Infect Dis Ther (2023) 12:2665–2689
https://doi.org/10.1007/s40121-023-00887-x

REVIEW

# New Insights into Biomarkers for Evaluating Therapy Efficacy in Pulmonary Tuberculosis: A Narrative Review

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Received: September 21, 2023 / Accepted: October 20, 2023 / Published online: November 8, 2023


## ABSTRACT

Evaluating therapy efficacy is crucial for patients with tuberculosis (TB), especially those with drug-resistant tuberculosis (DR-TB). The World Health Organization currently recommends sputum smear and culture as the standard methods for evaluating pulmonary tuberculosis (PTB) therapy efficacy. However, these approaches have limitations including low sensitivity, lengthy culture periods, and susceptibility to contamination. There is an urgent need for dependable biomarkers to evaluate therapy efficacy in patients with PTB. Numerous new biomarkers of *Mycobacterium tuberculosis* (MTB) and the host have been used in recent studies to evaluate PTB therapy efficacy. A systematic review and update of these biomarkers can facilitate the discovery of novel biomarkers and assessment models, as well as provide a solid scientific basis for alternative indicators of evaluating therapy efficacy. In this review we summarize the recent advancements and limitations of biomarkers used to monitor therapy efficacy, highlighting the importance of utilizing a combination of biomarkers. Although some biomarkers have potential in evaluating the efficacy of therapy in patients with PTB, they also have some limitations. Further research, validation, and optimization are required to identify the most reliable and effective alternative biomarkers and apply them to clinical practice.

**Keywords:** Pulmonary tuberculosis; Biomarkers; Therapeutics; Treatment response; Treatment outcome

Fuzhen Zhang and Fan Zhang contributed equally to this study and share first authorship.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s40121-023-00887-x.

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**Key Summary Points**

Evaluating the efficacy of pulmonary tuberculosis (PTB) therapy is vital for treatment decision-making, improving the cure rate, and preventing the transmission of tuberculosis.

The conventional methods for evaluating efficacy of therapy for PTB have limitations, including low sensitivity and a long culture period.

Host biomarkers, especially a combination of biomarkers, serve as valuable tools for evaluating the efficacy of PTB therapy, although certain biomarkers may have inherent limitations.

The changes in certain host immune markers vary between patients with drug-sensitive PTB and patients with drug-resistant PTB, and even within the same patient group, the same type of immune marker may exhibit different changes under different *Mycobacterium tuberculosis* (MTB) antigen stimuli.

Further studies are needed to identify the most reliable and effective alternative biomarkers and apply them to clinical practice.

## INTRODUCTION

The COVID-19 pandemic has adversely affected the progress of tuberculosis (TB) prevention and control programs [1]. The World Health Organization (WHO) 2022 report estimated that about 10.6 million people were living with PTB in 2021, a 4.5% increase from 10.1 million in 2020, and that the total number of deaths among HIV-negative and HIV-positive people rose to 1.6 million, up from 1.5 million in 2020. The period from 2015 to 2021 is only halfway to the first milestone of the End TB strategy. Moreover, the burden of drug-resistant tuberculosis (DR-TB) has also increased between 2020 and 2021 and has become a major public health concern [2]. Therefore, there is an urgent need for more effective interventions to prevent the further development of TB into DR-TB in the prevention and control of TB.

Conventional treatment for drug-sensitive TB (DS-TB) requires 6 months of therapy (2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol followed by 4 months of isoniazid and rifampicin), while treatment for DR-TB is much longer [3]. Prolonged therapy imposes a heavy burden on patients and health workers and increases the risk of poor adherence or treatment failure [4]. Poor adherence or treatment failure can lead to incomplete clearance of *Mycobacterium tuberculosis* (MTB), resulting in replication, transmission, and drug resistance mutations [5]. Therefore, early evaluation of TB therapy efficacy is beneficial for timely adjustment of therapy and improvement of cure rates, which can help control the spread of TB. Evaluating treatment outcomes, determining therapy efficacy, and adjusting treatment plans earlier are important steps in the course of therapy to prevent disease progression.

Currently, the conventional methods used to evaluate the efficacy of anti-tuberculosis (ATTB) treatment rely on monitoring the tubercle bacilli within sputum specimens by smear microscopy and mycobacterial culture. Sputum smear is a quick and cost-effective technique, but its sensitivity is limited to detecting $10^4$ bacteria per milliliter of sputum, and it cannot differentiate between live and dead bacteria [6, 7]. Sputum culture is considered the gold standard for evaluating ATTB therapy efficacy. Two consecutive negative sputum culture results in patients previously testing positive for MTB after intensive treatment are indicative of a favorable treatment outcome. However, sputum culture is prone to contamination, has a long culture time for MTB, and may take longer to determine a positive culture, especially for samples that are negative on sputum smear [8]. Traditional evaluation methods have certain drawbacks, which have led to the investigation of new biomarkers and imaging techniques for monitoring the efficacy of ATTB treatment.

These include studies focused on MTB and host biomarkers and imaging changes [9–11]. These novel biomarkers and imaging techniques can predict ATTB therapy efficacy earlier and faster, thereby improving clinical outcomes and controlling pulmonary tuberculosis (PTB) outbreaks.

In this review, we discuss and summarize the latest advancements in the application of novel biomarkers for evaluating the efficacy of ATTB therapy. This article does not require ethical approval because it is based on published research and does not involve any research conducted by the authors on humans or laboratory animals.

## BACTERIOLOGICAL BIOMARKERS

### Surveillance of MTB

Direct bacteriological test is the most reliable evidence to evaluate the efficacy of PTB therapy. The traditional monitoring techniques are sputum smear and sputum culture. Sputum smear monitoring is based on the conversion of acid-fast stain of MTB in sputum, indicating the clearance of PTB bacilli and predicting good treatment outcomes [12, 13]. However, sputum smear has low sensitivity and requires a high concentration of bacilli in sputum ($> 10^4$ bacilli/ml) to achieve a positive result [14–16]. For patients with poorly treated PTB, sputum culture is more predictive of treatment outcomes than sputum smear [17]. Sputum culture includes solid culture and liquid culture. Solid culture evaluates the efficacy by observing the change of colony numbers on the medium, while liquid culture evaluates the efficacy by measuring the time of conversion, which can predict the colony numbers in solid culture [18, 19]. It has been found that the sputum culture status at month 2 can predict treatment outcomes well [20]. For patients with multidrug-resistant tuberculosis (MDR-TB), some studies suggest that sputum culture conversion at month 2 of treatment can predict treatment success [21–23]. However, other studies show that the predictive effect of sputum culture conversion at different time points varies in different populations [24], which may be related to the composition of MDR-TB treatment regimen [25]. Nevertheless, the results of sputum culture conversion after 3, 6, and 24 months of treatment have a good predictive effect on the cure of MDR-TB [22, 26]. The drawback of sputum culture is that it has a long cycle and is prone to contamination [27], so it cannot reflect the treatment effect of PTB in a timely and rapid manner, and cannot prevent the further development and transmission of PTB effectively. To overcome the limitations of traditional sputum smear and sputum culture, fluorescence microscopy has been developed to detect MTB in sputum [28, 29], but its sensitivity is still very low [30], and its detection rate in patients with PTB and positive culture is only 68.7% [31]. Recent studies have shown that direct and rapid quantitative observation of viable MTB through fluorescent staining can be used to reflect the treatment effect of PTB [32], suggesting that this method has great potential for evaluating the treatment effect of PTB by detecting live MTB, but its accuracy needs to be further verified.

