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Tuberculosis Diagnosis in the Era of SARS-CoV-2

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Authors' contributions

This work was carried out in collaboration between both authors. Authors WRUAB and DNMA have equally contributed to manuscript preparation and have read the manuscript before publishing.

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Review Article

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ABSTRACT

Tuberculosis (TB) continues to be a global public health emergency responsible for approximately 1.3 million deaths annually. Enduring the existing TB challenges, the emergence of "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2), a similar respiratory infection threatened the success of TB control over the past few years. Contemplating the irreversible damage of the human immunodeficiency virus (HIV), one of the leading immune-suppressive conditions, a similar or worst expected with this synergism: TB-HIV-SARS-CoV-2. Therefore, an integrated approach is much demanded before the impending revolution, "Next Global Pandemic". The advancement of molecular diagnostic techniques, blood transcriptomics uncovered the importance of studying the cross-talk between host and pathogens. RNA-sequencing is a high-throughput sequencing technique allowing detailed characterization of gene expression profiles. With the impact of SARS-CoV-2 on host immunity, pathogen-derived biomarker identification is more disease-specific and constrains individual variations faced during host biomarker identification. However, several technical hurdles are encountered during the study of intracellular pathogens like *Mycobacterium tuberculosis*. The development of advanced RNA-sequencing techniques to tackle the issues targeting the host and pathogen interactions is in their infancy and restricted to in-vitro studies. Few studies on serum exosomal RNA-sequencing of active and latent TB patients enlightened the path of TB biomarker discovery urging the necessity of more studies. Thus, this review will explicitly discuss the existing TB diagnostic tools to understand where we stand in TB diagnosis and the recent advancements in blood transcriptomics emphasizing the importance of targeting the pathogen-derived biomarkers as a potential source for future diagnostics.

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1. INTRODUCTION

Mycobacterium tuberculosis (Mtb) continues to be a global challenge and a major public health concern. It is one of the most life-threatening airborne diseases caused by a single bacterial pathogen invading the respiratory tract. *Mycobacterium tuberculosis* infects both lungs, and other body sites referred to as pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (EPTB), respectively. When the tubercle bacilli enter the bloodstream, the condition is called "miliary TB". When it occurs in surrounding tissues of the spinal cord and brain, it is referred to as tuberculous meningitis [1,2]. According to World Health Organization (WHO) in the year 2020, TB is accountable for more than 10 million new cases and 1.3 million deaths per year worldwide, in which nearly 4.2 million (42%) patients are not officially reported or diagnosed [2].

Tuberculosis (TB) is a disease of poverty due to its extensive distribution among low to middle-income economically distressed countries. Although TB is preventable and curable, risk factors such as poverty, undernutrition, immune-suppressive conditions, diabetes, smoking, alcohol use, and use of other drugs exacerbate the condition. This will make it challenging to diagnose and treat with the existing procedures leading to multidrug-resistant (MDR) and extensively drug-resistant (XDR) variants [2–4]. In 2020, 132 222 cases with multidrug or Rifampicin (RIF)-resistant TB (MDR/RR-TB) and 25 681 cases of pre extensively drug resistant TB (pre-XDR-TB) or extensively drug resistant TB (XDR-TB) with a total of 157 903 total DR-TB were reported [3]. Additionally, 214,000 deaths were reported due to HIV infection, one of the leading immunosuppressive conditions [2].

Apart from that, 2 billion asymptomatic patients which are one-fourth of the world's population (21.2-24.8%) are accountable for latent TB infection (LTBI). Latent TB (LTB) patients do not explicitly show symptoms, which permits the bacteria to survive inside the host for years without causing any disease [5]. Presumably, an asymptomatic individual can carry the disease throughout their lifetime, and the lifetime risk of disease reactivation is estimated to be 5-10% [6]. The risk of reactivation is higher within the first 2- to 5 years and depends on the predisposing factors. Once the immunity is low and breachable, bacteria can multiply and transform into the active stage [7]. To prevent the spread of TB, it is critical to identify the asymptomatic latent TB patients which demand accurate and timely treatment before disease reactivation [8].

