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An overview on the Current Status of Tuberculosis and its Biomarkers

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Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) is one of the deadliest communicable tropical infectious diseases which is transmitted through inhalation of aerosolized droplets. This disease constitutes a serious global health issue with approximately 1.7 million mortalities every year. Furthermore, it is also estimated that two billion people live with latent Mtb infection and represent the possibility to develop active TB in future. At present, several effective therapeutic drugs are commercially available and few of the promising anti-tubercular agents from diversified sources are under clinical trial phases. The quest for developing a promising vaccine against TB is also an ongoing process. The currently used diagnostic tools for TB are time consuming, and sometimes are incapable in the diagnosis of infection. Hence, there is an urgency to identify new diagnostic tests which is not only sensitive and specific but also rapid in this prospect. Biomarkers are crucial to the development of new diagnostic tools, drugs, and vaccines against TB and could be a therapeutic weapon in reducing morbidity and mortality of TB. In fact, currently there is unavailability of sufficiently validated biomarkers to aid the evaluation of new TB vaccine, the improvement of TB diagnostics, and the development of shorter treatment regimens. The present chapter reviews on the current status of TB globally in terms of the availability of drugs/drugs in clinical trial phases and vaccine developmental pipeline. The study has been specially emphasized on the potentiality of biomarkers in the fight against TB. In view of several investigations reported over the past few years, biochemical markers have enormous contributions in the routine diagnosis of TB. But it is important to note that none of the existing markers so far are entirely satisfactory for us. In a nutshell, the interplay between the host immune system and Mtb may provide a platform for the identification of appropriate biomarkers through diversiform approaches in future.

Keywords: Anti-tubercular drugs; Biomarkers; *Mycobacterium tuberculosis*; Tuberculosis

Introduction

Undoubtedly, bacterial infections are significant threats worldwide, causing over a million mortalities and morbidities per annum. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) is one of the most severe, oldest, and deadliest airborne asymptomatic tropical diseases. Indeed, TB is a serious airborne fatal disease which infects one third of the world's populace. TB is a global pulmonary health problem that affects all age groups but it is quite common among men. TB can affect any parts of the body but generally infects the lungs. Development of TB outside the lungs is termed as extra-pulmonary TB and occurs mainly in young adults and immune suppressed persons. The "latent tuberculosis" is a special type of tuberculosis when the infections are devoid of any symptoms and progress to active disease if left untreated. In fact, in latent TB, Mtb remains inside the body in inactive state and becomes active later on. This type of TB is not contagious and persons affected with latent TB do not spread the disease and diagnosed by tuberculin skin test (TST) or blood tests. The active TB can be transmitted to other person and diagnosis of active TB is solely relied on chest X-rays, microscopic observation, and culturing the body fluids. The chronic cough, chillness, blood-containing sputum, fever, night sweats, chest pain, fatigue, nails clubbing, and weight loss are the classic symptoms of active TB. "Osseous tuberculosis" is another type of tuberculosis where

the infection spreads to the bones. The bursting of a tubercular abscess through skin causes tuberculous ulcer.

According to the World Health Organization (WHO) survey, there were 8.6 million new active cases of TB and 1.3 million mortalities during 2012 [1]. There were approximately 9.6 million cases of active TB estimated in 2014 which culminated in 1.5 million deaths. TB is an endemic disease in the developing countries and the majority of active TB cases were in the South-East Asia (29%), followed by African (27%), and Western Pacific (19%) regions. More than 95% of deaths occurred in developing countries and India alone represents about 25-30% of total TB cases. Understanding this devastating situation, the WHO developed the DOTS (direct observed therapy strategy) in the mid-1990 in order to control TB. Most of the countries adopted this unique strategy and confirms a short-course of chemotherapy treatment by administering crucial first-line drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide), followed by treatment with isoniazid and rifampicin for a period of four months. But, the development of multi-drug resistant (MDR)- Mtb and disease recrudesces were one of the rapid consequences of this strategy. Surprisingly, among the newly infected cases of TB, approximately 15% were co-infected with human immunodeficiency virus (HIV) due to the impairment of the immune system of the patient, leading to the delayed diagnosis and treatment of TB [2].