Early bactericidal activity (EBA) is the value of the average rate of decline of colony forming unit (CFU) per milliliter of sputum in the first 2 days of ATTB treatment, which is used to evaluate the efficacy of new ATTB drugs [33]. The current extension of EBA measurements to 14 days is mainly because some ATTB drugs, such as pyrazinamide, ethambutol, and bedaquiline, exhibit their bactericidal activity only after longer periods of administration [34–36]. EBA testing also relies on solid and liquid cultures of MTB, and studies have shown that early sustained rapid bactericidal activity is associated with treatment outcomes [37]. However, EBA has many limitations, such as frequent sampling, complex operation, high contamination risk, and low sensitivity for drugs with delayed bactericidal activity.

### Surveillance of Nucleic Acid of MTB

#### *DNA*

DNA detection of MTB is a rapid method to reflect the effect of PTB treatment. The Xpert

MTB/RIF technique recommended by WHO guidelines is a semi-nested real-time fluorescent PCR in vitro diagnostic technique that reflects the MTB load by outputting cycle threshold ($C_t$) value [38–40]. Recent studies have shown that Xpert MTB/RIF technology can be used to monitor the efficacy of PTB therapy [41–43], and that the $C_t$ value of Xpert MTB/RIF technology output has a strong correlation with the sputum culture time of PTB treatment [44], with the correlation reaching 86.0% and 90.2% at week 8 and week 24, respectively [45]. It has been shown that sputum conversion at month 2 and month 6 of treatment has a good predictive effect on the success of PTB treatment [20, 26]. Therefore, $C_t$ value can be used as a marker to predict treatment success and cure. Compared to Xpert MTB/RIF, the recently developed Xpert MTB/RIF Ultra offers further improvements in the detection sensitivity of PTB [46], However, it is important to note that Xpert MTB/RIF Ultra has a higher false positive rate compared to the gold standard sputum culture. This higher false positive rate can be attributed to the inclusion of DNA from dead MTB in the Xpert MTB/RIF Ultra detection [47], as well as the high sensitivity of the probe-based Taqman real-time quantitative PCR (RT-qPCR) in detecting MTB DNA [48]. In addition, the transrenal DNA (trDNA) of MTB in urine can also be detected by RT-qPCR to reflect the treatment effect. During the course of treatment, MTB trDNA will gradually decrease and become almost undetectable after 2 months of treatment. This biomarker has potential to be used as a prognostic marker for patients with PTB, especially for those with low bacterial load and extrapulmonary PTB [49]. Although DNA testing can quickly indicate MTB clearance and predict treatment effects, it cannot distinguish between dead and live MTB [50, 51], which may lead to false positive results in cured patients due to residual DNA.

### *RNA*

RNA detection from sputum can be used as an alternative marker to reflect the viability of MTB [52, 53], as mycobacterial RNA can rapidly respond to bacterial cell death [54, 55]. Studies have shown that the mRNA detection of MTB antigen 85B after ATTB treatment has 87.1% agreement with sputum culture, indicating that mRNA detection can quickly indicate the clearance of active MTB during ATTB treatment [56]. In addition, Stephen et al. used molecular bacterial load assay (MBLA) to detect 16S rRNA of MTB, which can quickly and accurately quantify the MTB load in sputum during treatment [57]. Compared with sputum culture, MBLA performed better in quantifying live MTB during treatment than GeneXpert and microscopy. It can be used to monitor bacterial load during PTB treatment, facilitating early detection of treatment failure and improving treatment outcomes [58, 59]. Many studies have used this method to evaluate the changes in viable bacterial counts during PTB treatment and the early efficacy of PTB therapy [59–62], and MBLA can also monitor the efficacy of PTB treatment by testing the stool of patients with sputum-negative PTB [63]. Recent studies have optimized RNA detection methods for MTB, including RNA extraction protocols and 16S rRNA primers, which have greatly improved the detection efficiency of MTB [64]. Although 16S rRNA detection can quickly reflect the effect of ATTB treatment, whether the measurement of 16S rRNA in the early stage of treatment can be used to predict the prognosis of PTB needs to be confirmed by more studies [57].

## Surveillance of Antigen Components of MTB

Detection of the cell wall lipoarabinomannan (LAM) component of MTB in urine can indicate the presence of MTB and has been commercialized. The LAM level in the urine of patients with culture-positive PTB decreases gradually after ATTB treatment, and the survival probability of patients with rapid LAM decline within 2 months of ATTB treatment is higher [65]. In addition, LAM can be used not only to predict the co-infection of PTB and HIV, with a positive predictive value of up to 80% for co-infected patients, but also to predict mortality, especially for patients with advanced HIV [66]. However, the sensitivity of LAM detection is very low (13–93%) [67, 68], and more studies are needed

to improve the sensitivity of LAM detection. Moreover, detection of MTB Ag85 antigen in sputum can reflect the early bactericidal effect of ATTB drugs, and its continuous presence in sputum indicates treatment failure or relapse [69–71]. Although the diagnostic accuracy of PTB is high [72, 73], the sensitivity of detecting Ag85 antigen in filtrate after 2 weeks of culture of MTB is only 80%. Interestingly, MPT64, a secreted protein of MTB, was found to be only secreted by viable MTB, with a sensitivity and specificity of 86.9% and 92.0%, respectively, which was similar to the diagnostic performance of GeneXpert for PTB [74] and was consistent with the 1+ positive predictive value of sputum smear [75]. Therefore, it is very promising to detect the expression of MPT64 secreted protein of MTB to monitor drug efficacy and ATTB therapy effect. Reduced or undetectable levels of serum culture filtrate protein-10 (CFP-10) and early secretory antigen target-6 (ESAT-6) concentrations after treatment can be used to monitor the efficacy of PTB therapy [76]. Studies have shown that the sensitivity and specificity of detection of MTB antigens CFP-10 and ESAT-6 in serum can be improved by using antibody-labeled and energy-focused porous disk silicon nanoparticles (nanodisks) and high-throughput mass spectrometry during ATTB treatment. However, this method is only preliminary at present, and more large-scale prospective studies are needed to verify and simplify the experimental process. Table 1 provides a summary of MTB pathogen and its components as biomarkers for evaluating the efficacy of PTB therapy.