Considering the prevailing challenges, the unexpected outbreak of new coronavirus, “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2), led the entire world into a tragic situation. Currently, the world is in terrible shape. On 11th March 2020, WHO declared coronavirus disease 2019 (COVID-19) as a global pandemic. According to the latest figures from WHO, the global coronavirus accounts for more than 440.8 million cases and 5.9 million deaths [9]. The numbers are increasing daily with the emergence of several new SARS-CoV-2 variants regardless of vaccination efforts (10,585,766,316 vaccine doses) [9]. A model estimated during the period of 2020 to 2025 an excess of 3.1-10.7% active TB (ATB) cases and 4-16% deaths are expected as a result of SARS-CoV-2 [10]. Not many countries are prepared for a pandemic situation. TB being the poor man’s disease, middle-low-income countries with deprived resources will undeniably struggle at the forefront of this pandemic [11,12]. The consequences will not only be limited to the developing countries but also a pandemic like COVID-19 can slowly spread to developed countries [13].

Recently much conversation is on the potential of reactivation of LTB in the presence of new SARS-CoV-2 [14,15]. TB bacterium is an opportunistic pathogen, waiting for the people’s immune system to become compromised to activate, which is previously proven by TB and HIV. A similar bidirectional synergy is expected to be observed from TB and SARS-CoV-2, declaring a new “perfect storm”. These two diseases share common social and biological risk factors encouraging disease transmission, progression, and poor TB treatment outcomes. These commonalities include, the disease transmission through aerosols, lungs as the primary infection site, overcrowding situation enhancing the disease spread, more risk is on immunocompromised individuals and shares similar symptoms [16]. TB being a long-standing disease, unless jointly managed, 8 years of global TB control efforts will go in vain and become a great threat to the global public health security regardless of the economic status of a country [13].

Currently, there is no effective diagnostic tool that can differentiate ATB from latent TB or the progression of LTB to ATB. The existing LTB tests show low efficacy when identifying HIV co-infected patients [8] and, BCG vaccinated children [17]. Further, LTB treatments are challenged by the unavailability of a gold standard diagnostic tool for emerging latent TB MDR Mtb strains [7]. Taking these into account, this review focused on the existing TB diagnostic tools to understand challenges faced over the past years and the existing knowledge on blood transcriptomics as a promising source of future pathogen-derived TB biomarker discovery to end TB in near future.

1.1 Current Approaches for Tuberculosis Diagnosis

Considering the existing diagnostic tests, detecting acid-fast bacilli (AFB) in sputum smears (sputum smear microscopy) is the most common method used in high TB burden countries and the gold standard culture method regardless of a lengthy and cumbersome process. Alternatively, molecular detection is more promising as a rapid diagnostic tool to facilitate the early detection of drug-resistant TB (DR-TB) and evaluate the treatment progression [18].

2. ACTIVE TUBERCULOSIS DIAGNOSIS

2.1 Microscopy-based Methods

The standard technique employed in high TB burden countries due to poor resource availability is the direct microscopic examination of AFB using pulmonary TB patients' sputum. Dr. Koch first introduced the principal staining and microscopic visualization of tubercle bacilli in 1882 [19]. Later it was modified by Franz Ziehl and Friedrich Neelsen in to Ziehl-Neelsen (ZN) acid-fast staining. However, this modification was first initiated by Paul Ehrlich, followed by Ziehl. The combined effort delivered the Ziehl-Neelsen acid-fast staining procedure to the world, widely used in low and middle income highly TB burdened countries [20].

Later, several modifications and improvements were introduced to the standard ZN staining. In 1914, Joseph J. Kinyoun modified the ZN staining into a cold staining procedure to avoid the heating stage of the traditional technique [21]. In 1938, Hagemann developed the first fluorescence staining using auramine as the fluorescent dye. This was later improved into auramine-rhodamine staining in 1962, examined under fluorescent microscopy (FM) using expensive halogen lamps and high compressed mercury lamps which is one of the drawbacks regardless being more sensitive [22]. Later, to make it affordable, this has been further improved with a replacement of a low cost Light Emitting Diode (LED). The most recent improvement of this technique introduced “SeeTB” as an alternative to conventional FM with improved sensitivity and deploy ability as a first line diagnostic test in high TB burden countries [23].

Overall, the microscopic examination of sputum is simple, inexpensive, and does not require sophisticated laboratory infrastructure to diagnose PTB rapidly. On the contrary, this method has lower sensitivity ranging from 20%-60% concerning detection of TB infected children [24] and immunocompromised individuals [25]. In addition, direct staining cannot distinguish between *M. tuberculosis* complex and other acid-fast organisms, such as the non-tuberculous mycobacteria (NTM) and *Nocardia* spp. [26].

