis, as evidenced by recent research findings. (12) These approaches encompass a range of techniques for screening and diagnosing multiple diseases, such as utilizing GeneXpert, a diagnostic tool used for detecting TBI and COVID-19. They use automated technology to analyze chest X-rays and detect cough patterns directly at the point of service. An evident transition may be seen from specialist methodology to integrated approaches, such as using community health workers to enhance early identification, diagnosis, and treatment of tuberculosis patients. A deliberate and coordinated process guarantees that susceptible demographics in industrialized countries can get preventative screening by the ideals of health care. The COVID-19 pandemic has prompted progress in infection prevention and control methods in healthcare systems, particularly in preventing TBI. This encompasses the increased use of masks by patients and personal protective equipment by healthcare workers. As a result, there has been a significant decrease in the spread of both COVID-19 and TBI (13). COVID-19 triggers diverse immune responses in individuals, ranging from asymptomatic to severe symptomatic cases marked by excessive cytokine release, potentially leading to fatal outcomes. The administration of immune-suppressing medications, like steroids, in COVID-19 treatment raises concerns about the potential reactivation of tuberculosis in the future. Although PCR and culture-based methods for TBI are considered the gold standard for diagnosing COVID-19, there is currently no ideal POC test available to determine active TBI infection. COVID-19 can manifest at any stage of TBI progression, posing higher risks for those with active pulmonary TBI. Identifying common signs and symptoms shared by both COVID-19 and TBI may contribute to the timely acquisition of imaging services, such as chest radiography or computed tomography, which can reveal evidence of pre-existing TBI before the onset of COVID-19. Key factors influencing mortality rates in COVID-19, such as age and comorbidities, such as HIV infection, poverty, diabetes, and malnutrition, contribute to tuberculosis mortality rates. Individuals with a compromised immune system, making them vulnerable to TBI, face an increased risk of contracting coronavirus. Implementing control measures for TBI has already been impacted by the ongoing COVID-19 pandemic, highlighting the importance of considering the potential for coinfection. Despite advancements, culture, and antibiotic susceptibility testing are the gold standard for diagnosing TBI. The COVID-19 outbreak presents an opportunity to explore the parallels between COVID-19 and TBI regarding transmission. Examining the challenges and insights gained from managing

both diseases can be mutually beneficial. Swift and accurate diagnosis and widespread public awareness are essential for effective management of both conditions (Figure 1).