## MTB-Specific Host Biomarkers

### *Cytokines*

Host biomarkers for assessing the response to ATTB treatment largely rely on cytokine balance [77], with cytokines being the most extensively studied biomarkers for monitoring treatment response. The most commonly used cytokine-specific assay for assessing the response to ATTB treatment is interferon gamma (IFNγ), which can be detected through T cell-based interferon-gamma release assays (IGRAs) using CFP-10 or ESAT-6 as stimulants. The number of spots produced by IFNγ-secreting T cells reflects the response of MTB-specific T cells to these antigens, and this number decreases after 2 months of ATTB treatment [78]. There are two forms of IGRA, including the ELISA-based QuantiFERON TB Gold test and the ELISPOT-based T-SPOT test [79]. While the number of spots produced by CFP-10- and ESAT-6-stimulated IFNγ-secreting T cells can be used to monitor the efficacy of ATTB treatment [80–83], and ELISPOT has shown potential as a surrogate marker of PTB treatment outcome [84], the utility of IGRA as a monitoring tool for therapy efficacy is limited. Most patients still test positive for IGRA even 6 months after they finish their treatment [85–88], primarily because MTB-specific T cells induced by MTB infection persist in the body after successful treatment of patients with active PTB, and these T cells can produce IFNγ upon stimulation with CFP-10 and ESAT-6. Owing to differences in study design, the results regarding MTB-specific IFNγ after ATTB treatment are inconsistent. Some studies have shown that CFP-10- and ESAT-6-stimulated whole blood IFNγ and interleukin-4 (IL-4) increase, while tumor necrosis factor-α (TNFα), IL-6, and IL-10 decrease after 2 months of ATTB treatment, with significant changes in IFNγ [89]. Moreover, IFNγ also increases under recombinant 32-kDa *Mycobacterium bovis* stimulation after 6 months of ATTB treatment [90]. However, other studies have shown that peripheral blood mononuclear cells (PBMCs) stimulated by CFP-10/ESAT-6 [91, 92] or CFP-10 [93] produce less IFNγ after 6 months of ATTB treatment. Some studies have also indicated that CFP-10/ESAT-6-stimulated whole blood IFNγ does not change after 3 months [94] and 6 months [95] of ATTB treatment, but IFNγ decreases under selected RD1 peptide stimulation [95]. Therefore, further investigation is needed to evaluate the use of extracellular MTB-specific IFNγ as a sole biomarker for assessing the efficacy of ATTB treatment.

Other MTB-specific cytokines, such as TNFα [91, 96], IL-1 receptor antagonist (IL-1ra) [97], IL-4 [89], IL-6 [91], IL-10, ratios of IFNγ to IL-10 [90], and IL-2/IFNγ ratios [98], have been

**Table 1** Bacteriological biomarkers for evaluating therapy efficacy of pulmonary tuberculosis

| | Biomarkers | Specimen | Index changes | Monitoring | Limitations |
|---|---|---|---|---|---|
| Surveillance of MTB | MTB | Sputum | AFB conversion from positive to negative | Treatment response, treatment outcome | Low sensitivity ($> 10^4$ bacilli/ml)<br>Not distinguishing between live and dead bacteria (conventional sputum smear microscopy)<br>Not distinguishing between MTB and NTM |
| | | | Culture conversion | Treatment response, treatment outcome, recurrence | Long turnaround time<br>Expensive<br>Easy to pollute<br>Requires containment facilities |
| | | | Culture time to positivity | Treatment response, treatment outcome | |
| | | | CFU/ml declining rate | Sterilizing activity | Long turnaround time<br>Cumbersome operation<br>Easy to pollute |
| Surveillance of nucleic acid of MTB | DNA | Sputum | Xpert MTB/RIF or Xpert Ultra or Taqman RT-qPCR $C_t$ value higher than threshold (e.g., $< 35$) | Treatment response, treatment outcome | Not distinguishing between live and dead bacteria |
| | trDNA | Urine | $C_t$ value higher than threshold (e.g., $< 35$) | Treatment response, treatment outcome | Low sensitivity<br>Not distinguishing between live and dead bacteria |
| | Ag85B mRNA | Sputum | $C_t$ value higher than threshold (e.g., $< 35$) | Treatment response | |
| | 16S rRNA | Sputum, stool | $C_t$ value higher than threshold (e.g., $< 35$) | Sterilising activity, treatment response | Larger longitudinal studies are needed to confirm the reliability |

**Table 1** continued

| | Biomarkers | Specimen | Index changes | Monitoring | Limitations |
|---|---|---|---|---|---|
| Surveillance of antigen components of MTB | LAM | Urine | Gradually reducing to undetectable levels | Treatment response, treatment outcome | Low sensitivity |
| | Ag85 antigen | Sputum, culture medium | Gradually reducing to undetectable levels | Sterilising activity, treatment outcome, recurrence | Low sensitivity |
| | MPT64 | Sputum | Gradually reducing to undetectable levels | Treatment response | Need to exclude the influence of blood in sputum on test results |
| | CFP-10, ESAT-6 | Serum | Gradually reducing to undetectable levels | Treatment response | Larger randomized prospective studies are needed to verify results<br>Complex operation process |

*AFB* acid-fast bacilli, *NTM* nontuberculous mycobacteria

associated with PTB treatment response. However, the results regarding certain cytokines after ATTB treatment are inconsistent. For example, MTB-specific TNFα and IL-10 have shown varying results. Some studies have reported an increase in TNFα and IL-10 after ATTB treatment in patients with MDR-TB stimulated with CFP [93], while other studies have shown a decrease in TNFα and IL-10 after ATTB treatment stimulated with CFP-10/ESAT-6 [89, 91, 97]. Additionally, stimulation with Bacillus Calmette–Guérin (BCG) or its recombinant 32-kDa antigen has been found to reduce IL-10 and increase IL-12 [99]. In cases of adverse treatment outcomes, low levels of TNFα, IL-1β, and IL-7 have been observed in whole blood stimulated with H37Rv [100]. MTB-specific IL-1ra (stimulated with CFP-10 and purified protein derivative (PPD)) [97] and IL-6 (stimulated with CFP-10 and ESAT-6) [89] have been shown to decrease after ATTB treatment, while MTB-specific IL-4 (stimulated with CFP-10 and ESAT-6) has been found to increase.