2.2 Culture-based Methods

To overcome the drawbacks of direct microscopic examinations, another essential diagnostic tool, the culture-based technique, was introduced by Dr. Koch [19]. The culture-based phenotypic identification of TB is the gold standard method for TB diagnosis, including DR-TB, and to evaluate the treatment response [27]. The original culturing media consisted of a simple cow or sheep serum [19] but was later modified into the most widely used Lowenstein-Jensen media (LJ media). The LJ media consists of agar and egg-based medium first proposed by Wessely and Lowenstein in 1931 and later modified by Jensen in 1932. This culture-based method can distinguish NTM from Mtb by their colony characteristics and only facilitate mycobacteria to be grown in the addition of malachite green [28].

Two modifications were made to the LJ medium to make it more affordable and penetrable: Ogawa medium and Ogawa-Kudoh medium [29]. Later, the most significant modification was introduced as a liquid culture medium: Middlebrook 7H11 and Middlebrook 7H9. The only disadvantage of culture media is that Mtb is a slow-growing bacterium; the liquid culture media may take 1-3 weeks, and solid media

growth will take longer than 4-6 weeks. On the bright side, this is the initial step towards semi-automated and fully automated Mtb detection systems. At present, BACTEC Mycobacterial Growth Indicator Tube-MGIT-960 is used in almost all hospital settings to perform drug susceptibility tests (DST) and remain a standard method [30]. New additions with low turnaround time (TAT) are available; they are expensive and require trained laboratory staff and specialized laboratories [18].

2.3 Molecular Biology-based Methods

With the profound understanding of molecular biology and drug resistance of TB, rapid and specific molecular detection methods were developed as a promising alternative to the extensive process involved in phenotypic culture methods. Genotypic-molecular detection helps to rapidly identify the drug resistance at an early stage to initiate treatment plans which can be directly applied to the clinical specimens. However, this technique's major drawback is that compared with the culture method, they are less sensitive and should consistently be jointly implemented in diagnosis.

2.4 Nucleic Acid Amplification Tests (NAAT)

Nucleic Acid Amplification (NAA) test is based on polymerase chain reaction, and it identifies the genetic material unique to Mtb. The U.S. Food and Drug Administration (FDA) approved two major tests for rapid diagnosis of TB, which are Amplified *Mycobacterium tuberculosis* Direct (MTD) test (Gen-Probe) and Amplicor *M. tuberculosis* test (Roche Diagnostics). FDA first approved the MTD test in 1995, which could be used for AFB smear-positive respiratory specimens. Later in 1999, an improvement for this technique was introduced, MTD 2, which could be used for AFB smear-negative respiratory specimens [31]. In addition, number of in-house NAA tests which are not approved by WHO is also used in many clinical settings to diagnose TB efficiently [32, 33]. The sensitivity and specificity of NAA tests are 96% and 99%, respectively. The positive predictive value against positive AFB smear is >95%, and the negative predictive value for negative AFB smear ranges from 50-80%. This concludes that the NAA test is a reliable diagnostic tool for AFB smear positive specimens, potentially reducing the unnecessary TB treatment duration. However, the final decision relies upon culture confirmation [34].

Gene Xpert MTB/RIF assay is one of the most widely used WHO-approved rapid NAA tests to detect Mtb and primary mutations responsible for Rifampicin resistance for the initial diagnosis of TB. Gene Xpert MTB/RIF assay is a fully automated real-time DNA-based molecular diagnostic technique in which the results can be obtained within 2 hours [35]. This assay is helpful for adults and children (<15 years) with smear-negative PTB, EPTB and HIV. The results are better than that of AFB microscopy but comparable to the solid culture method with more specificity. However, this technique could not detect drug resistance outside the 81bp Rifampicin resistance region and should be accompanied by conventional methods to assess the treatment response [36].

With the advancement of molecular biology, WHO introduced three NAA tests with moderate-high complexity levels for TB diagnosis, including different drug resistance sites. The NAA tests and products evaluated are as following [37],

(1) Moderate complexity: Detection of TB and resistance to Rifampicin and Isoniazid.

- Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott), FluoroType MTBDR and FluoroType MTB (Hain Lifescience), BD MAX™ MDR-TB (Becton Dickinson) Cobas MTB and Cobas MTB-RIF/INH (Roche).

(2) Low complexity: Detection of resistance to Isoniazid and second-line anti-TB Agents.

- Xpert MTB/XDR (Cepheid).

(3) High complexity: Detection of resistance to Pyrazinamide.

- Genoscholar PZA-TB II (Nipro).

The latter technique is based on a different molecular biology method discussed next under molecular line probe assay. All these techniques have shown higher sensitivity and specificity in identifying MDR-TB with the least hands-on time. However, difficulties associated with the diagnostic operation and the cost involved will limit the distribution of these tests in high TB burden countries apart from their high accuracy [38].

