Review Article

In the era of SARS-CoV-2: where we stand in tuberculosis diagnosis

Abstract

Tuberculosis (TB) continues to be a global public health emergency responsible for approximately 1.2 million deaths annually. Enduring the existing TB challenges, the emergence of "Severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2), a similar respiratory virus 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 are in their infancy and restricted to in-vitro studies. Few studies on exosomal RNA-sequencing on human sera 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.

Keywords: Biomarkers, Blood, Diagnostic tools, Tuberculosis, Transcriptomics

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. Mtb 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), tuberculosis is accountable for more than 10 million new cases and 1.2 million deaths per year worldwide. In which 2.9 million (29%) patients are not officially reported or diagnosed [3].

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 2019, 206 030 people with multidrug or rifampicin (RIF)-resistant TB (MDR/RR-TB) cases were reported, which is a 10% increase compared to 2018 TB cases (186 883). Additionally, 208,000 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%. 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

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active stage [6]. 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 [7].

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. 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) [8]. A model estimated during the period of 2020 to 2025 an excess of 3.1-10.7% active TB cases and 4-16% deaths is expected as a result of SARS-CoV-2 [9]. 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 [10, 11]. The consequences will not only be limited to the developing countries but also a pandemic like COVID-19 can slowly spread to developed countries [12].

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Recently much conversation is on the potential of reactivation of LTB in the presence of new SARS-CoV-2 [13, 14]. 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 [15]. 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 [12].

Currently, there is no effective diagnostic tool that can differentiate active TB from latent TB or the progression of LTB to active TB. The existing LTB tests show low efficacy when identifying HIV co-infected patients [7] and, BCG vaccinated children [16]. Further, LTB treatments are challenged by the unavailability of a gold standard diagnostic tool and moreover, emerging MDR Mtb strains [6]. Taking these into account, this review will focus on the existing tuberculosis 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.

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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 [17].

Active tuberculosis diagnosis

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 [18]. 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 burden countries [19].

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 [20]. 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 [21]. Later, to make it affordable, this has been further improved with a replacement of 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 deployability as a first line diagnostic test in high TB burden countries [22].

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 [23] and immunocompromised individuals [24]. 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. [25].

Culture-based methods

To overcome the drawbacks of direct microscopic examinations, another essential diagnostic tool, the culture-based technique, was introduced by Dr. Koch [18]. The culture-based phenotypic identification of TB is the gold standard method for TB diagnosis, including DR-TB, and to evaluate the treatment response [26]. The original culturing media consisted of a simple cow or sheep serum [18] 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 [27].

Two modifications were made to the LJ medium to make it more affordable and penetrable: Ogawa medium and Ogawa-Kudoh medium [28]. 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 [29]. New additions with low turnaround time (TAT) are available; they are expensive and require trained laboratory staff and specialized laboratories [17].

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 rapidly identify the drug resistance at an early stage to initiate treatment plans, and they 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.

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 [30]. Also, number of in-house NAA tests which are not approved by WHO is used in many clinical settings to diagnose TB efficiently [31, 32]. 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 [33].

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 [34]. 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 [35].

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 [36],

(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 [37].

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 is resistant to at least 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) [38].

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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 [38].

In 2015 the latest LPAs were introduced to the market 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 few additional mutations in gyrB and eis promoter region. WHO recommended these in 2016 [39].

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 and 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) [40]. 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.

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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) [41]. Detection is based on the gyrB and IS regions of the MTBC genome [42]. 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 [41]. 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 [17]. Still, discrepancies prevail not to use this method where HIV infection and drug resistance exist [42].

Lipoarabinomannan urine strip test (LAM)

The existing diagnostic tests for PTB rely on sputum samples, whilst some children cannot generate enough sputum and seriously ill HIV infected patients [43]. 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 active TB individuals, 40% is co-infected with HIV and at significant risk of death due to this problem [26].

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 [43, 44]. The same conditions apply to

HIV positive children [44]. There is supportive evidence that LAM assay can predict the mortality of HIV infected children missed during respiratory sampling [45].