### *Chemokines*

MTB-specific chemokines have not been extensively studied for monitoring PTB treatment. One of the most commonly studied chemokines is IFNγ inducible protein 10 (IP-10), which belongs to the CXC class of chemokines. IP-10 has been found to decrease in whole blood stimulated with QuantiFERON TB Gold In-Tube (QFT-GIT) antigens after ATTB treatment [101]. Some studies have shown that IP-10 decreases in whole blood stimulated with CFP-10 or ESAT-6 after 9 months of ATTB treatment [97], and with QFT-GIT antigens after 6 months of ATTB treatment, although the results were not statistically significant. However, IP-10 has been found to decrease significantly when stimulated with selected RD1 peptide [95]. Therefore, further studies are needed to determine whether

extracellular MTB-specific IP-10 is associated with the outcome of ATTB treatment.

## T Cell Features

The differentiation of T cell subsets upon completion of PTB treatment has been associated with a cure [102]. A study on PBMCs from patients with PTB undergoing ATTB treatment found that $IFN\gamma^+CD4^+$ T cells (stimulated with CFP10/ESAT-6) increased after 2 weeks of treatment, while $IFN\gamma^+CD8^+$ T cells (stimulated with CFP10/ESAT-6) [103] and total $TNF\alpha^+CD8^+$ T cells (stimulated with Ag85) decreased. $IFN\gamma^+TNF\alpha^+CD4^+$ T cells (stimulated with CFP10/ESAT-6) also decreased. Additionally, regulatory T cell (Treg) subsets $CD25^{hi}CD127^{low+}$, $CD25^{hi}CD147^{++}$, and $CD25^{hi}CD27^{low}CD161^+$ expanded significantly after in vitro PTB antigen stimulation, while $CD25^{hi}CD127^{low}CD39^+$ Treg remained unchanged [104]. After 8 weeks of treatment, the proportion of $CD4^+CD25^{hi}CD127^{low}$ Treg cells and $CD4^+CD25^{hi}CD127^{low}CD147^+$ Treg cells increased [105]. At 9 weeks of treatment, PPD stimulation-induced $IFN\gamma^+CD4^+$ T cells decreased in $HLA\text{-}DR^+$, $CD38^+$, and $Ki\text{-}67^+$ subsets [106]. Moreover, 1 month into treatment, the expression of $CD45RA^-CCR7^+$ central memory T cells ($T_{CM}$) in PBMCs of patients with PTB increased and correlated with sputum conversion [107]. After 2 months of treatment, the expression levels of $CD27^+CD38^+CD4^+$, $CD27^+HLA\text{-}DR^+CD4^+$, and $CD27^-HLA\text{-}DR^+CD4^+$ T cells increased, while the expression levels of $CD27\text{-}IFN\gamma^+CD8^+$, $CD27^-TNF\alpha^+CD8^+$, and $CD27^-Ki\text{-}67^+CD4^+$ T cells decreased. $CD27^-IFN\gamma^+CD4^+$ T cells (stimulated with PPD) increased in patients with a rapid response to ATTB treatment [108]. Therefore, changes in these T cell subpopulations can serve as early and rapid indicators for monitoring PTB treatment. Another study by Young et al. showed that stimulation of PBMCs from patients' peripheral blood with CFP-10/ESAT-6 and PPD after completing PTB treatment led to a decrease in the expression of $TNF\alpha^+CD4^+/CD8^+$ T cells (stimulated with PPD) and $CD107a^+CD4^+/CD8^+$ T cells (stimulated with CFP-10/ESAT-6 or PPD) [109]. Additionally, after stimulation of PBMCs with PPD, the expression of the CD25 marker and the percentage of T cell subsets $CD4^+CD25^+$ and $CD4^+CD25^+CD39^+$ were significantly reduced [110]. The study also found that a significant decline in $CD4^+CD25^+FoxP3^+$ Treg cells is a biomarker of a good outcome after treatment of PTB with extensive lung damage. Other studies have shown that a decline in MTB-specific $CD38^+IFN\gamma^+$, $HLA\text{-}DR^+IFN\gamma^+$, and $Ki\text{-}67^+IFN\gamma^+CD4^+$ T cells is a characteristic of treatment success [111]. Analysis of $CD8^+$ T cells showed a decrease in $CD95^+IFN\gamma^+$, $Ki\text{-}67^+IFN\gamma^+$, and $CD127^+IFN\gamma^+CD8^+$ T cells, while an increase was observed in other subsets [112]. Additionally, the co-expression of HLA-DR/CD38 and PD-1/CD38 on $CD4^+$ and $CD8^+$ T cells decreased in patients with successful treatment [105]. Studies have also shown that patients with cured PTB exhibit high expression of killer cell lectin-like receptor G1 (KLRG1), PD-1, and cytotoxic T lymphocyte (CTLA-4) in $CD4^+$ T cells after in vitro BCG stimulation of PBMCs [113]. These changes and expression levels of biomarkers have the potential to serve as indicators of a cure.

## Antibodies

The plasma of patients with PTB contains several MTB-specific antibodies, including anti-ESAT-6, Rv2626c, 38 kDa antigen, LAM and FdxA antibodies. Studies have demonstrated that during ATTB treatment, levels of anti-ESAT-6 and Rv2626c antibodies decrease, while levels of anti-38 kDa antigen and LAM antibodies increase. However, levels of anti-FdxA antibodies remain unchanged. These antibody levels are also correlated with disease severity and lung lesions [114]. Additionally, the study found that alanine dehydrogenase and malate synthetase antibodies were higher in patients who failed treatment compared to those who were cured at the start of treatment. After 6 months of ATTB treatment, co-incubation of CFP-10/ESAT-6 with patient serum revealed a decrease in serum IgG antibodies [91], while patients with cured PTB exhibited high

expression of serum IgG antibodies in response to PTB antigen Ag85 [115]. Therefore, these antibody titers have the potential to serve as biomarkers for monitoring treatment efficacy.

### Other Biomarkers

The neutrophil to lymphocyte ratio (NLR) has been observed to decrease after ATTB treatment, which can be used to assess the effectiveness of drug treatment [98]. Furthermore, MMP-8 levels at 2 months after ATTB treatment have been found to be associated with persistent positive sputum culture in patients, making it a potential biomarker for predicting poor response to ATTB treatment [116].

The results of these in vitro experiments suggest that monitoring changes in MTB-specific immune factors, intracellular immune factors, and T cell subsets during ATTB treatment can be useful for assessing therapy efficacy and predicting prognosis in the early stages. However, it should be noted that these in vitro PTB stimulation experiments require meticulous operations and some flow experiments can be expensive. Furthermore, the existing research findings have certain limitations. Therefore, further investigations with larger sample sizes are needed to gain a more comprehensive understanding.