Mtb is a weak Gram-positive, aerobic, rod-shaped, and non-motile bacterium that infects human macrophages, leading to TB. The generation time of bacterium is very slow (16-20 h). The bacterium is often stained with Ziehl–Neelsen stain (acid-fast stain) during its microscopic observation and its unique characteristic includes the high lipid content on the outer membrane of this bacterium. TB causing mycobacteria viz. *M. bovis, M. africanum, M. canetti*, and *M. microti* belong to *M. tuberculosis* complex (MTBC). Among them, *M. bovis* and *M. africanum* are not widespread at present. On the other hand *M. canetti* and *M. microti* are rare, limited to Africa only.

In spite of the discovery of streptomycin and first-line anti-TB drugs, TB continued to ravage the under-developed and developing countries. The rate of TB development rose once again with the onset of HIV infections, leading to the utilization of several drugs towards its cure and addressed the dominant cause of the development of MDR mycobacterium strains. In the current scenario, there is an emergence of multidrug resistant MDR strains, threatening global TB control. MDR-TB is a form of TB that is resistant to first-line anti-TB drugs, at least to isoniazid (INH) and rifampicin (RIF). The rise of MDR-TB led to the use of second-line drugs (para-aminosalicylic acid, cycloserine, terizidone, ethionamide, prothionamide, thioacetazone, linezolid, levofloxacin, moxifloxacin, ofloxacin, gatifloxacin, and capreomycin) that are very expensive, toxic and require long term treatment, causing the poor patient compliance. In 2014, 480 thousand cases were reported globally developing MDR-TB, with mortality counts of approximately 125 thousand. XDR-TB is a subset of MDR-TB which is resistant to the second-line anti-TB drugs, fluoroquinolones, and one of the three injectable anti-TB agents (e.g. amikacin, kanamycin, and capreomycin). Currently, the cases of MDR-TB and XDR-TB are continuously emerging and few countries reported at least one case of XDR-TB, which represented 10% of all MDR-TB cases.

Pathogenicity of Mtb

Macrophages are specialized cells of the immune system that engulf and remove the invading microbes through phagocytosis. Mtb primarily affects human pulmonary macrophage. Generally, during the process, microbes are trapped into the phagosomes which fuse with lysosome, and forms phagolysosome. Eventually, the digestive process occurring inside the phagolysosomes causes the destruction of invaded microbes. However, Mtb infects macrophages and after phagocytised escapes from this defence mechanism, and thus, survive within the drastic environment. Mtb utilizes macrophages for its own replication process (**Figure 1**).

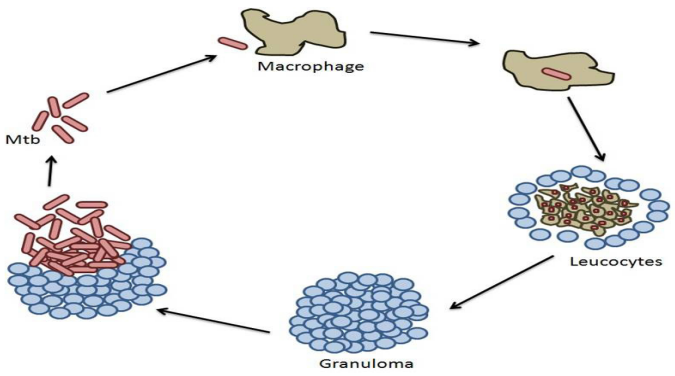

Figure 1: Mtb pathogenicity and utilization of macrophage

In fact, a number of strategies are involved in the defence property of Mtb. Unlike non-pathogenic mycobacterium strains, first of all the pathogenic Mtb arrests the maturation process of phagosomes and prevents the acidification of phagosomal compartments. It also inhibits the formation of the lysosomes and phagosomes complex. Further, the bacterium impairs the apoptosis of macrophage and suppresses the antimicrobial responses, thus helping the bacterium to escape from the phagosomes. The bacterium becomes undetectable to innate immune system because MHC class II antigen avoids its presentation. In this manner, Mtb is able to manipulate and survive in the adverse situation of pulmonary or any other parts of the host macrophages.