3.3 Diagnosis Improvement

The tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) are two critical diagnostic methods to detect tuberculosis infection (14). These tests indirectly detect the immune response to tuberculosis (TBI) and do not directly assess the existence or capacity to survive *M.tb* infection. Their capacity to differentiate between various phases of TBI is low, and they have a restricted ability to accurately anticipate the advancement of the infection to an active state of sickness (15). Therefore, a significant proportion of persons may need medical intervention to avoid a personal instance of active TBI. The IGRA QuantiFERON-TB Plus (QFT-Plus) includes recently developed antigens tailored to activate CD4+ and CD8+ T cells. QFT-Plus can detect recent infections and assess disease activity by measuring the intensity of the immune response. Compared to QFT-GIT, QFT-Plus emerges as a more accurate test for identifying TBI (16). The LIOFeron TB/TBI test, a novel IGRA test, potentially demonstrates greater sensitivity than the QFT-Plus assay. Due to their ability to assess a broader spectrum of immunological responses, IGRAs are less susceptible to the impact of immunosuppression. The choice of a diagnostic test for TBI is influenced by the clinical context, test availability, local TBI prevalence (whether high or low), and the fraction of the population vaccinated with BCG. Enhancing the deployment of TPT is a major challenge in streamlining and making screening procedures more accessible. The current hindrances include the absence of a widely accessible and easily obtainable test that demonstrates both high sensitivity and specificity for diagnosing TBI, particularly in circumstances with limited resources. The absence of a very precise diagnostic test for definitively excluding TBI is a substantial obstacle in finding the appropriate patient and choosing the ideal date for commencing TPT. The World Health Organization (WHO) proposes a set of algorithms that link different combinations of symptoms such as chest X-rays, and quick nucleic acid amplification tests to exclude the potential of TBI (17). The scarcity of these tests, particularly chest X-rays, along with the logistical difficulties in regularly implementing TST (such as storing tuberculin, requiring two visits, the possibility of false positives in individuals vaccinated with BCG, and the potential for negative results in immunocompromised individuals) and IGRA (which involves expenses and dependence on laboratory facilities), poses a significant hurdle for treatment programs, particularly in resource-constrained environments. The "Xpert Mycobacterium tuberculosis/resistance to rifampicin assay" is a fast nucleic acid amplification (NAA) method that is specifically developed to identify the existence of mycobacterium bacilli and evaluate its susceptibility to the antibiotic rifampicin. (18). The test is very sensitive but expensive, requiring extra resources such as manpower and time duration. This is critical in pediatric settings where acquiring sputum samples might provide difficulties. Imaging is crucial in the diagnosis of pulmonary TBI. The process entails the integration of two separate categories of information: structural data collected from CT scans, which identify lesions based on x-ray density, and functional data gained from PET scans utilizing ^{18}fluorodeoxyglucose (^{18}F-FDG), which highlight metabolic activity in inflammatory cells of mammals (19). In the rabbit model, the intensity of ^{18}F-FDG peaks five weeks after infection, stabilizing or decreasing in the subsequent month as the disease progresses to a chronic state. Administration of isoniazid or rifampicin during chemotherapy reduces the absorption of ^{18}F-FDG and diminishes the density and volume of CT lesions over time. Patients with active TBI exhibit elevated levels of Interleukin 18, directly correlating with the severity of the illness observed in radiographic imaging. Tuberculosis and Interleukin 18 promote T-cell activation and generate interferon in chronic inflammatory conditions (20). WHO advocates for advanced diagnostic techniques, including the use of liquid culture, drug susceptibility testing (DST), line probe assays (LPAs), and Xpert MTB/RIF that enables the rapid identification of multidrug-resistant tuberculosis (MDR-TBI). Several non-commercial techniques have been devised for culture and drug susceptibility testing (DST), including microscopic observation drug susceptibility (MODS), thin-layer agar (TLA), colorimetric redox indicator (CRI), and the nitrate reductase assay (NRA), which are recognized for their rapidity and cost-efficiency (21). Using cytospin slides and triton processing methods in the modified Ziehl-Neelsen (ZN) staining technique enhances its diagnostic efficacy and sensitivity for TBI meningitis, surpassing the conventional ZN staining technique. Fluorescence microscopy (FM) with auramine O staining provides a sensitivity of 10% greater than ZN but retains a similar level of specificity. This allows for rapid and accurate diagnosis (22). Using light-emitting diodes (LEDs) is an increasingly sophisticated and economical technique that has further improved the FM technique. To enhance the test sensitivity, substances such as bleach, sodium hydroxide, or a solution of N-acetyl L-cysteine and sodium hydroxide would facilitate the process of turning sputum into a liquid form, which in turn decreases the amount of time needed for collecting bacilli by centrifugation. The technician must analyze at least two or three slides for each patient on consecutive days. To establish a conclusive "smear-positive" diagnosis, both sets of evidence must demonstrate the existence of acid-fast bacilli (AFB). Confirming the infection conclusively takes more than two days. An innovative and efficient approach for TBI diagnosis with high capacity involves the use of a TBDx automated microscopic system, developed by Signature Mapping Medical Sciences in Herndon, VA, USA (23). This automated smear microscopy system can detect and diagnose TBI from sputum smears, saving time and improving diagnostic accuracy. Both the Tuberculin Skin Test (TST) and Interferon-Gamma Release Assay (IGRA) are diagnostic tests used to detect tuberculosis infection and find extensive use in diagnosing latent tuberculosis infection (LTBI) and active tuberculosis. TST entails injecting a tiny quantity of TBI-purified protein derivative (PPD) of the bacilli into the dermis of the lower arm. The person receiving the TST should return within 48 to 72 hours so that a healthcare provider can examine the arm for any immunological reaction. A positive reaction is an allergic reaction to a specific skin area that shows a measurable response to the antigen (24). The tuberculin skin test (TST) may provide erroneous positive outcomes in persons who have received the bacillus Calmette guérin (BCG) vaccine for TBI and false negative findings in patients with weakened immune systems. IGRA comprises the QuantiFERON-TBI Gold In-Tube test (QFT-GIT), which was created by Cellestis in Australia, and received FDA approval as indirect and adjunctive diagnostics for detecting TBI infection. The QFT-GIT test involves culturing a fresh blood sample that contains viable white blood cells with control samples and a unique mix of synthetic chemicals is analyzed using enzyme-linked immunosorbent assay (ELISA) to determine the quantity of IFN-α (25). Another diagnostic test, the T-Spot test (TST), involves placing peripheral blood mononuclear cells (PBMCs) in a controlled