### MTB-Nonspecific Host Biomarkers

#### *Cytokines*

IFNγ is widely used as a non-specific cytokine to assess the effectiveness of ATTB treatment. IFNγ levels decrease significantly in patients who experience sputum conversion [117–120], and these levels are also correlated with treatment success [121]. Other cytokines have also shown promising results in studies. For instance, soluble TNF (solTNF) and IL-12 levels decrease in patients with drug-sensitive PTB, while transforming growth factor-beta 1 (TGFβ1) and IL-35 levels increase in patients with DR-TB [120]. After 6 months of ATTB treatment, IL-1β, IL-9, IL-10, and IL-15 levels decrease in the saliva of patients with DR-TB [122]. IL-6 levels decrease and IL-1β levels remain unchanged after treatment, but IL-1β levels can differentiate between smear-positive and smear-negative patients after 6 months of treatment [123]. Furthermore, high levels of IL-10 and low levels of IL-8 after ATTB treatment indicate poor PTB treatment response and suggest a possible recurrence of the disease [124].

#### *Chemokines*

Serum levels of CXCR3 ligands (CXCL9, CXCL10 (IP-10), CXCL11) significantly decrease in patients who achieve sputum conversion to negative after 2 months of ATTB treatment [125]. Studies have also demonstrated that plasma levels of CXCL8, CXCL9, CXCL10, and CCL5 decrease after 6 months of ATTB treatment [126]. Among these, IP-10 has been extensively studied as a biomarker and has been found to decrease in plasma [127, 128] and urine [129, 130] after treatment. Furthermore, significantly lower levels of IP-10 have been observed in the plasma of patients with successfully cured PTB [131]. In patients with DR-TB, baseline plasma IP-10 levels have been positively associated with delayed sputum culture conversion [132]. Therefore, IP-10 is closely linked to PTB treatment success [121] and serves as a reliable biomarker for predicting treatment outcomes. In cohort studies, pre-treatment levels of CCL2, CCL3, CCL4, CXCL8, CXCL10, and CX3CL1 have been identified as risk factors for poor PTB treatment, while CXCL1 has shown the opposite effect [133]. Validation cohorts have also shown that CCL3, CXCL8, and CXCL10 are associated with an increased risk of adverse treatment outcomes [133]. Additionally, serum overexpression of eotaxin has been significantly observed in well-treated patients [134], indicating its importance as an efficacy monitoring indicator. Therefore, the combined use of chemokines may provide a better prediction of PTB treatment outcomes.

### T Cell Features

After 6 months of ATTB treatment, there is an increase in the number of $CD3^+$ cells and $CD4^+$ cells, while the number of $CD8^+$ cells decreases. Further analysis reveals that the number of

IFN$\gamma^+$ and IL-2$^+$CD4$^+$ T cells also increases [123], whereas the number of IL-10$^+$CD4$^+$ T cells decreases. A high Th1/Th2 ratio is identified as a biomarker for poor treatment outcomes [135]. Additional studies demonstrate a decrease in the frequency of CD25$^+$ markers and CD4$^+$CD25$^+$, CD4$^+$CD25$^+$FoxP3$^-$, and CD4$^+$CD25$^+$CD39$^+$ Treg cells in patients with PTB who have completed treatment, indicating their potential for monitoring treatment success. However, in the treatment failure group, there is a sharp increase in the frequency of the CD4$^+$CD25$^+$FoxP3$^+$ Treg subgroup, suggesting its potential for predicting treatment failure [136]. Other studies indicate that low PD-1 expression on CD25$^-$CD4$^+$ T cells and CD25$^+$Foxp3$^-$CD4$^+$ T cells during treatment is a positive indicator of ATTB treatment [137]. Additionally, studies show differences in the changes of T cell subsets between patients with drug-sensitive PTB and drug-resistant PTB after ATTB treatment. The expression of conventional Treg cells (cTreg), transmembrane form (tm) TNFR1$^+$, and tmTNFR2$^+$CD4$^+$ T cells decreases in both patients with drug-sensitive and patients with drug-resistant PTB after ATTB treatment. However, the decrease in unconventional tmTNFR2$^+$ Treg cells is observed only in patients with drug-sensitive PTB, and the decrease in tmTNFR2$^+$ activated CD4$^+$ (actCD4$^+$) cells is observed only in patients with drug-resistant PTB [120]. Therefore, the changes in T cell immune characteristics after ATTB treatment differ between patients with drug-sensitive and drug-resistant PTB, and further exploration is needed to study biomarkers for predicting therapy efficacy separately.

### Other Biomarkers

Serum C-reactive protein (CRP), intracellular adhesion molecule-1 (sICAM-1), urokinase-type plasminogen activator receptor (suPAR), and pentraxin 3 (PTX3) all decreased after treatment [138]. Additionally, Heslop's team discovered that high levels of fibroblast growth factor (FGF) in sputum after treatment were negatively correlated with reduced bacterial load [139], and a low expression of the antioxidant enzyme heme oxygenase-1 (HO-1) is also indicative of successful treatment [140]. Furthermore, plasma levels of extracellular matrix protein 1 (ECM1) are associated with rapid conversion of sputum to negative after 2 months of treatment, while levels of L-selectin (SELL) and CD14 decline after completion of treatment. These biomarkers can be used as indicators for therapeutic monitoring [141]. The failure of serum globulin levels to return to normal after 2 months of treatment can be used as a biomarker to predict the need for prolonged PTB treatment [131, 142].

Some immune molecules in other immune cells (monocytes and B cells) also change during ATTB treatment. For example, the high expression of PD-L1 in monocytes of patients with PTB after treatment is associated with worse treatment outcomes [143], while the increase of activated B cells is related to the success of PTB treatment, as indicated by the high expression of a proliferation-inducing ligand (APRIL), Fas-ligand (FASLG), IL-5 receptor alpha (IL5RA), and CD19 mRNA in B cells after ATTB treatment [144]. Moreover, the high expression of FASLG and IL5RA mRNA in B cells is a marker of successful ATTB treatment. Although there are few studies on the changes of immune molecules in other immune cells after ATTB treatment, these studies suggest that there are some immune molecules in other immune cells that can be used to predict the efficacy of PTB therapy. Table 2 provides a summary of the host biomarkers for evaluating the efficacy of PTB therapy, and Supplementary Table S1 presents a summary of the changes and monitoring outcomes of each host biomarker after ATTB treatment.