In spite of the digestive characteristics of macrophages, Mtb has developed multiple adaptive strategies to destroy the phagosomal pathways, and survive intracellularly in the host macrophages [3]. The virulent potency of Mtb is mainly due to its cell envelop constituting diverse lipid contents such as Sulfated glycolipid (SL), Trehalose dimycolates (TDM), Dimycocerosate phthiocerol (DIM), Lipoarabinomannan (LAM), Mannose capped lipoarabinomannan (Man-LAM), and Phosphatidylinositol mannoside (PIM). Among them, Lipoarabinomannan (LAM) and phosphatidylinositol mannoside (PIM) arrest phagosome fusion and acidification and have the potentiality to interfere with the host phagosome pathway [4, 5]. In contrary to this, SL and TDM prevent the fusion of lysosome [6]. However, DIM is involved in the formation of permeability barrier by cell envelops and inhibition of acidification [7].

The virulent Mtb prevents CR-mediated Ca^{2+} signalling during phagocytosis process and arrests the acidification and fusion mechanism [8]. Man-LAM may incorporate with Mtb-containing phagosomes membrane and avoids the macrophage sphingosine kinase (SK) or Ca^{2+}/calmodulin phosphatidylinositol (PI)3 kinase hVPS34 cascade and PIP3 (phosphatidylinositol 3,4,5-trisphosphate) production, causing the early endosome antigen 1 (EEA1) inactivation, a major component in phagosome maturation process [9]. LAM may also impair the host phagosome maturation mechanism by activating p38 mitogen-activated protein kinase (p38 MAPK), which are responsible for the down-regulation of EEA1 recruitment mechanism [5]. PIM has the potency to avoid phagosomal acidification by stimulating the fusion of Mtb-containing phagosome with early endosome compartments. It facilitates the access to nutrients necessary for the pathogen's survival strategy.

TACO (tryptophan aspartate coat protein) is a host protein that resides on the live and virulent Mtb constituting phagosomes. Unlike the uncoated phagosomes, TACO-coated Mtb-containing phagosomes will not be presented to lysosomes for degradation mechanism and thus prevents the phagosome fusion [10]. Mtb arrests apoptosis of the infected cells at the preliminary stage and induces necrosis-like cell death to escape from the hosts [11]. Currently, mycobacterial Lipoamide dehydrogenase C (LpdC), a TACO binding protein, has been reported to induce the Mtb virulence nature. This protein helps Mtb to escape from the host's reactive oxygen species (ROS) toxicity by forming a component of peroxynitrite reductase/peroxidase [4]. The pathogenicity process and virulence traits of Mtb mentioned above clearly depict that Mtb has developed adaptational strategies to evade phagocytosis mechanism.

At present, the emergence of multi- drug resistant tuberculosis (MDR-TB) and extensively-drug resistant tuberculosis (XDR-TB) has attracted attention to attain effective prevention, durable cure and treatment of the distending problems of TB. The major obstacle in the treatment of TB is the unique mode of pathogenicity of Mtb. It is totally clear that Mtb has potentiality to survive in the unfavourable conditions created by host macrophage during the infection. In addition to this, the association of TB with HIV infection is another considerable issue that caused 0.32 million mortality worldwide. These epidemiological problems shed light on the relevance of the immune system to control TB and create a public health problem among large population of human. From the last century, BCG vaccine has shown some protective efficacy in combating serious TB. But the lack of protecting pulmonary infections is the poor aftermath of this therapy and has been called into the million-dollar question. In this regard, several anti-tubercular drugs of distinct origins have been developed and used from last few decades.

Anti-Tubercular Drugs

At present, there are several therapeutic drugs available in the market for TB. There are few potent anti-tubercular drugs in clinical trials phases too. Among those drugs, only 5 are in Phase III while 7 are in Phase II trials. In addition to this, 8 drugs are under preclinical development phage, including 3 anti-tubercular drugs in Good Laboratory Practice toxicity evaluation. Surprisingly, there are no anti-tubercular drugs registered in Phase I. Table 1 shows not only the list of drugs that are commonly used to combat TB but also their mode of action as well as mode of administration.