environment with two peptide combinations representing ESAT-6 and CFP-10 and allowing them to incubate. This test measures the number of cells that produce IFN-γ (spots) in response to certain antigens. TST findings indicate the existence of TBI infection, without providing information on the present infection status of the person. Supplementary diagnostic techniques such as IGRAs or chest radiographs are used to verify the presence of an active TBI infection. This is required because there is a higher probability of obtaining incorrect positive or negative results from the TST. Nucleic acid amplification tests (NAAT) for TBI provide prompt and precise molecular diagnosis and the capability to anticipate medication resistance (26). Semi-automated NAAT often utilizes polymerase chain reaction (PCR) to amplify and identify mycobacterial rRNA or DNA directly from different clinical samples, including blood, sputum, bone marrow, and tissue. These techniques have been restricted due to the substantial expenses involved in resource-intensive environments. The Xpert MTB/RIF assay is a sophisticated instrument functioning as a fully automated and integrated NAAT instrument (27). It encompasses sample preparation, amplification, and DNA detection processes. This assay efficiently overcomes many constraints associated with current commercial NAAT techniques, such as concerns around cross-contamination, time consumption, and laboratory challenges. The Xpert MTB/RIF test, performed in a controlled environment, may rapidly amplify and confirm the presence of *M.tb* infection. It can also detect mutations that cause resistance to rifampicin within 2 hours. The GeneXpert Omni, created by Cepheid, is still in the developmental phase. This device aims to do POC testing for tuberculosis and MDR-TBI using Xpert MTBI/RIF cartridges. Identifying volatile organic compounds (VOCs) in exhaled breath shows potential for rapidly identifying active pulmonary TBI at the POC testing, where they discover many important breath biomarkers for TBI among VOCs. These biomarkers include oxidative stress products, such as alkanes and alkane derivatives, and volatile metabolites of the TBI bacilli, such as cyclohexane and benzene derivatives (28).

3.4 TBI biomarkers

RNA sequencing is presently applied to assess whole blood biomarkers designed to accurately forecast the probability of acquiring active TBI in individuals exposed to the disease. Recent research has successfully made genetic signatures indicative of the likelihood of developing tuberculosis within 6 to 12 months. A trio of genes has proven effective in distinguishing between active and latent TBI, with a correct classification rate of 91.5% for individuals. This marks an improvement compared to earlier genetic signatures, which achieved an accuracy of 80 to 85%. Currently, there are no available diagnostic tests capable of reliably detecting TBI or discerning between preclinical or early clinical illness and TBI. It cannot identify TBI caused by drug-resistant strains of M.tb (29). Analyzing TB pathways can reveal biomarkers associated with TBI in the blood of individuals with pulmonary TBI induced by interferon (IFN) and driven by neutrophils. This genetic profile involves type 2 (IFNγ) and type I (IFNαβ) IFN signaling. Unstimulated samples from children and adults with active TBI showed significantly increased interferon (IFN)γ inducible protein 10 (IP10) in their plasma. Several approaches have assessed this rise. The presence of immunological activation markers CD38, HLA-DR, and the proliferation marker Ki-67 on Mtb-specific CD4+ T-cells showed a direct relationship with the amount of Mtb present. In addition, multiparametric flow technology investigated polyfunctional T-cells as prospective biomarkers. The frequency of polyfunctional CD4+ T-cells in mice is highly linked with the level of protection against Mtb infection caused by the vaccination. Automated liquid culture equipment, such as the mycobacterial growth indicator tube, is widely used to diagnose TBI. These standardized approaches serve as promising frameworks for biomarker exploration, as they consistently yield information about the time needed for growth detection. This timeframe is significantly and inversely associated with the size of the initial sample, as observed in assessments with laboratory stock cultures (30). PCR-based techniques offer a precise means of quantifying viable mycobacteria. A notable tool in this realm is the GeneXpert MTB/RIF test, which is an automated molecular diagnostic technique and is very sensitive and efficient, specifically designed to diagnose pulmonary TBI. This technique utilizes real-time PCR amplification using molecular probes to identify MTBI DNA specifically. Lipoarabinomannan, an important component of the mycobacterial cell wall, can be identified in a patient's urine using a commercially accessible enzyme-linked immunosorbent test (ELISA). The sensitivity of the test is most effective for identifying TBI in individuals who have advanced HIV infection. Significantly, the levels of lipoarabinomannan progressively decrease after 1-2 months of concurrent TBI and HIV therapy, indicating the presence of urine lipoarabinomannan at the beginning of the treatment. Exploring lipoarabinomannan as a potential biomarker warrants further investigation, especially considering the potential of novel assays with improved sensitivity to broaden its applicability (31).