Host-nonspecific immune factor changes after ATTB treatment are widespread, so recent studies have begun to conduct multi-immune factor screening to identify representative biomarkers. Vladyslav et al. conducted a multicenter cohort study and discovered significant changes in Toll-like receptor (TLR) and aptamer gene expression, along with cytokine and chemokine levels, in patients with PTB following 2 months of ATTB treatment [145]. It was found that the changes of Toll interaction protein (TOLLIP), TLR9, TLR7, Toll-like receptor

**Table 2** Host biomarkers for evaluating therapy efficacy of pulmonary tuberculosis

| | Biomarkers | Stimulators | Specimen | Monitoring | Limitations |
|---|---|---|---|---|---|
| MTB-specific host biomarkers | | | | | |
| Cytokines | IFNγ | CFP, CFP-10, ESAT-6, CFP-10/ESAT-6, Recombinant 32-kDa *M. bovis*, selected RD1 peptides | Whole blood, PBMC | Treatment response, treatment outcome | Limited monitoring capacity<br>Inconsistent results |
| | TNFα | CFP, ESAT-6, CFP-10/ESAT-6, H37Rv | Whole blood, PBMC | Treatment response, treatment outcome | Inconsistent results<br>Larger cohorts are needed to confirm the consistency of these results |
| | IL-10 | CFP, ESAT-6, CFP-10/ESAT-6, Recombinant 32-kDa *M. bovis* | Whole blood, PBMC | Treatment response, treatment outcome | Inconsistent results<br>Related to the severity of tuberculosis |
| | IL-1ra | CFP-10/PPD | Whole blood | Treatment response | Not directly affected by the immune response of antibiotics against MTB |
| | IL-1β, IL-7 | H37Rv | Whole blood | Treatment outcome | Require containment facilities<br>Larger prospective immunological studies are needed to confirm the results |
| | IL-4, IL-6 | CFP-10/ESAT-6 | Whole blood | Treatment response | Small size sample<br>Only applicable to DS-TB |
| | IL-12 | Recombinant 32-kDa *M. bovis* | PBMC | Treatment response | Further studies are needed |
| | IFNγ/IL-10 ratio | Recombinant 32-kDa *M. bovis* | PBMC | Treatment response, treatment outcome | Small size sample |
| | IL-2/IFNγ ratio | QFT-GIT antigens | Whole blood | Treatment response | |

**Table 2** continued

| | Biomarkers | Stimulators | Specimen | Monitoring | Limitations |
|---|---|---|---|---|---|
| Chemokines | IP-10 | CFP-10/ESAT-6, QFT-GIT antigens, selected RD1 peptides | Whole blood | Treatment response | |
| T cell features | $IFN\gamma^+CD4^+$ | CFP-10/ESAT-6, Ag85 | PBMC | Treatment response | Small size sample<br>Expensive |
| | $IFN\gamma^+CD8^+$, $IFN\gamma^+TNF\alpha^+CD4^+$, $CD4^+CD25^{hi}CD127^{low+}$, $CD4^+CD25^{hi}CD147^{++}$, $CD4^+CD25^{hi}CD27^{low}CD161^+$, $CD4^+CD25^{hi}CD127^{low}CD39^+$, $CD4^+CD25^{hi}CD127^{low}CD147^+$, $CD27^+CD38^+CD4^+$, $CD27^+HLA\text{-}DR^+CD4^+$, $CD27^-HLA\text{-}DR^+CD4^+$, CD27-$IFN\gamma^+CD8^+$, $CD27^-TNF\alpha^+CD8^+$, $CD27^-Ki\text{-}67^+CD4^+$, $CD95^+IFN\gamma^+/Ki\text{-}67^+IFN\gamma^+CD8^+$, $CD127^+IFN\gamma^+CD8^+$ | CFP-10/ESAT-6 | PBMC | Treatment response | Significant individual differences in patient population<br>Lack of available disease severity assessment<br>Lack of longitudinal data<br>Need to be substantiated by further studies |
| | $HLA\text{-}DR/CD38^+CD4^+/CD8^+$, $PD\text{-}1/CD38^+CD4^+/CD8^+$ | CFP10/ESAT-6 | PBMC | Treatment outcome | |
| | $HLA\text{-}DR/CD38/Ki\text{-}67^+IFN\gamma^+CD4^+$, $TNF\alpha^+CD4^+/CD8^+$, $CD4^+CD25^+$, $CD4^+CD127^+$, $CD4^+CD25^+CD39^+$, $CD4^+CD25^+FoxP3^+$ | PPD | PBMC | Treatment response | |
| | $T_{CM}$ | BCG, PPD, ESAT-6 | PBMC | Treatment response | |
| | $CD107a^+CD4^+/CD8^+$ | PPD, CFP-10/ESAT-6 | PBMC | Treatment response | |
| | $CD38^+IFN\gamma^+/HLA\text{-}DR^+IFN\gamma^+/Ki\text{-}67^+IFN\gamma^+CD4^+$ | MTB, CFP-10/ESAT-6 | PBMC | Treatment outcome | |
| | $KLRG1^+/PD\text{-}1^+/CTLA\text{-}4^+CD4^+$ | BCG | PBMC | Treatment outcome | |
| Antibodies | Anti-ESAT-6 antibody, anti-Rv2626c antibody, anti-38 kDa antigen antibody, anti-LAM antibody, alanine dehydrogenase antibody, malate synthetase antibody | | Plasma | Treatment outcome | Small size sample<br>Need to be substantiated by further studies |
| | Anti-CFP-10/ESAT-6 antibody, anti-Ag85 antibody | | Serum | Treatment outcome | Low sensitivity |
| Other biomarkers | NLR, MMP-8 | QFT-GIT antigens | Whole blood | Treatment response | Small size sample<br>Low positive predictive value (24%) |

**Table 2** continued

| | Biomarkers | Stimulators | Specimen | Monitoring | Limitations |
|---|---|---|---|---|---|
| MTB-nonspecific host biomarkers | | | | | |
| Cytokines | IFNγ | | Sputum, plasma, serum | Treatment response, treatment outcome | Only suitable for evaluating early anti-TB treatment in sputum<br>Limited monitoring capacity<br>Rather heterogeneous in cohort of certain studies |
| | TNFα | | Sputum | Treatment response | Only suitable for evaluating early anti-TB treatment in sputum |
| | solTNF, IL-6, IL-12, IL-35, TGFβ1 | | Plasma | Treatment response | Small size sample |
| | IL-1β | | Plasma, saliva | Treatment response, treatment outcome | Only distinguish between AFB (+) and AFB (−) TB patients after 6 months of anti-TB treatment<br>High-quality requirements for saliva samples |
| | IL-8 | | Sputum, serum | Treatment response, treatment outcome, relapse | Small size sample<br>Only suitable for evaluating early anti-TB treatment in sputum |
| | IL-9, IL-15 | | Saliva | Treatment response | High quality requirements for saliva samples |
| | IL-10 | | Serum, saliva | Treatment response, treatment outcome, relapse | Small size sample<br>High-quality requirements for saliva samples |