Table 1: Mechanism of action as well as mode of administration of commonly used anti-tubercular drugs

Anti-tubercular drugs	Mechanisms of action	Administration	References
Bedaquiline	Electroneutral uncoupling of respiration-driven ATP synthesis and blocking ATP synthase in Mtb. Binding and perturbing the a-c subunit interface of the Fo, corresponding to futile proton cycle.	Oral	[12]
Capreomycin	Inhibiting protein synthesis by interrupting the interaction between ribosomal proteins L12 and L10	Intramuscular	[13]
Cycloserine	Interfering with early step bacterial cell wall formation inside the cytoplasm by competitive inhibition of two enzymes, L-alanine racemase and D-alanylalanine synthetase.	Oral	[14]
Ethambutol	Disrupting arabinogalactan synthesis by blocking the arabinosyl transferase that leads to the increased permeability of the cell wall.	Oral	[15]
Isoniazid	Blocking the natural enoyl-AcpM substrate and thus, inhibiting the fatty acid biosynthesis.	Oral	[16]
Kanamycin	Inhibiting protein synthesis by irreversible binding to the cytosolic as well as membrane-associated bacterial ribosome.	Intravenous	[17]
Pyrazinamide	Disrupting the membrane transport.	Oral	[18]
Rifampin, Rifabutin, Rifalazil and Rifapentine	Inhibiting protein synthesis inhibition as well as inducing programmed cell death.	Oral	[19]
Nitroimidazoles	Inhibiting DNA synthesis.	Oral	[20]
AZD5847	Mycobacterial 50S ribosomal subunit impairment.	Oral	[21]
Fluoroquinolones	DNA replication inhibition.	Intravenous	[22]
Isoxyl	Mycolic acids and fatty acids biosynthesis inhibition.	Pulmonary	[23]
Ethionamide and Prothionamide	Inhibiting the mycoloic acids synthesis.	Oral	[24]
Erythromycin and Roxithromycin	Protein synthesis inhibition.	Oral	[25]
p-aminosalicylic acid	Thymine nucleotides biosynthetic pathways inhibition.	Oral	[26]
Riminophenazines	Electron transport chain inhibition.	Oral and parenteral	[27]
Thiacetazone	Disruptive characteristics of the permeability of cell envelope and host immunomodulation.	Oral	[28]
Delamanid	Mycolic acid synthesis inhibition.	Oral	[29]
Sutezolid	Preventing the initiation of protein synthesis in bacteria by binding to 23S RNA.	Oral	[30]
Streptomycin	Binding with 30S subunit of the ribosome and interfering with protein synthesis.	Intramuscular	[31]
Amikacin	Binding with the small subunit 30S of the ribosome.	Intramuscular	[32]
Ciprofloxacin	Targeting the DNA gyrase.	Intravenous	[33]

Current Status of TB Vaccine Development

The quest for a promising vaccine against TB has been ongoing, yet a successful potent candidate has been elusive. The only successful vaccine is Bacillus Calmette–Guerin (BCG), which is developed from the bacterium *Mycobacterium bovis* [34]. The development of an effective vaccine against TB is a huge hurdle for researchers today. One major problem with vaccination is the enigmatic nature of Mtb infection, which is characterized as chronic rather than acute [35]. Over the last two decades, much has been learned about the immune response to Mtb both ineffective clearances of the pathogen and in the development of disease, which often continues unabated. The results of these studies, while complex and at times contradictory do offer a clearer path towards a vaccine that will be effective. In fact, no new vaccines have entered clinical testing since TAG's 2015 Pipeline Report, although many of the 14 candidates in the pipeline have initiated new trials (**Table 2**).

