

Novel Biomarkers for Tuberculosis: Towards Affordable and Reliable Diagnostic Solutions

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ABSTRACT

Tuberculosis (TB) remains a significant global health challenge—a situation further exacerbated by the COVID-19 pandemic, which has disrupted diagnosis and treatment efforts. Despite being preventable and curable, TB continues to be a major threat, ranking among the leading causes of infectious disease mortality. The unreliability and unaffordability of current diagnostic procedures underscore the need for new biomarkers and point-of-care testing solutions. Recent research has identified several potential biomarkers for diagnosing TB and differentiating between active and latent infections. This review aims to identify and evaluate a comprehensive set of TB-related biomarkers, including those associated with vaccine-induced protection, active disease, infection status, TB risk, and treatment response. Articles were sourced from the PubMed database using the keywords “tuberculosis,” “biomarker,” “diagnosis,” and “kit,” based on defined inclusion and exclusion criteria. The identified biomarkers include Rv1566c-444 (vaccine-induced); IP-10, IFN- γ , ferritin, 25(OH)D, IL-1ra, IL-1 β , GM-CSF, fibronectin, HMGB1, Tpx-IgA, NCAM-1, vitronectin, CFH, α -2-macroglobulin, and *M. tuberculosis* β -lactamase (infection and disease-related); IFN- γ (TB risk); and IL-1 β , IP-10, and suPAR (treatment response). Among these, IP-10 and IFN- γ stand out as the most promising host biomarkers, while Ag85B is highlighted as a key pathogen-specific marker. The results of this review offer valuable insight for the development of effective, low-cost TB diagnostic kits and provide scientific support for public health policy aimed at accelerating TB detection and control, especially in resource-limited settings.

Keywords: biomarkers, diagnosis, TB risk assessment, TB treatment monitoring, tuberculosis

INTRODUCTION

Tuberculosis (TB) continues to exert a major global impact, presenting significant public health challenges due to its high incidence, prevalence, and mortality rates. Despite extensive control efforts, many countries have yet to meet the targets outlined in the End TB Strategy. Although TB is both preventable and curable, it remains the second leading cause of death from infectious diseases globally—surpassing HIV/AIDS mortality by nearly twofold in 2023 (World Health

Organization, 2024). The persistent global burden of TB underscores the urgent need for accessible, affordable, and accurate point-of-care diagnostic tools, particularly in high-burden and resource-limited settings. While current diagnostic methods are essential, they face critical limitations in sensitivity, cost, infrastructure requirements, and feasibility for widespread use (Varughese *et al.*, 2023; Walzl *et al.*, 2018). For instance, traditional sputum smear microscopy is inexpensive but has poor sensitivity, especially in children and people living with HIV. Molecular diagnostics such as the

Xpert MTB/RIF assay offer improved sensitivity (World Health Organization, 2022; Lombardi *et al.*, 2017; Detjen *et al.*, 2015) and drug resistance detection, yet remain expensive and infrastructure-dependent (Dorman *et al.*, 2018). Similarly, immunological tests like interferon-gamma release assays (IGRA) are useful for detecting latent TB infection (LTBI) but cannot distinguish it from active TB disease and require advanced laboratory facilities (Teklu *et al.*, 2018; Teo *et al.*, 2024). These diagnostic gaps highlight the pressing need for innovative approaches that can address these shortcomings. In this context, the use of biomarkers has gained increasing attention due to their potential to transform TB diagnosis and management. Biomarkers are not only useful for detecting TB, but also for assessing disease progression, identifying LTBI (Carranza *et al.*, 2020; Zhou *et al.*, 2015), and monitoring treatment efficacy (Zhang *et al.*, 2023). They also enable the study of host-pathogen interactions, inform drug resistance mechanisms, and support the development of new therapeutic strategies. Advances in high-throughput omics technologies, such as metabolomics and lipidomics, have further accelerated the discovery of novel diagnostic biomarkers (Bisht *et al.*, 2019; Li *et al.*, 2022; Yu *et al.*, 2023). TB biomarkers can be broadly categorized into two types: pathogen-based and host-based. Pathogen-based biomarkers involve direct detection of *Mycobacterium tuberculosis* components, such as antigens or nucleic acids. Host-based biomarkers, on the other hand, encompass a wide array of immune and molecular indicators, including hematological parameters, antibodies, cytokines (Li *et al.*, 2019; Nguyen *et al.*, 2020; Nyoman *et al.*, 2023), chemokines, RNA transcripts (Yan *et al.*, 2021), proteins, and integrated multi-marker signatures (Baquero-Artigao *et al.*, 2023; Nogueira *et al.*, 2022). Despite the promise of biomarker-based diagnostics, several challenges remain—particularly in ensuring their practicality in low-resource settings. Detecting *M. tuberculosis* antigens or immune responses often requires sophisticated tools, and many candidate biomarkers have yet to be validated in field settings. Establishing robust proof-of-concept for both TB infection and its progression could accelerate industrial investment in diagnostic kit development. Factors such as sample type, turnaround time, cost-efficiency, and ease of use must be carefully considered.