**Table 2** continued

| | Biomarkers | Stimulators | Specimen | Monitoring | Limitations |
|---|---|---|---|---|---|
| Chemokines | IP-10 | | Plasma, serum, urine | Treatment response, treatment outcome | Difference between DR-TB and DS-TB<br>Need to be substantiated by further studies |
| | CXCL9 | | Plasma, serum | Treatment response | Small size sample<br>Lack of the correlation to disease severity<br>Not exclude changes caused by other infections |
| | CXCL8 | | Plasma | Treatment response, treatment outcome | |
| | CXCL11 | | Serum | Treatment response | Not exclude changes caused by other infections |
| | CCL2, CCL3, CCL4, CX3CL1, CXCL1 | | Plasma | Treatment outcome | Moderate sample size<br>Need to be substantiated by further studies |
| | CCL5 | | Plasma | Treatment response | Need to be substantiated by further studies |
| T cell features | $CD3^+$, $CD4^+$, $CD8^+$, $IFN\gamma^+$/IL-2 $^+CD4^+$, IL-$10^+CD4^+$, $tmTNF^+$ cTreg, $tmTNFR1^+$/$tmTNFR2^+CD4^+$, $tmTNFR2^+$uTreg, $tmTNFR2^+$ activated $CD4^+$ | | Whole blood | Treatment response | Small size sample<br>Expensive |
| | Th1/Th2 ratio, $CD25^+$ marker, $CD4^+CD25^+$, $CD4^+CD25^+FoxP3^-$, $CD4^+CD25^+CD39^+$Treg, $CD4^+CD25^+FoxP3^+$ Treg | | Whole blood | Treatment outcome | Need to be substantiated by further studies |

**Table 2** continued

| | Biomarkers | Stimulators | Specimen | Monitoring | Limitations |
|---|---|---|---|---|---|
| Other biomarkers | CRP, sICAM-1, suPAR | | Serum | Treatment response | Lack of further studies to verify |
| | HO-1, PTX3 | | Plasma | Treatment outcome | |
| | ECM1, SELL, CD14 | | Plasma | Treatment response | |
| | Globulin | | Serum | Treatment extension | |
| | PD-L1 of $CD14^+$ monocytes, FASLG and IL5RA of B cell | | PBMC | Treatment outcome | |

adaptor molecule 1 (TICAM1), IL-1 receptor-associated kinase 4 (IRAK4), CD14, and cytokines vascular endothelial growth factor (VEGF), macrophage inflammatory protein (MIP)-1β, IL-7, IFNγ, and granulocyte colony-stimulating factor (G-CSF) after 2 months of ATTB treatment were statistically significant. TOLLIP, lymphocyte antigen 96 (LY96), and nine cytokines, including TNFα, MIP-1α, monocyte chemoattractant protein-1 (MCP-1/CCL2), IL-8, IL-5, IL-15, IFNα, granulocyte–macrophage colony-stimulating factor (GM-CSF), and G-CSF, were found to be associated with successful treatment outcomes. Serum TLR2 levels decreased in successful patients, while failed patients showed the opposite trend. Another clinical trial screening 70 host biomarkers associated with PTB severity and treatment response demonstrated that most biomarkers decreased after 8 weeks of treatment, with serum amyloid A1 (SAA1), procalcitonin (PCT), IL-1β, IL-6, CRP, PTX3, and MMP-8 showing strong associations with disease severity [146]. In the case of patients with DR-TB, plasma levels of CRP, SAA, VEGF-A, soluble interleukin-2 receptor alpha (sIL-2Rα), and IP-10 at baseline treatment were positively correlated with delayed sputum culture conversion and a combination of MCP-1, sIL-2Rα, and SAA could distinguish patients with PTB with a fast response to ATTB treatment [132]. Additionally, significant changes were observed in the concentrations of granzyme A, MCP-1, IL-1β, IL-9, IL-10, IL-15, MIP-1β, ferritin, and serum amyloid A in the saliva of patients with drug-resistant PTB after 2 or 6 months of treatment [122]. Among these, only MCP-1 increased after treatment, while the others decreased. These findings suggest that a combination of multiple immune factors may be useful for monitoring PTB treatment, but further research is needed to determine the optimal combination for predictive purposes.

## Host Biomarkers for Omics Analysis

Recent study has summarized biomarkers in omics for monitoring PTB treatment [147]. Transcriptomics revealed that complement

C1q, C2, BF, and serpin in whole blood, as well as G1, UCP2 (involved in fatty acid metabolism), IFNα signaling pathway-related molecules IL15RA, UBE2L6 (a member of the ubiquitin family), guanylate binding protein 4 (GBP4), GBP5, dual-specific phosphatase 3 (DUSP3), and kruppel-like transcription factor 2 (KLF2), decreased after treatment. Serum miRNAs, including miR-21-5p, miR-92a-3p, and miR-148b-3p, also decreased after treatment, and high expression of Pragmin (Src kinase regulator) after treatment was a risk factor for relapse. Proteomics showed that the serum complement C7 and angiotensinogen, innate and adaptive immune-related proteins, such as coagulation factor V and serum amyloid protein, decreased after treatment, while the phosphoserine-tRNA kinase of infected primary human leukocytes increased after treatment. In addition, metabolomics showed that pyridoxine and bradykinin decreased after treatment, and the combination of L-histidine, arachidonic acid, biliverdin, and cysteine glutathione disulfide could be used as markers to cure PTB. Exploring the biomarkers in the host after ATTB treatment by omics methods has great application potential, but the sample size of the current omics studies is limited. It is necessary to further increase the sample size and reduce the biological differences of individuals to verify whether they are suitable for the monitoring of clinical efficacy. In addition, the Opti-4TB study, a "proof of concept" method, may predict the prognosis of PTB based on multi-omics combined detection of host immune markers [148]. Although it has a broad clinical application potential, one still needs to obtain more accurate biomarkers that reflect the effect of ATTB treatment and develop a simple prognostic prediction and evaluation system to achieve clinical translation.

## CONCLUSIONS

Despite the limitations of traditional biomarkers, they are still widely used owing to their simplicity, cost-effectiveness, and suitability for resource-limited settings. However, the lack of timeliness and low sensitivity of these biomarkers has led to the further spread of PTB, resulting in significant health risks and economic losses. Therefore, there is an urgent need for improved biomarkers that can accurately reflect therapy efficacy and guide treatment decisions to control PTB outbreaks. The emergence of new biomarkers, including those derived from MTB itself and its host, as well as advancements in detection methods, offer the possibility of rapid, accurate, and efficient monitoring of PTB therapy efficacy. However, as a result of the diversity of these biomarkers, variations in study designs, small sample sizes, and inconsistent reports of certain biomarkers [149], it is necessary to expand the sample size to identify important biomarkers for monitoring ATTB therapy efficacy.