Table 2: List of TB vaccines in developmental pipeline

S. No.	Agent	Type	Clinical trial phase
1	*M. vaccae*	Whole-cell M. vaccae	Phase III
2	M72/AS01	Protein/adjuvant	Phase IIb
3	H4 + IC31	Protein/adjuvant	Phase IIa
4	H56 + IC31	Protein/adjuvant	Phase IIa
5	MTBVAC	Live genetically attenuated *M. tuberculosis* (MTB)	Phase IIa
6	VPM1002	Live recombinant Rbcg	Phase IIa
7	Dar-901	Whole-cell M. obuense	Phase IIa
8	ID93 + GLA-SE	Protein/adjuvant	Phase IIa
9	RUTI	Fragmented MTB	Phase IIa
10	Ad5Ag85A	Viral vector	Phase I
11	ChAdOx1.85A + MVA85A	Viral vector	Phase I
12	MVA85A (aerosol)	Viral vector	Phase I
13	MVA85A-IMX313	Viral vector	Phase I
14	TB/FLU-04L	Viral vector	Phase I

Biomarkers of TB

Mtb has infected the human populace for hundreds of years and today still remains one of the leading causes of morbidity and mortality globally. Mtb infection can depict a wide range of clinical properties that challenge existing diagnostic tools. Unfortunately, the highest rate of Mtb infections is found in the developing countries, and they have financial burden to overcome this situation. Improving the rate of case detection and the treatment of infective individuals in terms of 'End TB Strategy' is the prime focus to control TB globally. How¬ever, successful implementation of the End TB Strategy relies on accurate diagnostics and identification of biomarkers for important stages of Mtb infection.

A biomarker is defined as a characteristic that is objectively measured and assessed as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [36]. It can be either host- or pathogen-specific. It helps to provide information about the current as well as future health status of the patient. Therefore, there is an urgency to identify new biomarkers for TB also in order to monitor the risk and eradication of latent Mtb infection [37]. At present, despite the process in developing specific TB markers is very slow, several studies are underway using proteomics, transcriptomics, and metabolomics with multiplexed assays to compare a variety of gene expression profiles among patients with TB [38].

Biomarkers for active TB

Mtb could be detected directly in blood, sputum or urine. Likewise, Mtb DNA can be detected in blood and urine of pulmonary TB patients with a better sensitivity than Mtb culture from the same biological fluid [39]. LAM has been proposed

as TB biomarker; however the available commercial test on urine has a poor sensitivity that can be partly increased by other LAM assays [40].

The Mtb Ag85 complex is a 30-32 kD family, comprised of three proteins viz. Ag85A, Ag85B, and Ag85C with enzymatic mycolyl transferase activity involved in the coupling of mycolic acids to the arabinogalactan of the cell wall [41]. The detection of Ag85 in blood and urine, however, shows highly variable performance in various reports [42]. There are diversified non-sputum based-assays for active TB diagnosis, relying on serum, plasma, urine or stimulated or unstimulated blood (Figure 2). Mtb specific antibody detection is not a pronounced diagnostic approach because of the heterogeneity of the response to Mtb [43].

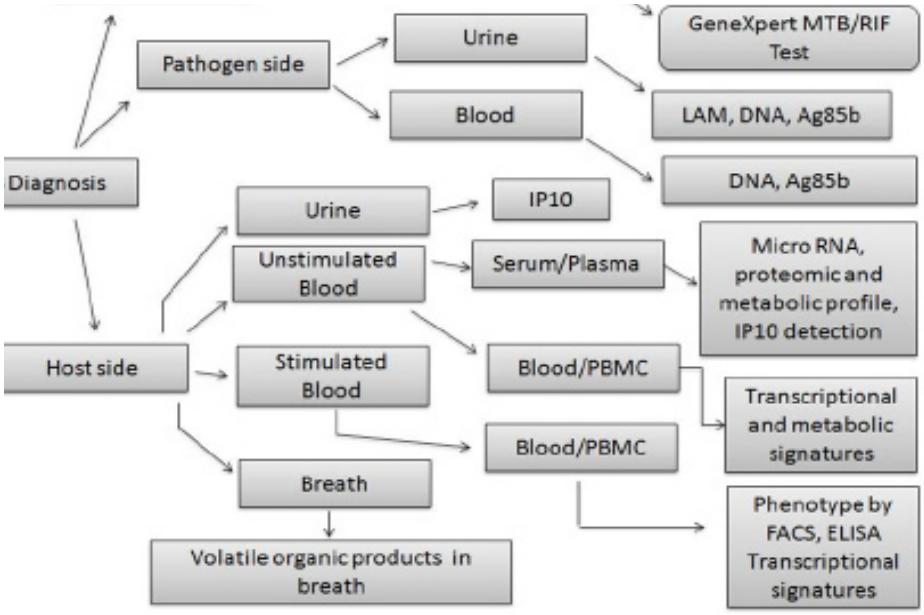

Figure 2: Biomarkers for active TB diagnosis.