However, many data suggest low specificity and unacceptability of LAM assay for HIV positive children [46], severely acutely malnourished children [47], as well as for some HIV infected adults [48]. 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 [44]. Further modifications and extensive research are required to the LAM assay [49].

Latent tuberculosis diagnosis

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 [50]. 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 [51] 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 [52].

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 [53].

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) [54] 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 [55].

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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 [55]. The most recent systematic review and a meta-analysis among sub-populations of adults and children with HIV, children, individuals diagnosed with tuberculosis, 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 [55].

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* [56].

IGRA is based on the cell-mediated immune response, a measure of T cell mediated interferon-gamma (IFN-ɤ) 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) [57]. 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) [56].

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 [58].

Concerning the disease progression to active TB, 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 [59]. A recent systematic review revealed a positive IGRA, indicating

positive LTBI and the IGRA value that helps determine the risk of progression to active TB infection [60].

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 [61]. 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 [59].

Biomarker discovery

UpToDate, none of the existing diagnostic tools can discriminate active TB from latent TB and progression of latent TB to active TB. 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 [62]. 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" [63]. 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 [64].

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 [65]. 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 [64, 66].

Host-derived blood transcriptomic biomarkers

Over the past years, several host-derived blood transcriptomic biomarkers have been identified in different populations to differentiate active TB (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) ~~was~~ were identified in

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peripheral blood mononuclear cells (PBMCs), which allowed to discriminate between TB individuals and healthy individuals combining qPCR with microarray analysis [67]. 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 [68]. 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.* [69] validated several biosignatures that have been identified from previous studies [67, 68, 70]. Further, they have identified new biosignatures similar to an autoimmune disease called systemic lupus erythematosus (SLE) [69]. A case-control study done by Kaforou et al. [71] identified a 27-gene whole blood signature that can distinguish TB and LTB, regardless of HIV infection status in an African adult population. Similarly, Anderson et al. [72] identified TB-specific transcriptional signatures for African children irrespective of HIV status.

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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 [73]. Similarly, Zak et al. [74] 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 [75–77].

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 active TB diagnosis [78, 79]. 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 [80–87]. 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 tuberculosis, irrespective of HIV status, was identified by Turner et al. [88]. Later, Gupta et al. [89] performed the largest meta-analysis using 17 pooled datasets, achieving eight gene signatures following WHO TPP standards to identify the risk of developing tuberculosis 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 [90]. However, further host biomarker validation remains a primary necessity due to biological variation among individuals in different geographic locations.

Pathogen-derived blood transcriptomic biomarkers

Unlike host-derived biomarkers, pathogen-derived biomarkers are disease-specific and less prone to individual variations [91]. 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 [92, 93], targeted RNA sequencing [94], single-cell RNA sequencing [95, 96], and exosomal RNA sequencing [97–99].

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 latent tuberculosis where the Mtb is restricted to the lungs [100]. Therefore, additional sophisticated and costly steps should be conjoined 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 [65, 101, 102].

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%) [103]. 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 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 [104]. 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 [97–99, 105]. Considering cumbersome, costly, and time-consuming host-pathogen RNA sequencing techniques, exosomal RNA sequencing is a promising technique that needs further studies for potential biomarker identification.

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Conclusions and future directions

The upsurge of challenges encountered by the global tuberculosis control with the new SARS-CoV-2 pandemic, coupled with the HIV pandemic, the emergence of MDR-TB and XDR-TB, and reactivation of latent tuberculosis ascended the pressing need for accurate, rapid, and, a cost-effective diagnostic tools for active and latent TB individuals. Apart from the costly, time-consuming, laborious tools which require specialized laboratories and trained staff, developing simpler and cost-effective POC tests are demanded. As a solution, renewed hope in advanced research on the host and pathogen-derived transcriptomics together with sophisticated bioinformatics will pave the way to new biomarker identification. Considering the existing evidence, future studies based on pathogen-derived biomarkers will be of much helpful due to their high specificity to eradicate TB in near future.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