Cytokines and chemokines play a crucial role in evaluating the efficacy of PTB therapy [150]. Zimmer et al. conducted a meta-analysis of biomarkers associated with treatment response to active PTB, summarizing 81 biomarkers from 77 relevant studies. Despite the heterogeneity in the design of surveillance studies for PTB treatment, studies involving a large number of biomarkers have shown that cytokines (IL-6 and TNFα), chemokine (IP-10), and non-specific inflammatory marker (CRP) can serve as biomarkers for early monitoring of ATTB treatment [151]. Moreover, the assessment of therapy efficacy in patients with PTB through the use of MTB-specific and nonspecific host biomarkers encompasses a wide range of cytokines and chemokines. During ATTB treatment, changes in MTB-specific biomarkers produced through in vitro stimulation can reflect the therapeutic efficacy in patients with PTB. These biomarkers can improve the specificity of prediction, although they may involve complex procedures and have high requirements. Monitoring MTB-nonspecific host biomarkers during ATTB treatment, including those identified through omics analysis, is a promising approach. Although the changes in MTB-nonspecific biomarkers of ATTB treatment are complex, more accurate prediction models can be developed by combining multiple immune factors [152]. Additionally, the alterations in host immune markers differ between patients with drug-sensitive PTB and patients with drug-resistant PTB, and even within the same patient

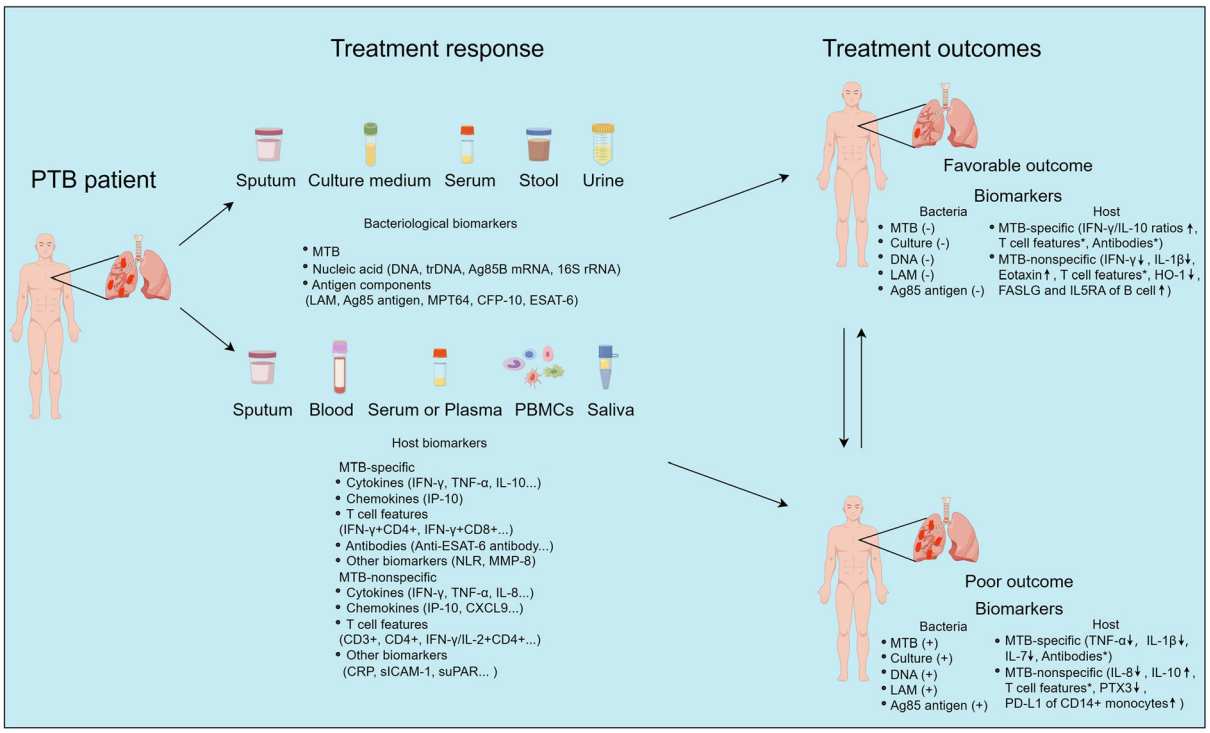


**Fig. 1** Biomarkers monitor the efficacy of ATTB treatment in patients with PTB. Biomarkers from bacteria and hosts in patient samples may vary during ATTB treatment, and certain biomarker changes can indicate treatment outcomes. *The changes in these biomarkers can be obtained in supplementary Table S1. This image was drawn using Figraw

group, the same type of immune marker may display distinct changes in response to different MTB antigen stimuli. Hence, it is crucial to investigate and analyze the biomarkers of these two patient groups separately.

Certain biomarker changes during PTB treatment can reflect the efficacy of ATTB treatment (Fig. 1). This review provides an updated summary of biomarkers for monitoring ATTB therapy efficacy and suggests new research directions for further validation studies. It is hoped that through these biomarkers, a simple and feasible tool for evaluating therapy efficacy can be established, thereby reducing the incidence and mortality of PTB and achieving the goal of ending tuberculosis as soon as possible.

***Author Contribution.*** The initial draft of the manuscript was prepared by Fuzhen Zhang and Yu Pang. Fuzhen Zhang, Fan Zhang, Yu Dong, Liang Li and Yu Pang contributed to the writing of the manuscript. Fuzhen Zhang, Fan Zhang, Yu Dong, Liang Li and Yu Pang have reviewed and approved the manuscript's results and conclusions. All authors have read and confirmed that they meet the criteria for authorship as outlined by the International Committee of Medical Journal Editors (ICMJE).

***Funding.*** This work was supported by the Beijing Hospitals Authority Ascent Plan from Yu Pang (DFL20191601) and Liang Li (DFL2022 1401) and Beijing Hospitals Authority Clinical Medicine Development of Special Funding from Yu Pang (ZYLX202122), and Yu Pang funded the journal's Rapid Service fee.

***Data Availability.*** Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

### *Declarations*

***Conflict of Interest.*** Fuzhen Zhang, Fan Zhang, Yu Dong, Liang Li and Yu Pang have nothing to declare.

***Ethical Approval.*** This article does not require ethical approval because it is based on published research and does not involve any research conducted by the authors on humans or laboratory animals.



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## Publisher's Note