Progress has been made in this direction. Commercially available kits such as the Erythra TB-KIT and the GBTsol Latent TB Test Kit (Glory Biotechnologies, Korea) utilize immune-based methods to detect TB-specific responses (Gill *et al.*, 2022; Goletti *et al.*, 2022). The former employs a lateral flow assay (LFA) with PPD stimulation, while the latter detects ESAT-6/CFP-10-specific T cells through microfiltration. However, these kits are still limited by high costs, laboratory dependencies, and suboptimal ability to distinguish latent from active TB (Alonzi *et al.*, 2023; Seele *et al.*, 2023).

To ensure broader diagnostic access and improved disease surveillance, the identification of optimal biomarkers remains a critical goal. Ideal biomarkers should be affordable, reliable, detectable from minimally invasive samples such as blood or urine, and compatible with portable diagnostic platforms. This review aims to evaluate a spectrum of TB biomarkers—including those related to vaccine-induced protection, disease activity, infection status, risk of progression, and treatment response—with strong potential for integration into practical, point-of-care diagnostic tools that meet WHO target profiles and support global TB elimination goals.

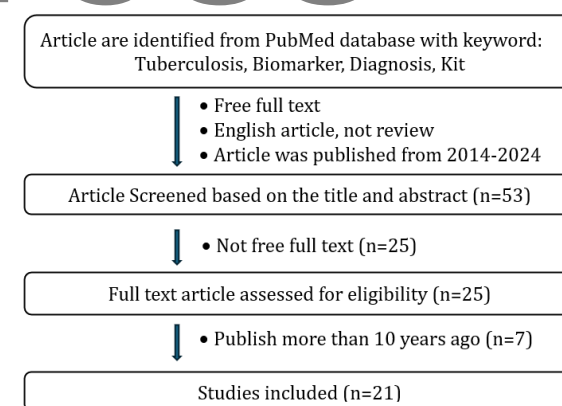


Figure 1. Article collected and screened method flow chart

METHODS

The review was conducted over the course of several phases in January 2024. Article are identified from PubMed database with keyword tuberculosis, Biomarker, Diagnosis, Kit. Inclusion criteria is free full text, English article, not review and article was published from 2014-2024.