2.5 Molecular Line-probe Assays (LPA)

Molecular line-probe (MLP) assay is a WHO recommended rapid detection method for MDR-TB based on reverse hybridization of DNA on a strip. Patients belong to MDR-TB are at least resistant to Rifampicin (RIF) or Isoniazid (INH), the two most potent first-line drugs. XDR-TB is resistant to INH and RIF plus any Fluoroquinolone (Ofloxacin, Levofloxacin, Moxifloxacin and Gatifloxacin) and at least one of the three injectable second-line drugs (Amikacin, Kanamycin, or Capreomycin) [39].

In 2008, WHO recommended using first-line (FL) line probe assay (LPA), GenoType MTBDRplus V1 and INNO-LiPA Rif.TB assay, for the rapid detection of MDR-TB, which are not currently used in clinical settings. Later in 2011, new advancements were made with enhanced sensitivity and to detect RIF and Isoniazid (INH) resistance using GenoType MTBDRplus version 2 and Nipro NTM+MDRTB detection kit 2. These LPAs were unveiled together with UNITAID and the Foundation for Innovative New Diagnostics (FIND) to Stop TB plan. The main target of this is to diagnose TB quickly and increase the availability of the drug to high TB burden countries [39].

In 2015 the latest LPAs were introduced to market, the GenoType MTBDRsl version 1.0, which can detect second-line TB drug resistance in gyrA and rrs regions. Later, GenoType MTBDRsl V2 similarly detects second-line TB drugs and injectables and a few additional mutations in gyrB and eis promoter region. These assays were recommended by WHO in 2016 [40].

The latest systematic review and a meta-analysis combining 74 studies evaluated the RIF and INH resistance in three LPAs commissioned by WHO to update a policy guide: Hain Genotype MTBDRplusV1, MTBDRplusV2 and Nipro NTM+MDRTB revealed a higher sensitivity and specificity for both RIF and INH resistance in smear-positive samples (RIF- 96.7%, 98.8% and INH- 90.2%, 99.2% sensitivity and specificity respectively, smear negative- 44% sensitivity in composite reference standard) [41]. Overall, LPAs accurately detect RIF resistance in MDR-TB and XDR-TB for smear-positive, adult PTB patients in high TB burden settings and minimal accuracy in smear-negative samples. But few discrepancies are present in detecting INH resistance to the best accuracy, and conventional culture-based DST is approved for INH resistance.

2.6 Loop-mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification is a commercial molecular assay developed by Eiken Chemical Company Ltd. (Tokyo, Japan) for resource-poor settings to detect *Mycobacterium tuberculosis* (MTB) complex (MTBC) [42]. Detection is based on the gyrB and IS regions of the MTBC genome [43]. This assay will only take one hour to give the results and could be visualized by the naked eye. This will only require a heating block and an Ultraviolet light visualizer [42]. WHO has introduced the LAMP method as an alternative to the AFB microscopy for adult PTB diagnosis and could be used in intermediate to high TB burden settings [18]. Still, discrepancies prevail not to use this method where HIV infection and drug resistance exist [43].

2.7 Lipoarabinomannan Urine Strip Test (LAM)

The existing diagnostic tests for PTB rely on sputum samples, whilst some children and seriously ill HIV infected patients cannot generate enough sputum. [44]. The current diagnostic methods are futile as sputum generated by these individuals does not have sufficient bacterial load (paucibacillary) to give a positive culture or a positive smear. In Africa, out of all ATB individuals, 40% is co-infected with HIV and at significant risk of death due to this problem [27].

Lipoarabinomannan (LAM) urine test is an alternative method approved by WHO that could be used for smear-negative, HIV infected both adult and young individuals. LAM assay is based on LAM antigen, a lipopolysaccharide present in mycobacterial cell walls, released from metabolically active or degenerating bacterial cells. LAM is effective when diagnosing HIV positive adults with PTB and EPTB, whose CD4+ cell count is less than or equal to 100 cells/µL, or HIV positive patients who are seriously ill [44, 45]. The same conditions apply to HIV positive children [45]. There is supportive evidence that LAM assay can predict the mortality of HIV infected children missed during respiratory sampling [46].

However, many data suggest low specificity and unacceptability of LAM assay for HIV positive children [47], severely acutely malnourished children [48], as well as for some HIV infected adults [49]. Also, the LAM assay is only suitable for Mtb endemic areas as it cannot distinguish Mtb from various other *Mycobacterium* sp. Considering both the positive and negative outcomes, LAM assay could be used as an inexpensive point of care platform, but this alone cannot be used as a screening or diagnostic test [45]. Further modifications and extensive research are required to the LAM assay [50].