References

1. Centres for Disease Control and Prevention (2013) To View or Order the Core Curriculum on Tuberculosis. 19–43
2. World Health Organization (2021) Tuberculosis
3. Chakaya J, Khan M, Ntoumi F, et al (2021) Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. Int J Infect Dis 113:S7–S12. https://doi.org/10.1016/j.ijid.2021.02.107
4. Silva DR, Marcela Muñoz-Torrico RD, Tatiana G, et al (2018) Risk factors for tuberculosis: diabetes, smoking, alcohol use, and the use of other drugs. Boletín UNAM-DGCS-187bis Ciudad Univ 44:145–152
5. Ai JW, Ruan QL, Liu QH, Zhang WH (2016) Updates on the risk factors for latent tuberculosis reactivation and their managements. Emerg Microbes Infect 5:e10. https://doi.org/10.1038/emi.2016.10
6. Knight GM, McQuaid CF, Dodd PJ, Houben RMGJ (2019) Global burden of latent

multidrug-resistant tuberculosis: trends and estimates based on mathematical modelling. Lancet Infect Dis 19:903–912. https://doi.org/10.1016/S1473-3099(19)30307-X

7. Kasprowicz VO, Churchyard G, Lawn SD, et al (2011) Diagnosing latent tuberculosis in high-risk individuals: Rising to the challenge in high-burden areas. J Infect Dis 204:. https://doi.org/10.1093/infdis/jir449

8. World Health Organization WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/. Accessed 5 Mar 2022

9. Finn McQuaid C, McCreesh N, Read JM, et al (2020) The potential impact of COVID-19-related disruption on tuberculosis burden. Eur Respir J 56:. https://doi.org/10.1183/13993003.01718-2020

10. Gupta A, Singla R, Caminero JA, et al (2020) Impact of COVID-19 on tuberculosis services in India. Int J Tuberc Lung Dis 24:637–639. https://doi.org/10.5588/ijtld.20.0212

11. Soko RN, Burke RM, Feasey HRA, et al (2021) Effects of coronavirus disease pandemic on tuberculosis notifications, malawi. Emerg Infect Dis 27:1831–1839. https://doi.org/10.3201/eid2707.210557

12. Nikolayevskyy V, Holicka Y, van Soolingen D, et al (2021) Impact of the COVID-19 pandemic on tuberculosis laboratory services in Europe. Eur Respir J 57:. https://doi.org/10.1183/13993003.03890-2020

13. Lee G, Stoll JJ, El Husseini I (2021) A Rare Case of Latent Tuberculosis Reactivation in the Setting of COVID-19 Infection. Am J Respir Crit Care Med 203:

14. Khayat M, Fan H, Vali Y (2021) COVID-19 promoting the development of active tuberculosis in a patient with latent tuberculosis infection: A case report. Respir Med Case Reports 32:101344. https://doi.org/10.1016/j.rmcr.2021.101344

15. Aguillón-Durán GP, Prieto-Martínez E, Ayala D, et al (2021) COVID-19 and chronic diabetes: the perfect storm for reactivation tuberculosis?: a case series. J Med Case Rep 15:1–5. https://doi.org/10.1186/s13256-021-03193-7

16. Hamzaoui A (2015) Childhood tuberculosis. Rev Pneumol Clin 71:168–180. https://doi.org/10.1016/j.pneumo.2014.03.006

17. Zijenah LS (2018) The World Health Organization Recommended TB Diagnostic Tools. Tuberculosis. https://doi.org/10.5772/intechopen.73070

18. Formad HF (1885) The etiology of tuberculosis. J Am Med Assoc IV:312–316. https://doi.org/10.1001/jama.1885.02390870004001a

19. Bishop PJ, Neumann G (1970) The history of the Ziehl-Neelsen stain. Tubercle 51:196–206. https://doi.org/10.1016/0041-3879(70)90073-5