3.5 Prophylactic therapy

Isoniazid has traditionally been the primary medication prescribed for tuberculosis preventive therapy. The isoniazid regimen lasting from 6 to 9 months faces challenges related to limited acceptability and completion rates. This is mainly attributed to the extended length of therapy, inadequate compliance, and acceptability (32). Research indicates that rifampicin, or rifapentin, is a long-acting rifamycin in shorter treatment regimens and is equally effective compared to isoniazid-based regimens. These abbreviated regimens exhibit higher treatment completion rates and enhanced safety. Administering rifampicin daily for 4 months has demonstrated efficacy comparable to a 9-month course of isoniazid in preventing the onset prevalence of active TBI in adults and children (33). Both approaches showed superior completion rates and reduced incidence of severe adverse effects. In specific cases, preventive therapy might be recommended for household contacts with a high risk of contracting MDR-TBI), which should be identified as a personalized risk. The process of prophylactic therapy for TB involves a systematic approach to evaluating and treating individuals at risk of TB infection. Positive results prompt further examination to rule out active TB and assess risk factors. If prophylaxis is warranted, a suitable medication regimen, such as Isoniazid (INH) monotherapy or combination therapy, is selected, considering treatment duration and patient-specific factors. Patient education, monitoring for adverse effects, and regular follow-ups are crucial throughout treatment. Post-therapy assessments and reinforcement of preventive measures follow the completion of therapy. If TB disease signs arise, comprehensive evaluation and treatment referrals are essential. This systematic approach ensures proper

evaluation, treatment initiation, monitoring, and completion, aiming to mitigate TB infection risks and prevent disease progression in high-risk individuals (Figure 2).