Note: Ag: antigen; LAM: lipoarabinomannan; BAL: broncholavage; IP: Interferon- γ inducible protein; FACS: Fluorescence-activated cell sorting.

The serum micro-RNAs have depicted various levels of accuracy for diagnosing active TB in drug-sensitive and drug resistant TB [44]. Modular and pathway analysis showed that the neutrophil driven interferon (IFN)-inducible gene profile, consisting of both Type 2 (IFNγ) and Type I (IFNαβ) IFN signalling represented a significant TB signature detectable in the peripheral blood from pulmonary TB patients [45]. The complex analysis and the costly molecular tools related to the transcriptional profiles make it currently difficult to be applied as routine diagnostic tests. The interferon (IFN)γ inducible protein 10 (IP10) was observed to be enhanced in the unstimulated plasma of children and adults with active TB, and has been evaluated by different methodologies including also innovative technologies based on lateral flow assays using the interference-free, fluorescent up converting phosphor (UCP) labels in multicenter studies conducted in Africa [46]. Interestingly, IP10 can be also detected in the urine of adult patients [47]. Flow-cytometry has been found to be a promising tool in order to improve the diagnosis of TB. In addition to this, advancement in flow cytometry allows the simultaneous assessment of cytokine production and memory status. Active TB is associated with a reduction in CD27 surface expression on circulating Mtb-antigen stimulated CD4+ T-cells [48]. Currently, a novel T-cell activation marker-TB (TAM-TB) assay was identified for the diagnosis of active TB in children [49]. The transcriptomic, proteomic, or metabolomic approaches have been used to identify new markers for TB patients' stratification [50, 51]. Tientchieu et al. [50] evaluated the transcriptomic and metabolic profiles of subjects infected with two different lineages of Mtb. Sputum may also be used to analyze proteomic profiles, as data on smear-negative vs. smear-positive TB patients were significantly different from those found in control subjects.

Volatile organic compounds (VOCs) in breath may contain biomarkers of active pulmonary TB derived directly from Mtb. A breath test is identified as potential biomarkers of active pulmonary TB [52]. However, detection of VOCs is technically difficult because most breath VOCs is excreted in picomolar concentrations, and most analytical instruments currently used cannot detect VOCs at this concentration.

Cytokines and chemokines as biomarkers

Cytokines and chemokines are small protein molecules that are involved in regulating immunological responses at cellular level and stimulate wide range of cells causing immunity and inflammation. The actions of cytokines could be pleiotropic where one cytokine has the potentiality to act on different cell types where multiple cytokines have the same functional effect. They may also have antagonistic property where the impact of one cytokine opposes the action of others or synergistic effects where two different cytokines work together. The actions of chemokines could be homeostatic where they guide cells during immune surveillance for pathogens by interacting with antigen presenting cells. Some chemokines have roles in promoting angiogenesis or guide cells to tissues that provide specific signals critical for cellular maturation. On the other side, few chemokines are inflammatory and they function mainly as chemo attractants for leukocytes.

Extensive investigations have been carried out on biomarkers that result from the immune response to the infection of Mtb. These in vitro tests have been developed considering T- cell based immuno assays which measures IFN gamma (IFN-γ) response against Mtb specific antigens, early secreted antigenic target 6 (ESAT-6), and culture filtrate protein 10 (CFP-10). T-cell-based Interferon Gamma Release Assays (IGRAs) are commercially available and widely used for diagnostic purposes but the sensitivity of FDA approved IGRAs is less than ideal. At present, extensive researches are in process for biomarkers that might be used to indicate Mtb infection. In this regard, few studies have reported IP-10 as a better alternative marker for latent TB infection diagnosis among immune-compromised patients [53-55]. Others reported measurement of IL-2, IL-6, IP-10, and MIP-1β may enhance diagnostic sensitivity for Mtb infection compared with assessment of IFN-γ alone [56]. The enhancement of IP-10, MCP-1, MCP-2, MCP-3, and IL-1RA in supernatants from whole blood stimulated with Mtb-specific antigens was also reported to be a marker of TB infection [57]. Another study also showed IP-10 as a novel diagnostic marker for Mtb infection and its level was less influenced by infections other than TB [58].