20. Kinyoun JJ (1915) a Note on Uhlenhuths Method for Sputum Examination, for Tubercle Bacilli. Am J Public Health 5:867–870. https://doi.org/10.2105/ajph.5.9.867

21. McCLURE DM (1953) The development of fluorescence microscopy for tubercle bacilli and its use as an adjunct to histological routine. J Clin Pathol 6:273–281.

https://doi.org/10.1136/jcp.6.4.273

22. Pandey V, Singh P, Singh S, et al (2019) SeeTB: A novel alternative to sputum smear microscopy to diagnose tuberculosis in high burden countries. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-52739-9

23. Kunkel A, Abel zur Wiesch P, Nathavitharana RR, et al (2016) Smear positivity in paediatric and adult tuberculosis: Systematic review and meta-analysis. BMC Infect Dis 16:1–9. https://doi.org/10.1186/s12879-016-1617-9

24. Tiamiyu AB, Iliyasu G, Dayyab FM, et al (2020) A descriptive study of smear negative pulmonary tuberculosis in a high HIV burden patient's population in North Central Nigeria. PLoS One 15:1–9. https://doi.org/10.1371/journal.pone.0238007

25. Olson ES, Simpson AJH, Norton AJ, Das SS (1998) Not everything acid fast is Mycobacterium tuberculosis - A case report. J Clin Pathol 51:535–536. https://doi.org/10.1136/jcp.51.7.535

26. World Health Organization (2020) Global Tuberculosis report 2020. Geneva, Switzerland

27. Central TB Division Directorate (2009) Revised National TB Control Programme Training Manual for Mycobacterium tuberculosis Culture & Drug susceptibility testing. Cent TB Div Dir Gen Heal Serv Minist Heal Fam Welfare, Nirman Bhawan, New Delhi 76

28. Kudoh S, Kudoh T (1974) A simple technique for culturing tubercle bacilli. Bull World Health Organ 51:71–82

29. Moreira ADSR, Huf G, Vieira MAMDS, et al (2015) Liquid vs solid culture medium to evaluate proportion and time to change in management of suspects of tuberculosis - A pragmatic randomized trial in secondary and tertiary health care units in Brazil. PLoS One 10:1–11. https://doi.org/10.1371/journal.pone.0127588

30. CDC (2009) Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. MMWR 58:7–10

31. Magana-Arachchi DN, Perera J, Gamage S, Chandrasekharan V (2008) Low cost in-house PCR for the routine diagnosis of extra-pulmonary tuberculosis. Int J Tuberc Lung Dis 12:275–280

32. Wei Z, Zhang X, Wei C, et al (2019) Diagnostic accuracy of in-house real-time PCR assay for Mycobacterium tuberculosis: A systematic review and meta-analysis. BMC Infect Dis 19:1–11. https://doi.org/10.1186/s12879-019-4273-z

33. the Blue Cross, Association BS (2021) Testing for Diagnosis of Active or Latent Tuberculosis AHS –G2063

34. WHO (2016) Automated Real-Time Dna Amplification Test for Rapid and Simultaneous Detection of Tb and Rifampicin Resistance

35. Hopmeier D, Lampejo T, Rycroft J, et al (2020) The limitations of the Cepheid GeneXpert® Mtb/Rif assay for the diagnosis and management of polyresistant pulmonary

tuberculosis. Clin Infect Pract 7–8:100038. https://doi.org/10.1016/j.clinpr.2020.100038

36. World Health Organization (2021) Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB : rapid communication

37. de Vos M, Scott L, David A, et al (2021) Comparative analytical evaluation of four centralized platforms for the detection of mycobacterium tuberculosis complex and resistance to rifampicin and isoniazid. J Clin Microbiol 59:1–11. https://doi.org/10.1128/JCM.02168-20

38. Tagiliani DCE (2018) Line probe assays for drug- resistant tuberculosis detection. 34

39. World Health Organization (2016) End tb. use Mol line probe assays Detect Resist to Second anti-tuberculosis drugs Policy Guid 1–40