3.6 Combination therapies

Clinical trials have provided substantial evidence indicating that the most effective strategy for tuberculosis treatment involves a prolonged course of a combination of medications. Achieving the desired outcome requires administering multiple drugs over an extended period. The current protocol for treating drug-sensitive tuberculosis spans six months, comprising two phases of drug therapy. The initial treatment consists of a first phase of months with four medicines (isoniazid, rifampicin, pyrazinamide, and ethambutol), followed by a second phase of months with two medicines (isoniazid and rifampicin). This approach has been used for over four decades and established an estimated efficacy rate of 85% (34). However, the prevalence of tuberculosis cases resistant to existing medications, encompassing drug-resistant and multidrug-resistant cases, remains significant, exceeding 500,000 annually. Traditionally, treating drug-resistant tuberculosis involves administering more than four medications for up to 24 months due to the limited bactericidal capacity of the drugs, posing potential drug adverse reactions. The lack of treatment approach consistency has resulted in a suboptimal success rate of less than 50% in curing patients (35). Introducing innovative clinical trial designs and positive outcomes opens avenues for advancing more effective tuberculosis treatments. The pathogenesis of pulmonary TBI involves a synchronized immunological response, including several cell populations that undergo alterations over the course and treatment of the illness. The intricate and ever-changing characteristics of the infections necessitate that Mtb must withstand various stresses and possess physiological adaptability to cope with changing conditions. Elevated concentrations of extracellular Mtb are associated with necrotic and cavitating lesions, presenting significant challenges for treatment in both humans (36) and animal cases. Research in humans indicates that different lesions exhibit diverse responses to pharmacological therapy, potentially influenced by the amount of medication reaching Mtb. The response of Mtb to medications varies based on the lesion's location, mainly due to varied drug concentrations at specific sites. These findings influence the rationale for prolonged treatment duration and the enhanced effectiveness of therapy involving multiple medications and/or higher dosages. Certain antibiotics specifically target replicating cells, potentially limiting their efficacy against infections caused by non-replicating Mtb bacteria. However, additional medications in the treatment plan demonstrate the ability to eliminate actively proliferating and non-replicating Mtb. This attribute may contribute to the reduced length of therapy reported when integrating these medicines into the existing standard care regimen (37). Bedaquiline and pretomanid have shown good treatment effects in vitro against replicating and non-replicating Mtb. Tackling medication resistance is a formidable obstacle in managing several other ailments, such as bacterial infections and cancer. Prolonged exposure to medicine increases the probability of microbes acquiring resistance. Given the protracted duration of tuberculosis therapy lasting several months, the risk of resistance development is increased (38). Drug resistance may emerge when cells acquire specific factors that allow them to survive drug doses that would otherwise inhibit or kill cells lacking these factors. These traits have a genetic foundation, rendering the medicine ineffective at therapeutically beneficial quantities if resistance develops, enabling the resistant group to survive. A strategic approach involves employing a combination of medications that target distinct cellular mechanisms, proving effective in impeding resistance development in both experimental and clinical contexts. Multidrug therapies for tuberculosis have demonstrated improved efficacy compared to single-drug therapy, resulting in a decreased incidence of relapse with drug-resistant Mtb. Therefore, multidrug treatments comprising more than three medications are considered productive. This anticipation rests on the assumption that there would be fewer than one naturally occurring Mtb cell resistant to triple-drug therapy in severely infected patients, with infection severity measured by the bacillary load in cases of illness. The formulation of the selection of the three-drug combination consisting of pretomanid, moxifloxacin, and pyrazinamide was based on the encouraging outcomes obtained in preclinical models (39) and later assessed in a clinical study. The favorable clinical effectiveness of this three-drug combination led to the commencement of the current SimpliciTB clinical trial. The objective of this clinical trial is to assess the impact of adding bedaquiline to the treatment protocol that includes pretomanid, moxifloxacin, and pyrazinamide for both drug-susceptible (DS) and drug-resistant (DR) tuberculosis. By adding and substituting medications, researchers have adapted these therapeutic frameworks, leading to the identification of treatment regimens that have either been demonstrated to enhance treatment outcomes.

3.7 Artificial intelligence in TBI therapy

Developing and implementing innovative and sustainable strategies is crucial for overcoming healthcare resource constraints linked to tuberculosis screening. Utilizing computer-aided detection and machine learning is a promising approach to address resource and diagnostic obstacles. The WHO has recently revised its recommendations for TBI screening. The amended guidelines now recommend using computer-aided detection tools to analyze digital chest radiography pictures in patients 15 years old and above. These software applications provide a numerical score that indicates the probability of tuberculosis infection (40). The "Stop TB" partnership and the "Foundation for Innovative New Diagnostics" (FIND) have created an online resource center for computer-aided detection methods in the diagnosis of TBI as a means of supporting this campaign. With advancements in technology and appropriate clinician training, artificial intelligence (AI) holds the potential to address lingering clinical challenges constrained by limited resources or technological barriers. The cost-effective nature of chest radiography provides a highly accurate means of screening patients. Furthermore, it is an easily accessible tool for detecting pulmonary tuberculosis, especially in cases where bacterial confirmation is challenging while utilizing minimal radiation. Common radiographic indicators of TBI include the presence of cavities, nodules, consolidation, pleural effusion, and enlarged mediastinal lymph nodes (41). Enlarged mediastinal lymph nodes are the prevalent manifestation. Most research investigating the recognition of certain radiographic findings by imaging modalities has shown a lack of agreement among observers. The diagnostic accuracy of mediastinal lymphadenopathy was poor, likely due to overlapping structures, even when lateral views were utilized. This issue persisted despite attempts to improve the accuracy (42).