Previous findings have indicated that IGRAs have stronger responses in patients with active TB than in those with latent TB [59]. Others researchers reported that IL-2, IFN-γ, and TNF-α expression profile of CD4+ T- cells hold promise in detecting active TB disease [60]. Pro inflammatory cytokines viz. TNF, IL-12(p40), and IL-17 are enhanced in TB cases and can discriminate active TB disease from latent infection [61]. High serum concentration of IL-8, IP-10, MCP-1, and MIP-1 β are observed in TB patients in comparison with non-TB cases [62]. According to Chegou et al. [63], detection of single or combinations of three host markers (selected from EGF, sCD40L, MIP- 1β, VEGF, TGF-α or IL-1α) by utilizing an adaptation of the commercial QFT assay may be accurately identified as active TB within 24 h. RNA expression level of CXCL-8, FoxP3, and IL 12β differentiates latent TB infection from disease [64]. The relative mRNA level of IFN-γ, IL-4, and IL-4δ2 has been identified as a suitable and better marker than IFN-γ alone, since ratios of IFN-γ to IL-4 and IL-4δ2 to IL-4 decreased when contacts developed TB and increased in cured TB cases. Chegou et al. [63] reported that the levels of IFN-α2, IL-1Ra, sCD40L, IP-10, and VEGF in QFT-IT supernatants have potential to support the diagnosis of TB. In another study, Goletti et al. [65] reported that IFN-gamma response to RD1 selected peptides is associated with active TB with a higher specificity than QFT-IT and TST. The levels of IL-6 and IL-9 were observed to be significantly high in plasma and after Mtb antigen stimulation in active TB patients and the levels of CCL1, CCL21, and IL-6 were specifically increased in pleural effusions of tuberculous pleurisy patients [66]. Table 3 enlists the few potential host biomarkers and their respective indicated process [67].

Table 3: Few potent host biomarkers and their respective indicated process

S.No.	Biomarker	Indicated process
1	Elevated IL-4 expression	Risk of subsequent TB
2	IFN-γ /IL-4 ratio	Disease status
3	IL-4δ2/IL-4 ratio	Infection status and degree of disease
4	CRP	Degree of disease
5	Granzyme B	Degree of disease
6	LAG-3	Degree of disease
7	Neopterin	Response to therapy and degree of disease
8	SuPAR	Degree of disease
9	sICAM-1	Infection status and degree of disease
10	IFN-γ induced by ESAT-6	Mtb infection and risk of subsequent TB

11	IL-8, IL-12β, FOXP3	Infection status
12	TNFα/TNFR1 ratio	Degree of disease
13	Lactoferrin, CD64 and Rab33A	Mtb infection and disease status
14	Volatile components in breath	Mtb infection

The stage of Mtb infection is mainly determined by the potentiality of the host innate and adaptive immune systems to eradicate the bacteria. Specific and non-specific host immune responses contribute to the differential outcomes of exposure and infection, although the detailed understanding of the underlying mechanisms is lacking. The host response at the various infection stages shows opportunities to measure markers that have diagnostic potential. The first three phases of Mtb infection are asymptomatic. Only some TB patients develop immunological signs of infection; others can eliminate the bacteria during the innate immune phase, without generating T-cell memory. T-cells are engaged by antigen-presenting cells in the adaptive immune phase, and this generates effector and memory T-cells. B-cells are also activated and Mtb specific antibodies are produced. In quiescent phase, the bacteria are contained inside granulomas, which consist of a central area containing infected macrophages and giant foam cells, surrounded by TCM and B-cells. An optimal T-helper (TH) cell balance is required to control Mtb while limiting immunopathology. This includes pro-inflammatory TH1-type responses, tumour necrosis factor (TNF), interleukin-12 (IL 12) production), and TH17-type responses (characterized by IL 17 production). The replicating phase is symptomatic and at this stage the bacteria have escaped immune control, granulomas are disrupted, the acute-phase response is activated, and the levels of pro-inflammatory markers are enhanced (**Figure 3**).