40. Nathavitharana RR, Cudahy PGT, Schumacher SG, et al (2017) Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: A systematic review and meta-analysis. Eur Respir J 49:. https://doi.org/10.1183/13993003.01075-2016

41. WHO (2016) Fact sheet-Tb-Lamp/Loop-Mediated Isothermal Amplification for the Detection of M. Tuberculosis. Tuberc Diagnostics

42. Shete PB, Farr K, Luke S, et al (2019) Diagnostic accuracy of TB-LAMP for pulmonary tuberculosis: a systematic review and meta-analysis. BMJ Open 9:1–11. https://doi.org/10.1136/bmjopen-2019-033084

43. Sabur NF, Esmail A, Brar MS, Dheda K (2017) Diagnosing tuberculosis in hospitalized HIV-infected individuals who cannot produce sputum: Is urine lipoarabinomannan testing the answer? BMC Infect Dis 17:1–6. https://doi.org/10.1186/s12879-017-2914-7

44. World Health Organization (2016) Lateral Flow Urine for the Diagnosis and Screening of Active Tuberculosis in People Living With Hiv Who Recommendations. World Heal Organ

45. Lacourse SM, Cranmer LM, Njuguna IN, et al (2018) Urine Tuberculosis Lipoarabinomannan Predicts Mortality in Hospitalized Human Immunodeficiency Virus-Infected Children. Clin Infect Dis 66:1798–1801. https://doi.org/10.1093/cid/ciy011

46. Nicol MP, Allen V, Workman L, et al (2014) Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: A prospective study. Lancet Glob Heal 2:e278–e284. https://doi.org/10.1016/S2214-109X(14)70195-0

47. Schramm B, Nganaboy RC, Uwiragiye P, et al (2021) Potential value of urine lateral-flow lipoarabinomannan (LAM) test for diagnosing tuberculosis among severely acute malnourished children. PLoS One 16:1–13. https://doi.org/10.1371/journal.pone.0250933

48. Thit SS, Aung NM, Htet ZW, et al (2017) The clinical utility of the urine-based lateral flow lipoarabinomannan assay in HIV-infected adults in Myanmar: An observational study. BMC Med 15:1–11. https://doi.org/10.1186/s12916-017-0888-3

49. MacLean E, Pai M (2018) Urine lipoarabinomannan for tuberculosis diagnosis: Evolution and prospects. Clin Chem 64:1133–1135. https://doi.org/10.1373/clinchem.2018.286625

50. Daniel TM (2006) The history of tuberculosis. Respir Med 100:1862–1870. https://doi.org/10.1016/j.rmed.2006.08.006

51. Marwa K, Kondamudi NP (2021) Type IV Hypersensitivity Reaction. In: StatPearls Publ.

52. Walker H, Hall W, Hurst J (1990) Clinical Methods: The History, Physical, and Laboratory Examinations., 3rd Editio. Butterworth, Boston

53. Aggerbeck H, Ruhwald M, Hoff ST, et al (2018) C-Tb skin test to diagnose Mycobacterium tuberculosis infection in children and HIV-infected adults: A phase 3 trial. PLoS One 13:1–16. https://doi.org/10.1371/journal.pone.0204554

54. Hamada Y, den Boon S, Cirillo DM, et al (2021) Framework for the evaluation of new tests for tuberculosis infection. Eur Respir J 58:. https://doi.org/10.1183/13993003.04078-2020

55. Krutikov M, Faust L, Nikolayevskyy V, et al (2022) The diagnostic performance of novel skin-based in-vivo tests for tuberculosis infection compared with purified protein derivative tuberculin skin tests and blood-based in vitro interferon-γ release assays: a systematic review and meta-analysis. Lancet Infect Dis 22:250–264. https://doi.org/10.1016/S1473-3099(21)00261-9

56. Lalvani A, Pareek M (2010) Interferon gamma release assays: principles and practice. Enferm Infecc Microbiol Clin 28:245–252. https://doi.org/10.1016/j.eimc.2009.05.012