3. LATENT TUBERCULOSIS DIAGNOSIS

3.1 Tuberculin Skin tests

The tuberculin skin test is one of the oldest immunology-based methods first developed by Dr. Robert Koch in 1890 and later modified by Van Pirquet and Charles Mantoux. The final composition of Purified Protein Derivatives (PPD) injected in TST was confirmed by Florence Seibert in 1934 [51]. TST is involved in Type-IV delayed hypersensitivity reaction mediated by CD4+ lymphocytes. Once the PPDs are injected, a local immune response will be triggered, causing a skin induration and erythema [52] which is precisely measured to check the status of the infection, whether it is “positive” or “negative”. However, after the first PPD administration, it will take 48-72 hours to obtain the results, making the patient visit the clinic multiple times. Also, reading should be taken by well-trained medical staff to avoid imprecise judgments [53].

The cocktail of Mtb antigens injected in PPD is typical to Mtb, *Mycobacterium bovi*s BCG, and NTM, thus leading to low specificity when differentiating Mtb infection from NTM infection and BCG vaccinated children giving false-positive results. Additionally, the delayed hypersensitive reaction involves CD4+ lymphocytes. As a result, immunocompromised individuals experiencing lower CD4+ lymphocytes will poorly respond to TST, giving false-negative results. Even though lower specificity may result in a diagnosis, TST shows higher sensitivity than IGRA, thus remaining as WHO-recommended diagnostic tool for LTBI in all persons living in low and middle-income countries and children <5 years [54].

In recent years, conventional TST was modified into four newer simple skin-based test strategies: Diaskintest (Generium Pharmaceutical, Moscow, Russia), C-Tb skin test (Statens Serum Institut, Copenhagen, Denmark), EC-skin test (Zhifei Longcom Biologic Pharmacy Co., Anhui, China) [55] and the DPPD test (Host Directed Therapeutics Bio Corp, Seattle, WA, USA). All these tests utilize recombinant ESAT-6 (dimer) and CFP-10 (monomer) antigens derived from *M. tuberculosis* and modified to obtain better specificity, sensitivity, and accuracy, except in the DPPD test which is a recombinant protein based on amino acids from the N-terminus sequence [56].

Diaskintest (DST) is the first novel skin test introduced to the market after approval. According to a meta-analysis, DST showed 86% sensitivity regardless of age and the accuracy is 95.1%. More importantly, DST showed its highest sensitivity (100%) among children and 60% sensitivity among HIV-positive patients [56]. The most recent systematic review and a meta-analysis among sub-populations of adults and children with HIV, children, individuals diagnosed with TB, and those exposed to the disease for all four tests; Diaskintest, C-Tb skin test, EC-skin test, and DPPD test, had a similar agreement with IGRA and TST (80% and more). The sensitivity reported is 91.18% for Diaskintest, 74.52% for the C-Tb skin test, and, 86.06% for the EC-skin test. The test specificity was only assessed for C-Tb, which is 99.15% for IGRA and 93.31% for TST. Overall, with the available data, all novel skin tests show similar diagnostic performance with IGRA and TST proving the possibility to replace the expensive skin tests with simpler skin-test platforms. However, more studies are demanded on both the EC-skin test and DPPD test [56].

3.2 Interferon Gamma Release Assay (IGRA)

Interferon Gamma Release Assay (IGRA) is an immunodiagnostic approach to detect Mtb. It is an improvement to the TST to minimize the false-positive results when diagnosing BCG vaccinated individuals, and most NTM infected cases except from *M. Kansasi*, *M. szulgai*, *M. marinum*, *M. flavescens*, and *M. gastrii* [57].

IGRA is based on the cell-mediated immune response, a measure of T cell mediated interferon-gamma (IFN-ϒ) release in response to Mtb specific antigens, namely, ESAT-6, CFP-10 & TB7.7. Currently, there are two commercially available tests approved by U.S. FDA and WHO which are, QuantiFERON-TB® assay (Cellestis

Limited, Australia) and T-SPOT-TB® (Oxford Immunotec, UK) [58]. Both these assays utilize two different techniques to measure the IFN- γ released by T cells. The QuantiFERON-TB® assay is based on enzyme-linked immunosorbent assay (ELISA), which measures the production of IFN-γ by circulating T-cells in whole blood, and T-SPOT-TB® is based on the Elispot technique, which measures the production of IFN-γ by peripheral blood mononuclear cells (PMBCs) [57].