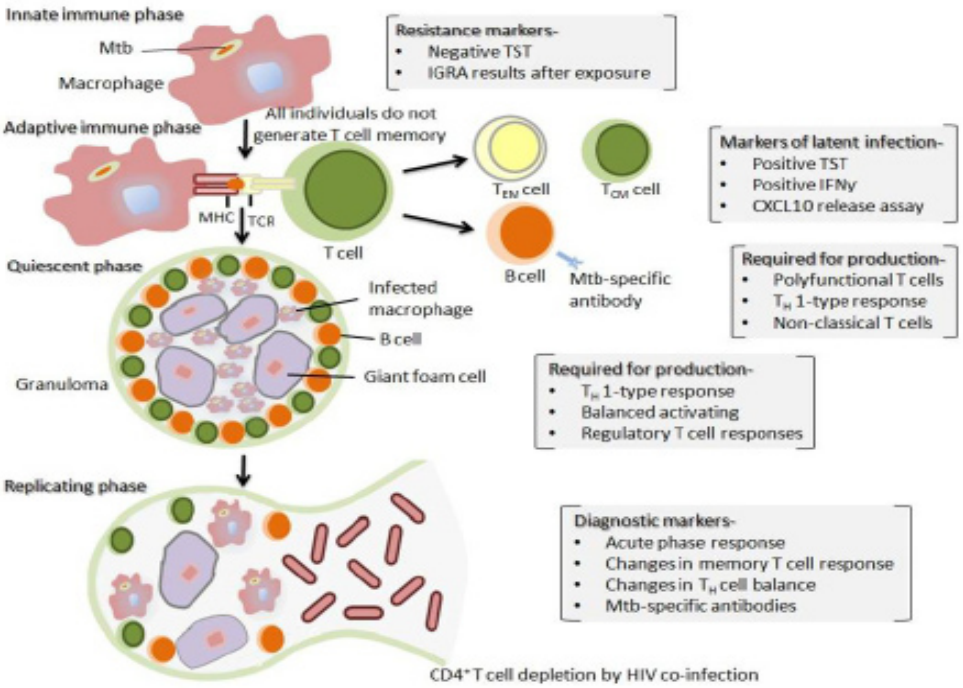

Figure 3: Immune responses and host biomarkers of Mtb exposure and infection (Adapted from Walzl et al. [68]).

Pediatric biomarkers

Children have increased risk of progression from infection to active disease in a comparison with adults. Despite the possibility to diagnose TB infection in children using biomarker-based tuberculin skin testing (TST) and interferon-gamma release assays (IGRAs), these tests have shortcomings such as inability to differentiate between TB infection and disease, cross-reactivity with other mycobacteria, and increased false-negative tests among immune-compromised children. Therefore, efforts to identify and validate biomarkers of TB in adults and children are ongoing and, if proven, will improve the reliability of TB diagnosis. Few of pediatric biomarker research highlights are mentioned below.

LAM assay

The lateral flow urine LAM assay is supposed to work well in children too because children and people with HIV tend to have higher rates of extra-pulmonary TB. The WHO recommendation for LAM in people with CD4 counts <100 does

extend to children, based on the generalization of data from adults, while acknowledging very limited data in children.

C-reactive protein

C-reactive protein (CRP) is a non-specific marker of inflammation detectable in blood. It has shown ability for screening for TB and indicating response to TB treatment in adults. A study to identify the expression patterns of biomarkers in the plasma of HIV-negative children in India with pulmonary and extrapulmonary TB found that children with active TB showed significantly elevated levels of CRP. CRP should be evaluated for use in TB diagnostic algorithms in larger pediatric cohorts.

TAM-TB assay

T-cell activation marker-tuberculosis assay (TAM-TB) demonstrated 83.3% sensitivity and 96.8% specificity among children with TB symptoms compared with culture. The combined use of the TAM-TB assay and Xpert MTB/RIF demonstrated 94% sensitivity compared with culture. TAM-TB is a rapid blood-based test with the potential to improve the detection of active TB in children.

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