57. Centres for Disease Control and Prevention (2016) TB Elimination Interferon-Gamma Release Assays (IGRAs)-Blood Tests for TB Infection What are they? How do they work? 3–5

58. ECDC (2011) Use of interferon-gamma release assays in support of TB diagnosis

59. World Health Organization (2018) Latent tuberculosis infection Updated and consolidated guidelines for programmatic management

60. Ledesma JR, Ma J, Zheng P, et al (2021) Interferon-gamma release assay levels and risk of progression to active tuberculosis : a systematic review and dose-response meta-regression analysis. 1–10

61. Gualano G, Mencarini P, Lauria FN, et al (2019) Tuberculin skin test – Outdated or still useful for Latent TB infection screening? Int J Infect Dis 80:S20–S22. https://doi.org/10.1016/j.ijid.2019.01.048

62. McNerney R, Maeurer M, Abubakar I, et al (2012) Tuberculosis diagnostics and biomarkers: Needs, challenges, recent advances, and opportunities. J Infect Dis 205:147–158. https://doi.org/10.1093/infdis/jir860

63. Atkinson AJ, Colburn WA, DeGruttola VG, et al (2001) Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther 69:89–95. https://doi.org/10.1067/mcp.2001.113989

64. Sandvik AK, Alsberg BK, Nørsett KG, et al (2006) Gene expression analysis and clinical diagnosis. Clin Chim Acta 363:157–164. https://doi.org/10.1016/j.cccn.2005.05.046

65. Westermann AJ, Gorski SA, Vogel J (2012) Dual RNA-seq of pathogen and host. Nat Rev Microbiol 10:618–630. https://doi.org/10.1038/nrmicro2852

66. Burel JG, Babor M, Pomaznoy M, et al (2019) Host transcriptomics as a tool to identify diagnostic and mechanistic immune signatures of tuberculosis. Front Immunol 10:1–12. https://doi.org/10.3389/fimmu.2019.00221

67. Jacobsen M, Repsilber D, Gutschmidt A, et al (2007) Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. J Mol Med 85:613–621. https://doi.org/10.1007/s00109-007-0157-6

68. Berry MPR, Graham CM, McNab FW, et al (2010) An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466:973–977. https://doi.org/10.1038/nature09247

69. Maertzdorf J, Ota M, Repsilber D, et al (2011) Functional correlations of pathogenesis-driven gene expression signatures in tuberculosis. PLoS One 6:1–8. https://doi.org/10.1371/journal.pone.0026938

70. Maertzdorf J, Repsilber D, Parida SK, et al (2011) Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun 12:15–22. https://doi.org/10.1038/gene.2010.51

71. Kaforou M, Wright VJ, Oni T, et al (2013) Detection of Tuberculosis in HIV-Infected and -Uninfected African Adults Using Whole Blood RNA Expression Signatures: A Case-Control Study. PLoS Med 10:. https://doi.org/10.1371/journal.pmed.1001538

72. Anderson ST, Kaforou M, Brent AJ, et al (2014) Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa. N Engl J Med 370:1712–1723. https://doi.org/10.1056/nejmoa1303657

73. Sloot R, Schim van der Loeff MF, van Zwet EW, et al (2015) Biomarkers Can Identify Pulmonary Tuberculosis in HIV-infected Drug Users Months Prior to Clinical Diagnosis. EBioMedicine 2:172–179. https://doi.org/10.1016/j.ebiom.2014.12.001

74. Zak DE, Penn-Nicholson A, Scriba TJ, et al (2016) A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 387:2312–2322. https://doi.org/10.1016/S0140-6736(15)01316-1

75. Suliman S, Thompson EG, Sutherland J, et al (2018) Four-gene pan-African blood signature predicts progression to tuberculosis. Am J Respir Crit Care Med 197:1198–1208. https://doi.org/10.1164/rccm.201711-2340OC