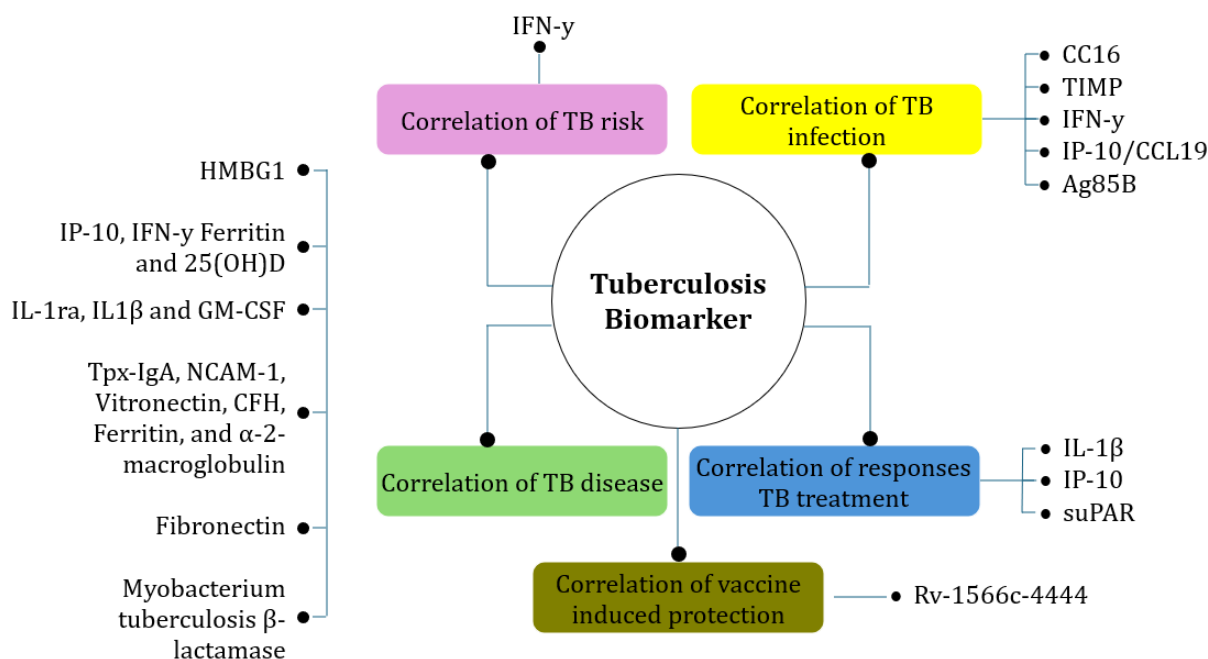


Figure 2. Biomarkers tuberculosis correlation classification

Articles screened based on title and abstract as much 53 articles. Exclusion criteria is not free full text (25 articles) and publish more than 10 years ago (7 articles). Studies included as much 21 articles (Figure 1).

RESULTS AND DISCUSSION

Key biomarkers relevant to TB diagnosis and management can be broadly classified into five categories: biomarkers associated with vaccine-induced protection, active TB disease, risk of disease progression, TB infection, and response to TB treatment (Goletti *et al.*, 2018). Biomarkers for vaccine-induced protection are essential for evaluating the effectiveness of TB vaccines by indicating the presence of a protective immune response post-vaccination. Disease-related biomarkers help differentiate active TB from latent TB infection and other respiratory conditions, thereby enhancing diagnostic precision and assessing disease severity (Cheng *et al.*, 2022; Nemes *et al.*, 2022). Risk biomarkers predict the likelihood of progression from latent infection to active disease, enabling identification of individuals who may benefit from preventive interventions (Petruccioli *et al.*, 2016). Infection biomarkers detect current or past infection with *M. tuberculosis*, serving as useful tools for TB screening and latent infection diagnosis (Carranza

et al., 2020; Zellweger *et al.*, 2020). Lastly, treatment-response biomarkers are applied to monitor therapeutic efficacy and identify patients at risk of relapse or treatment failure (Zimmer *et al.*, 2022). Integrating these biomarker categories into clinical practice can significantly enhance TB control strategies—by enabling early and accurate diagnosis, identifying high-risk populations, guiding timely treatment, and evaluating treatment outcomes. In line with the World Health Organization (WHO) target product profiles (TPP) for TB diagnostics, ideal biomarkers should meet minimum performance thresholds: sensitivity ranging from 79% to 96% and specificity ranging from 72% to 89%, depending on the test application (triage, confirmatory, or predictive testing) (Figure 2).

Biomarkers that correlate to vaccine induction

The primary goal of vaccination is to establish long-lasting immunological memory capable of providing rapid protection upon re-exposure to a pathogen (Li *et al.*, 2024). This response is mediated by memory B cells and long-lived plasma cells, which can continuously produce antibodies even in the absence of antigen stimulation (Stewart *et al.*, 2023; Prezzemolo *et al.*, 2014). While current TB vaccines primarily stimulate cell-mediated immunity, emerging