IGRA shows higher specificity than TST (absence of BCG vaccination) with the available data, but both assays share similar sensitivity. Still, IGRA is recommended as an aid in diagnosing the infection but not to be performed routinely. Therefore, IGRA is preferred as a secondary test in the following circumstances, for individuals vaccinated with BCG, immunosuppressed patients (HIV, especially if CD4+ <200/mmc, or taking immunosuppressive drugs) and, children >5 years [59].

Concerning the disease progression to ATB, WHO reported neither IGRA nor TST is adequate, given the fact that both tests showed a Risk Ratio of 1.49 and 2.03, respectively, in a TB-risk population [60]. A recent systematic review revealed a positive IGRA, indicating positive LTBI and the IGRA value that helps determine the risk of progression to ATB infection [61].

UpToDate, there is a deficit of scientific publications to compare the efficacy of IGRA over TST due to limitations in methodologies, small sample size and inadequate statistical power [62]. Therefore, currently, there is no gold standard method for LTBI diagnosis. Both TST and IGRA could be used depending on the clinician's opinion, population risk category and cost involved. Regardless of these factors, treatments are initiated in HIV contacts and children <4 years as a step towards end TB strategy [60].

4. BIOMARKER DISCOVERY

UpToDate, none of the existing diagnostic tools can discriminate ATB from latent TB and progression of latent TB to ATB. With the rapid advancement of research and innovations, biomarker-based assays peaked in the arena as a solution for accurate, rapid point-of-care (POC) diagnostic tests [63]. According to the Office of Science Policy-National Institutes of Health (NIH), "Biological marker (biomarker) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [64]. These diagnostic biomarkers originate either from the host or the pathogen. TB biomarkers have been identified in different biological fluids such as blood, plasma, serum, urine, saliva, etc. However, it is crucial to choose a readily available and accessible biological sample that could be collected from all individuals regardless of their age, sex and pathological condition, or risk factors associated with the disease. Considering this fact, biomarker studies based on blood are popular among researchers [65].

MTB is an extremely successful intracellular pathogen invading the human lungs. Therefore, understanding the intricate transcriptomic cross-talk between the host and pathogen is greatly benefited by biomarker identification [66]. Transcriptomic is based on the gene transcriptional process. The changes in RNA expression (messenger RNA, microRNA, long noncoding RNA, small RNA) are identified and quantitatively measured compared to regular gene expression. The variable expression of RNA depicts the biological state of a cell, tissue, or organ, which can be either temporary or permanent. Therefore, transcriptomic studies are helpful in both diagnostic and prognostic aspects for, 1) TB biomarker identification for diagnosis purposes, 2) to evaluate the treatment success, 3) to identify the risk of progression from LTBI to ATB and, 4) for new drugs and vaccine development [65, 67].

4.1 Host-derived Blood Transcriptomic Biomarkers

Over the past years, several host-derived blood transcriptomic biomarkers have been identified in different populations to differentiate ATB from LTBI, and ATB and LTBI from healthy individuals, mainly with the use of microarray analysis and to a fewer extent RNA sequencing. In 2007, three gene biosignatures (CD64, LTF, and Rab33A) were identified in peripheral blood mononuclear cells (PBMCs), which allowed to discriminate between TB individuals and healthy individuals combining qPCR with microarray analysis [68]. A comprehensive blood transcriptional profiling of ATB, LTBI, and healthy controls generated 393-gene whole blood signature that can discriminate ATB from LTBI,

as well as an 86-gene set that can distinguish TB disease from other bacterial and inflammatory infections [69]. They also noted that ATB biosignatures were diminishing in patients following anti-TB treatment after two months and ultimately diminishing after 12 months. Another significant finding is that neutrophil-driven interferon-inducible genes primarily lead to ATB, consisting of both IFN-γ and type I IFN-αβ signaling. Later, Maertzdorf *et al.* [70] validated several biosignatures that have been identified from previous studies [68, 69, 71]. Further, they have identified new biosignatures similar to an autoimmune disease called systemic lupus erythematosus (SLE) [70]. A case-control study done by Kaforou et al. [72] identified a 27-gene whole blood signature that could distinguish TB and LTB, regardless of HIV infection status in an African adult population. Similarly, Anderson et al. [73] identified TB-specific transcriptional signatures for African children irrespective of HIV status.