76. Warsinske HC, Rao AM, Moreira FMF, et al (2018) Assessment of Validity of a Blood-Based 3-Gene Signature Score for Progression and Diagnosis of Tuberculosis, Disease Severity, and Treatment Response. JAMA Netw open 1:e183779. https://doi.org/10.1001/jamanetworkopen.2018.3779

77. Thompson EG, Du Y, Malherbe ST, et al (2017) Host blood RNA signatures predict the outcome of tuberculosis treatment. Tuberculosis 107:48–58. https://doi.org/10.1016/j.tube.2017.08.004

78. Lesho E, Forestiero FJ, Hirata MH, et al (2011) Transcriptional responses of host peripheral blood cells to tuberculosis infection. Tuberculosis 91:390–399. https://doi.org/10.1016/j.tube.2011.07.002

79. Bloom CI, Graham CM, Berry MPR, et al (2013) Transcriptional Blood Signatures Distinguish Pulmonary Tuberculosis, Pulmonary Sarcoidosis, Pneumonias and Lung Cancers. PLoS One 8:. https://doi.org/10.1371/journal.pone.0070630

80. Sambarey A, Devaprasad A, Mohan A, et al (2017) Unbiased Identification of Blood-based Biomarkers for Pulmonary Tuberculosis by Modeling and Mining Molecular Interaction Networks. EBioMedicine 15:112–126. https://doi.org/10.1016/j.ebiom.2016.12.009

81. Sweeney TE, Braviak L, Tato CM, Khatri P (2017) Tuberculosis: a Multicohort Analysis. Lancet Respir Med 4:213–224. https://doi.org/10.1016/S2213-2600(16)00048-5.Genome-wide

82. Petrilli JD, Araújo LE, da Silva LS, et al (2020) Whole blood mRNA expression-based targets to discriminate active tuberculosis from latent infection and other pulmonary diseases. Sci Rep 10:1–9. https://doi.org/10.1038/s41598-020-78793-2

83. Lu C, Wu J, Wang H, et al (2011) Novel biomarkers distinguishing active tuberculosis from latent infection identified by gene expression profile of peripheral blood mononuclear cells. PLoS One 6:1–10. https://doi.org/10.1371/journal.pone.0024290

84. Wang S, He L, Wu J, et al (2019) Transcriptional Profiling of Human Peripheral Blood Mononuclear Cells Identifies Diagnostic Biomarkers That Distinguish Active and Latent Tuberculosis. Front Immunol 10:. https://doi.org/10.3389/fimmu.2019.02948

85. Dupnik K, Bean J, Lee M, et al (2018) Blood transcriptomic markers of Mycobacterium tuberculosis load in sputum. 22:950–958. https://doi.org/10.5588/ijtld.17.0855.Blood

86. Leong S, Zhao Y, Joseph NM, et al (2018) Existing blood transcriptional classifiers accurately discriminate active tuberculosis from latent infection in individuals from south India. Tuberculosis 109:41–51. https://doi.org/10.1016/j.tube.2018.01.002

87. Estévez O, Anibarro L, Garet E, et al (2020) An RNA-seq Based Machine Learning Approach Identifies Latent Tuberculosis Patients With an Active Tuberculosis Profile. Front Immunol 11:1–12. https://doi.org/10.3389/fimmu.2020.01470

88. Turner CT, Gupta RK, Tsaliki E, et al (2020) Blood transcriptional biomarkers for active pulmonary tuberculosis in a high-burden setting: a prospective, observational, diagnostic accuracy study. Lancet Respir Med 8:407–419. https://doi.org/10.1016/S2213-2600(19)30469-2

89. Gupta RK, Turner CT, Venturini C, et al (2020) Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. Lancet Respir Med 8:395–406. https://doi.org/10.1016/S2213-2600(19)30282-6

90. Perumal P, Abdullatif MB, Garlant HN, et al (2021) Validation of Differentially Expressed Immune Biomarkers in Latent and Active Tuberculosis by Real-Time PCR. Front Immunol 11:1–19. https://doi.org/10.3389/fimmu.2020.612564