evidence suggests that humoral responses may also contribute to protection against *M. tuberculosis* (Rijnink *et al.*, 2021; Dutt and Correno-Parra, 2025). One approach to enhancing vaccine-induced protection involves conjugating mycobacterial polysaccharides to immunogenic proteins. For example, Prados-Rosales *et al.* (2017) developed polysaccharide-conjugate vaccines by linking arabinomannan (AM)—a capsular polysaccharide present on the *M. tuberculosis* surface—to Ag85B or protective antigens from *Bacillus anthracis*. Immunization with these constructs in mice elicited robust antibody responses, reduced bacterial loads, and significantly improved survival, indicating the potential of AM-conjugated proteins as vaccine candidates. Among protein-based vaccine antigens, Rv1566c (RipD) has emerged as a promising biomarker candidate. Rv1566c is a member of the NlpC/p60 family, known for its non-catalytic peptidoglycan-binding ability that may stabilize the mycobacterial cell wall (Both *et al.*, 2014; Chen *et al.*, 2022). A recombinant truncated form, Rv1566c-444, contains immunodominant T-cell epitopes and has demonstrated high immunogenicity in murine models. Luan *et al.* (2022) reported that immunization with Rv1566c-444 in BALB/c mice elicited a strong Th1 immune response, evidenced by elevated IFN- γ /IL-4 ratios and increased CD4+ T cell proliferation. Although the diagnostic sensitivity (44.44%) of Rv1566c-444 is lower than commercial kits using ESAT-6, CFP-10, and Rv3615c, its specificity reached 94.40%. These findings suggest that while Rv1566c-444 alone may not meet WHO's minimum sensitivity requirement for a standalone TB diagnostic test (TPP \geq 65–80%), its high specificity and immunogenicity support its potential as a vaccine antigen or component in multi-antigen diagnostic kits. Furthermore, *ex vivo* analyses in human TB patients revealed that Rv1566c-444 induced a greater number of spot-forming cells (SFCs) than in healthy donors, supporting its relevance in human immune responses. Taken together, the dual role of Rv1566c-444—as an immunogenic vaccine antigen and a potential diagnostic biomarker—makes it a compelling target for further translational development.

Biomarkers that correlate to tuberculosis disease

Biomarkers that correlate with TB disease are primarily used to differentiate active TB from latent TB infection (LTBI) and other non-TB

conditions. These biomarkers play a crucial role in establishing accurate diagnosis and monitoring disease progression. Several host-derived protein markers have shown diagnostic potential. For instance, high mobility group box-1 (HMGB1) levels in cerebrospinal fluid (CSF) were significantly elevated in patients with tuberculous meningitis (TBM), as reported by Chen *et al.* (2016). Using a cut-off of 3.80 ng/mL, HMGB1 demonstrated a sensitivity of 79.49% and specificity of 94.52% for TBM diagnosis in patients with extra-neural TB values that fall within the WHO's recommended TPP range. This supports the use of HMGB1 as a promising biomarker for severe forms of TB involving the central nervous system.

In pediatric populations, Comella-del-Barrio *et al.* (2019) evaluated combinations of IP-10, IFN- γ , ferritin, and 25(OH)D to distinguish active TB from LTBI. The pairing of IP-10 and IFN- γ achieved excellent diagnostic accuracy, with an area under the ROC curve (AUC) of 0.955, and sensitivity of 93.20% for active TB and 90.00% for LTBI. However, sensitivity declined to 76.20% in children with mediastinal TB, suggesting that biomarker performance may vary with TB manifestations. In contrast, not all biomarkers meet diagnostic standards. Blok *et al.* (2014) assessed the urine-based lipoarabinomannan (LAM) ELISA, which yielded a sensitivity of only 4.80% for pediatric TBM, despite specificity of 93.10%. Due to its poor sensitivity, the LAM assay is not recommended for TBM detection in children.

Sariko *et al.* (2019) explored a biosignature combining multiple cytokines with the antibody in lymphocyte supernatant (ALS) assay. While the cytokine panel alone (IL-2, TNF- α , VEGF) yielded moderate accuracy (sensitivity 82.20%, specificity 76.20%), the ALS assay alone performed better, achieving 92.00% sensitivity and 96.00% specificity—exceeding WHO diagnostic targets. Antibody-based diagnostics have also gained traction. Jacobs *et al.* (2022) evaluated IgA and IgM responses against antigens such as APA, Rv3019c, PstS1, and LAM. The combination of seven IgA antibodies yielded an AUC of 0.80, while integrating these with five cytokine biomarkers boosted accuracy to an AUC of 0.97, with sensitivity 95.00% and specificity 88.50%. These findings highlight the advantage of multiplexed approaches in improving TB diagnosis. However, Broger *et al.* (2017) noted that no single IgG-based assay met WHO TPP criteria for use in HIV-uninfected adult populations, indicating limitations in standalone antibody-based tests.