A small-scale study (14 HIV infected TB patients and 15 controls) reported combined IL13-AIRE biomarkers could identify the HIV infected TB individuals eight months before ATB progression [74]. Similarly, Zak et al. [75] identified 16 gene signatures that can predict the progression of LTBI to ATB 12 months before disease activation with a 53.7% sensitivity and 82.8% specificity. This was later validated using fewer gene panels comprising three to four gene signatures to facilitate it as a point of care test [76–78].

Over time, “omics” based studies have been conducted to identify differentially expressed genes (DEGs) among ATB, LTBI, and healthy individuals to pave a way to generate a global set of biosignatures for ATB diagnosis [79, 80]. Up-to-date several validations have been performed using machine learning techniques along with datasets from different geographic locations to link the DEGs by several studies and to identify the simpler and lesser number of gene signatures that can discriminate various TB stages: ATB, LTBI, HIV infected TB patients, PTB, EPTB and Household contacts [81–88]. Recently, a four-gene signature that met the minimum WHO technology product profile (TPP) standards with a sensitivity of 90%, and 70% specificity for a triage test to discriminate patients with and without TB, irrespective of HIV status, was identified by Turner et al. [89]. Later, Gupta et al. [90] performed the largest meta-analysis using 17 pooled datasets, achieving eight gene signatures following WHO TPP standards to identify the risk of developing TB within 3-6 months; the most recent study successfully validated immune gene biomarkers to identify a minimum set of biosignatures suitable for TB diagnosis and progression. The GBP1+ IFITM3 panel met the minimum and optimal performance criteria for the ATB and LTBI groups [91]. However, further host biomarker validation remains a primary necessity due to biological variation among individuals in different geographic locations.

4.2 Pathogen-derived Blood Transcriptomic Biomarkers

Unlike host-derived biomarkers, pathogen-derived biomarkers are disease-specific and less prone to individual variations [92]. Therefore, attention towards pathogen-based biomarker studies will open up a novel pathway for TB biomarker studies. Over the past few years, major advancements were introduced into the field of RNA sequencing which is capable of analyzing the molecular interplay between the host and the pathogen simultaneously. These advanced technologies include; dual-RNA sequencing [93, 94], targeted RNA sequencing [95], single-cell RNA sequencing [96, 97], and exosomal RNA sequencing [98–100].

With TB, worryingly, all four RNA sequencing techniques are at their infancy. This is because, the detection of a sufficient amount of bacterial RNA in all biological fluids, importantly in peripheral blood is challenging (host RNA >98% and bacterial RNA <1%) and especially in a condition like LTB where the Mtb is restricted to the lungs [101]. Therefore, additional sophisticated and costly steps should be co-joined to target and sort the pathogen-infected cells or cellular particles and enrich the pathogen transcripts during the RNA extraction or analysis process making it complex. Due to these limitations, most of the mentioned RNA sequencing is conducted in-vitro utilizing different cell sorting techniques and transcript enriching techniques [66, 102, 103].

Overcoming this limitation, recently much attention is driven to exosomal RNA sequencing utilizing a small nanosized extracellular vesicle called exosomes in aid of clinical diagnostics and biomarker discovery. Exosomes are biological shuttles carrying proteins, lipids, DNA, and RNA

from the parent cell to neighboring or distant cells. The content transported via exosomes depends on the cell type and the pathological state of the cell. In a diseased cell, exosomes carry both pathogen and host-derived molecules, in which the pathogen-derived molecules for a lesser extent (1%) [104]. Mtb is an intracellular respiratory pathogen invading the lung tissues and surviving inside macrophages. Macrophages play an essential role against bacteria initiating the innate host immunity. As a result, mycobacteria ingested by macrophages, dendritic cells, and neutrophils reside in phagolysosomes later undergo autophagy, and the resulting extracellular vesicles (EVs) are released into the external environment. EVs can be released into various biological fluids in different ways. It could be either from host cells harboring degraded/undegraded bacterial molecules or EVs directly resulting from mycobacteria [105]. Therefore, either way, EVs carry both host and pathogen molecules and are potential biomarkers for disease diagnosis and therapeutics. Lately, more attention has been driven towards messenger RNA (mRNA), microRNA (miRNA), long noncoding RNA (lncRNA), circular RNA (circRNA), and bacterial small RNA (sRNA) for TB biomarker identification [98–100, 106]. Considering cumbersome, costly, and time-consuming host-pathogen RNA sequencing techniques, exosomal RNA sequencing is a promising technique that need further studies for potential biomarker identification.