AI and computer-aided detection tools are vital in advancing and automating the analysis of digital chest radiography for TBI screening. AI involves the application of programming, training, and testing methods to empower computers with the ability to think and learn. Machine learning, a branch of AI, employs statistical methods to enhance the capabilities of robots and allow them to improve their performance. Deep learning, a specialized branch of machine learning, becomes particularly useful when dealing with vast amounts of data that require processing of input data. Deep learning networks employ artificial neural networks, which consist of multiple layers, to analyze data comprehensively. These networks, sometimes featuring multiple layers, possess the capability to autonomously learn from extensive datasets, enabling them to provide precise predictions for unfamiliar incoming data. Integrating machine learning and deep learning algorithms aims to enhance the efficiency of radiology operations, automating tasks such as lesion identification and providing valuable support to radiologists. While AI-driven computer-aided detection algorithms demonstrate enhanced efficacy, further clinical investigations are essential to address potential biases and validate results in real-world scenarios beyond controlled research environments. A groundbreaking research conducted by Mouton, pitcher, and Douglas pioneered the examination of AI for identifying anomalies in chest radiographs of pediatric patients within a population with an increased susceptibility to TBI cases. The application of AI in TBI imaging, particularly in pediatric cases, faces various challenges commonly encountered in the context of other diseases. These challenges encompass the scarcity associated with AI models, which includes the use of varied training data, the absence of external validation, the possibility of biases, reliance on subjective reference standards (such as human interpretation of radiographic diagnosis instead of correlation with microbiological references), and a scarcity of real-world implementation data. To properly train AI models for TBI imaging, gathering a broad training dataset from many medical institutions, including equipment and modalities from different vendors and manufacturers, is critical. The focus on impartial training highlights the need to use initial data devoid of other influences that might conceal information, such as demographics or medical particulars influencing certain diagnoses. In the future, it will be essential to carry out randomized controlled trials across multiple centers, employing AI models and comparing their outcomes with those of trials without AI integration. This approach is instrumental in gaining a comprehensive understanding of the potential advantages and efficiencies that AI could offer to patients. Advancements in computer-aided design (CAD) have made notable progress, particularly in developing a TBI detection algorithm. This algorithm initially segments lung areas and extracts specific characteristics, such as distinctive shapes, from the images. A classifier then evaluates these extracted features to determine the presence of TBI. Currently, the commercially available program for tuberculosis detection based on computer-aided design is CAD4TB, created by Delft Imaging Systems in Veenendaal, Netherlands (43). The algorithm's area under the curve (AUC) falls between 0.71 and 0.84, suggesting that commercially accessible products may not keep up with the latest breakthroughs in AI. The integration of clinical data into CAD4TB, along with its reconstruction, has resulted in its transformation from a CAD application to a deep learning model. AI deep learning networks, such as AlexNet, which were particularly developed for TBI detection, have become crucial. The AlexNet, a pre-trained deep learning network renowned for its efficacy in the ImageNet Large Scale Visual Recognition Competition, was first developed to categorize non-medical photos. The pretraining of AlexNet for picture recognition has been fully finished, necessitating modifications for medical imaging rather than starting the model training process again. Both the trained and untrained versions of GoogLeNet and AlexNet, which are highly acclaimed deep learning networks, have shown remarkable achievements in the ImageNet challenge, and a combination of both models, together with a radiologist, was used to address contradicting cases, leading to the most accurate approach (44). The method achieved a sensitivity of 97.3%, a specificity of 100%, and an AUC (area under the curve) of 0.99. Researchers are actively investigating AI for supplementary information analysis that surpasses human comprehension in several applications. The progress in tuberculosis detection models results in higher accuracy and effectiveness. The previously stated deep learning models, namely AlexNet and GoogLeNet, undergo pretraining on many pictures and can differentiate between several categories of images even before their application in radiology. Therefore, they need significant computer memory and hardware prerequisites to operate efficiently. Artificial Intelligence (AI) revolutionizes healthcare, aiding in early detection and treatment of diseases. In tuberculosis, AI contributes to diagnosing both active and latent infections through advanced imaging analysis. Chronic cough, weight loss, night sweats, and fatigue are common symptoms. AI's role lies in predictive analytics, helping healthcare professionals identify patterns and assess risks promptly. By leveraging technology, we enhance our ability to address tuberculosis and other health challenges, ultimately improving patient outcomes and streamlining healthcare processes (Figures 3 and Figure 4).