91. Tucci P, González-Sapienza G, Marin M (2014) Pathogen-derived biomarkers for active tuberculosis diagnosis. Front Microbiol 5:1–6. https://doi.org/10.3389/fmicb.2014.00549

92. López-Agudelo VA, Baena A, Barrera V, et al (2022) Dual RNA Sequencing of Mycobacterium tuberculosis-Infected Human Splenic Macrophages Reveals a Strain-Dependent Host–Pathogen Response to Infection. Int J Mol Sci 23:. https://doi.org/10.3390/ijms23031803

93. Pisu D, Huang L, Grenier JK, Russell DG (2020) Dual RNA-Seq of Mtb-Infected Macrophages In Vivo Reveals Ontologically Distinct Host-Pathogen Interactions. Cell Rep 30:335-350.e4. https://doi.org/10.1016/j.celrep.2019.12.033

94. Cornejo-Granados F, López-Leal G, Mata-Espinosa DA, et al (2021) Targeted rna-seq reveals the m. Tuberculosis transcriptome from an in vivo infection model. Biology (Basel) 10:1–18. https://doi.org/10.3390/biology10090848

95. Gideon HP, Hughes TK, Wadsworth MH, et al (2020) Single-cell profiling of tuberculosis lung granulomas reveals functional lymphocyte signatures of bacterial control. bioRxiv 2020.10.24.352492

96. Cai Y, Dai Y, Wang Y, et al (2020) Single-cell transcriptomics of blood reveals a natural killer cell subset depletion in tuberculosis. eBioMedicine 53:102686. https://doi.org/10.1016/j.ebiom.2020.102686

97. Lv L, Li C, Zhang X, et al (2017) RNA profiling analysis of the serum exosomes derived from patients with active and latent Mycobacterium tuberculosis infection. Front Microbiol 8:1–10. https://doi.org/10.3389/fmicb.2017.01051

98. Singh, Pratap, Schorey J (2015) Exosomal RNA from Mycobacterium tuberculosis infected cells is functional in recipient macrophages. Traffic 16:555–571. https://doi.org/10.1117/12.2549369.Hyperspectral

99. Lyu L, Zhang X, Li C, et al (2019) Small RNA profiles of serum exosomes derived from individuals with latent and active tuberculosis. Front Microbiol 10:1–10. https://doi.org/10.3389/fmicb.2019.01174

100. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres M (2020) Diagnosis for Latent Tuberculosis Infection: New Alternatives. Front Immunol 11:1–13. https://doi.org/10.3389/fimmu.2020.02006

101. Marsh JW, Humphrys MS, Myers GSA (2017) A laboratory methodology for dual RNA-sequencing of bacteria and their host cells in vitro. Front Microbiol 8:. https://doi.org/10.3389/fmicb.2017.01830

102. Sivakumaran D, Ritz C, Gjøen JE, et al (2021) Host Blood RNA Transcript and Protein Signatures for Sputum-Independent Diagnostics of Tuberculosis in Adults. Front Immunol 11:1–10. https://doi.org/10.3389/fimmu.2020.626049

103. Diaz G, Wolfe LM, Kruh-Garcia NA, Dobos KM (2016) Changes in the Membrane-Associated Proteins of Exosomes Released from Human Macrophages after Mycobacterium tuberculosis Infection. Sci Rep 6:. https://doi.org/10.1038/srep37975

104. Wang J, Wang Y, Tang L, Garcia RC (2019) Extracellular vesicles in mycobacterial infections: Their potential as molecule transfer vectors. Front Immunol 10:. https://doi.org/10.3389/fimmu.2019.01929

105. Lu G, Jiang XR, Wu A, et al (2021) Two Small Extracellular Vesicle sRNAs Derived From Mycobacterium tuberculosis Serve as Diagnostic Biomarkers for Active Pulmonary Tuberculosis. Front Microbiol 12:1–8. https://doi.org/10.3389/fmicb.2021.642559

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