4.3 Impact of SARS-CoV-2 Pandemic on Tuberculosis Diagnosis

TB is a longstanding disease due to the social stigma among people and tagged as the poor man's disease, thus making it difficult to diagnose and treat [107]. Still, TB is an underfunded area lacking proper diagnostic tests. One of the biggest challenges to overcome in TB diagnosis is the gap in new TB case notification. With much effort, the numbers were significantly improved from 5.8 million to 7.1 million from 2012 to 2019. With the onset of the SARS-CoV-2 pandemic, the numbers drastically declined to 5.8 million by deteriorating the success of TB control and elimination back to the year 2012 [2].

Globally we are battling with two severe respiratory infections SARS-CoV-2 and TB sharing common clinical symptoms making it difficult for early diagnosis based on clinical characteristics. Both SARS-CoV-2 and TB share fever, cough, fatigue, and difficulty in breathing. The most significant difference is the onset of symptoms, where TB will gradually develop the symptoms such as night sweating, gradual weight loss, hemoptysis, and loss of appetite taking several weeks to months, while SARS-CoV-2 will develop symptoms in a few (5-7) days with more non-respiratory symptoms such as fatigue, myalgia, headache, and gastrointestinal symptoms [108]. Several studies have reported simultaneous detection of TB and SARS-CoV-2 once a patient is referred to a hospital [109–113]. In many cases, TB co-infection is due to the reactivation of LTB, exacerbation of mild PTB [114], or co-infection with HIV conditions [113, 115] in which the host immunity is compromised [116]. Nonetheless, this suggests the initiation of differential diagnosis to identify TB in risk populations to reduce the new ATB cases that will ultimately count for the annual death toll.

The initial allotment of existing TB public health tools, personnel, and infrastructure to SARS-CoV-2 distressed the TB management. However, with time more funds dedicated to the pandemic and emerging novel diagnostic strategies have given an opportunity to simultaneously reach out to individuals with SARS-CoV-2 and TB following integrated testing recommended by the US Agency for International Development (USAID) and Stop TB partnership [117]. Currently, a wide range of molecular diagnostic tests is available for TB and SARS-CoV-2 diagnosis. The common platforms utilized by both diseases are, GeneXpert (Cepheid, USA), Truenat (Molbio, India), RealTime (Abbott, USA), BD MAX (BD, USA), Hain FluoroType (Brukner, Germany), Loopamp (Eiken Chemical Co., Japan), Cobas 6800/8800 (Roche, Switzerland), Standard M (SD. Biosensor, Republic of Korea) and EasyNAT (UStar Biotechnologies, China) [108, 118]. The disparity between the two diseases using two different biological samples; sputum in TB and most commonly nasopharyngeal swabs in SARS-CoV-2, and the cost involved with the cartridges for these molecular diagnostic tests are questionable when implemented as an integrated test in low and middle-income countries. A recent study reported that the simultaneous detection of SARS-CoV-2 and TB with throat swabs increases the chance of developing an integrated system to tackle both infections [110].

5. CONCLUSIONS AND FUTURE DIRECTIONS

Starting from 1993, by which TB is declared as a global health emergency the evolution of TB diagnosis is slow and the innovations are inadequate to tackle TB. The existing global TB challenges; HIV pandemic, the emergence of MDR-TB and XDR-TB, and reactivation of LTBI remain unresolved with no gold standard method for both ATB and LTB diagnosis. With the advent of SARS-CoV-2, TB became non-urgent emergency by hindering its' control and elimination efforts, risking thousands of lives whom had the potential of curing the disease with ATB treatment or stopping the initiation of LTB activation by proper and early diagnosis. After 2 years from the starting of this pandemic, even though high income countries could handle the up surging economic crisis, all the middle-low-income countries face a huge economic devastation which will eventually drag the TB control in to a tragedy. The damage done by SARS-CoV-2 is profound and irreversible for many years. Taking all these into account, there is a pressing need for accurate, rapid and, cost-effective diagnostic tools for active and latent TB diagnosis, apart from the costly, time-consuming, laborious tools which require specialized laboratories and trained staff. Developing a simpler and cost-effective point-of-care test using novel specimens is critical for a higher deploy ability. This has been achieved by SARS-CoV-2 with a record breaking time, ultimately leading to home-based diagnostic kits. Similar efforts are demanded with active public awareness and attracting more funds towards research innovations for TB. In future, renewed hope in advanced research on host and pathogen-derived transcriptomics together with sophisticated bioinformatics will pave a way to new biomarker identification. Considering the existing evidence, future studies based on exosomal transcriptomics for pathogen-derived biomarker identification have much more potential, minimizing individual variations rendering high disease specificity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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