3.8 Challenges

While TPT has demonstrated progress in specific countries and high-risk populations, it has yet to attain the targeted levels essential for fulfilling the objectives outlined in the end-TB plan. Regions with a high prevalence of TBI and limited resources tend to prioritize TBI treatment over prevention. A key obstacle to initiating isoniazid preventative treatment (45) is the concern about potential medication shortages, particularly in remote hospitals. The need for qualified healthcare professionals or inadequate training in prescribing TPT and encouraging patients with no symptoms to undergo screening or treatment for TBI might diminish confidence in healthcare practitioners. Regular assessment for TBI might lead to stigmatization among persons who do not exhibit any symptoms. The current diagnostic assays for TBI have intrinsic limitations and are not widely available in areas with limited resources. These obstacles hinder the broad control of TBI and might even lead to drug resistance if the administration of TPT needs to be done accurately. While immunotherapy enhances the immune system to fight cancer (46), it can pose challenges for individuals with latent tuberculosis, potentially reactivating the infection due to immune activation. Balancing these treatments requires careful monitoring and possibly adjusting immunotherapy regimens to prevent tuberculosis reactivation. Sputum analysis aids tuberculosis (TB) diagnosis through microscopy, culture, and molecular tests (47). AI faces challenges in TB detection due to sample variability, low bacterial load, and diverse strains. Interpreting complex sputum images accurately remains a hurdle, necessitating AI algorithms' robustness to detect TB features amidst contaminants. Integration with rapid, cost-effective testing methods is crucial to enhance AI's role in early TB diagnosis, treatment monitoring, and curbing. Extra-pulmonary TB's diverse manifestations beyond the lungs

complicate diagnosis, leading to delayed treatment. Challenges for AI lie in analyzing atypical data sources, diverse clinical presentations, and limited datasets to accurately detect and manage extra-pulmonary TB (48). Treatment challenges also include drug resistance, lengthy treatment regimens, and the need to modulate the immune response effectively (49). The relevance of serological detection of animal viruses to tuberculosis lies in the broader context of infectious disease surveillance and control in animal populations (50). While these viruses primarily affect livestock, the principles and techniques used in serological detection can inform similar approaches for tuberculosis surveillance in animals, which are known reservoirs for Mycobacterium tuberculosis. Understanding the prevalence and spread of these viral infections can provide insights into managing tuberculosis ultimately benefiting both animal and human health.

3.9 Future scope

Enhanced POC diagnostic methods that accurately differentiate between inactive and active TBI while determining the probability of developing active TBI would undeniably constitute significant progress in global TBI prevention endeavors. IGRA testing is more effective than the TST in confirming and controlling TBI. Nevertheless, the present utilization of recent technology is restricted, and more investigation is crucial to improve the distinction between active and inactive TBI while uncovering prognostic indicators for the advancement of the illness. There is an ongoing need to further the progress of creating a TPT that is shorter or ultra-short in duration (<1 month) while being safe and well-tolerated. This therapy should be implemented without any potential medication interactions. Simultaneously, optimizing the utilization of existing technologies and strategically allocating resources to individuals at the highest risk of developing or reactivating active tuberculosis will significantly reduce the global tuberculosis burden.

4 Conclusion

Each year, technological progress facilitates the development of more efficient diagnostic techniques for identifying active tuberculosis in patients. Failure to identify drug-resistant TBI may result in inefficient treatment and the possible dissemination of drug-resistant strains throughout the wider population. Consequently, the foremost requirement for tuberculosis diagnosis involves bacteriological confirmation of the disease and the identification of medication resistance. Many diagnostic methods for tuberculosis, which rely on antibody detection or assessment of immune system reactivity, prove unsuccessful in individuals with compromised immune systems. Given the prevalent physical and clinical conditions in regions affected by the TBI pandemic, there is an urgent demand for a highly effective diagnostic approach. From a clinical perspective, it is crucial to pinpoint both the existence of TBI and any potential genetic alterations that could impede successful therapy to shorten the duration of therapy. Treatments need to eliminate the entire population of Mtb through sterilization rapidly. The diverse nature of TBI lesions and bacterial populations results in distinct subgroups of Mtb, each requiring specific antibiotic penetration.

Acknowledgment

The authors would like to thank all authors of the review paper included in this review.

Authors' Contribution

VIR and PY designed the project and wrote the manuscript text. AARM prepared figures. SSP supervised the project. All authors reviewed the manuscript.

Ethics

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

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