Fibronectin, a structural protein, has been investigated as a disease severity marker. Kulkarni *et al.* (2024) found that TB patients with more severe disease showed significantly lower serum fibronectin levels. Cut-offs of <109.39 ng/mL and <99.32 ng/mL were associated with higher sputum smear grades and extensive pulmonary lesions, respectively, indicating its potential as a biomarker for disease progression. Lastly, Sule *et al.* (2019) introduced the REFtb assay, based on the detection of the BlaC enzyme using a fluorogenic substrate (CDG-3). The assay achieved sensitivity of 89.00% for smear-positive and 88.00% for smear-negative culture-positive TB cases, with specificity of 82.00%. With a turnaround time of only 10 minutes and no need for technical expertise, REFtb holds great promise as a point-of-care diagnostic tool in resource-limited settings, aligning well with WHO's practical and performance criteria.

Biomarkers that correlate to tuberculosis risk

Biomarkers indicating the risk of TB progression are essential for identifying individuals who are likely to develop active disease, especially from latent TB infection. These biomarkers are crucial in guiding preventive therapy and targeted monitoring. A study by Kim *et al.* (2024) utilized serial [18F] fluorodeoxyglucose (FDG)-PET/CT scans to monitor metabolic changes in TB-infected individuals. These scans successfully detected early, asymptomatic stages of TB by revealing metabolic activity and structural alterations in the lungs, which conventional radiography often fails to identify. Microbiological sampling further confirmed the presence of active *M. tuberculosis* in local lymph nodes and infection sites, validating the imaging findings. Complementing imaging tools, the Actiphage assay, a bacteriophage-based molecular method, was used to detect *M. tuberculosis* DNA in blood samples. The study showed a significant association between Actiphage positivity and progressive TB infection (Namuganga *et al.*, 2021). This underscores the value of pathogen-derived biomarkers in enhancing early risk assessment. Importantly, 38.00% of QuantiFERON-TB Gold Plus (QFT)-positive TB contacts showed signs of incipient TB based on PET-CT findings, despite being asymptomatic, emphasizing the importance of early intervention (Goletti *et al.* 2022; Petruccioli *et al.* 2016). In addition to IFN- γ (Table I), several host-derived biomarkers, such as IP-10/CCL19, have also demonstrated strong predictive power. The IP-10/CCL19 ratio in particular achieved

100% sensitivity and specificity in identifying individuals with short-term TB progression risk (Daniel *et al.* 2023). This highlights its potential role as a soluble biomarker for early TB prediction. Furthermore, IFN- γ release assays (IGRAs), though widely used, may benefit from being integrated with novel tools like Actiphage or cytokine profiling to improve specificity in TB risk stratification.

Overall, combining advanced imaging, molecular diagnostics, and immune biomarkers—as detailed in Table 1—provides a comprehensive and specific approach to predicting TB progression. These strategies align with WHO recommendations that emphasize early detection and intervention in high-risk populations.

Biomarkers that correlate to tuberculosis infection

Accurate biomarkers for TB infection are essential for early diagnosis, monitoring disease progression, and evaluating treatment efficacy (Yerlikaya *et al.* 2023). The study by Nandi *et al.* (2021) focuses on the use of Club Cell protein 16 (CC16) as a biomarker for the early detection of silicosis—a condition that significantly increases the risk of TB. CC16—an anti-inflammatory protein predominantly secreted by non-ciliated bronchiolar epithelial cells—plays a protective role in the respiratory tract (Arliny and Mursalin, 2021). The study indicates that individuals with silicosis or other chronic respiratory diseases exhibit reduced levels of CC16. This reduction suggests the potential of CC16 as an early indicator of lung injury caused by silica dust exposure. Consequently, CC16 can serve as a valuable biomarker for the early detection of conditions that predispose individuals to TB, such as silicosis and silico-tuberculosis (Arliny and Mursalin, 2021).

The study investigated the creation of a rapid point-of-care lateral flow assay designed for the semi-quantitative estimation of serum CC16 levels to identify early silicosis in workers exposed to silica dust. This assay utilized gold nanoparticles conjugated with anti-CC16 monoclonal antibodies to detect CC16 concentrations in serum samples. A total of 106 serum samples were tested, revealing that CC16 levels below 9.0 ng/mL indicated moderate to severe silicosis, while levels between 6.1 and 9.0 ng/mL suggested early silicosis. The assay demonstrated high sensitivity (100%) and specificity (95.00%), confirming its effectiveness as a reliable screening tool for the early detection of silicosis (Arliny and Mursalin, 2021).

Table I. Potential biomarkers diagnosis kit based on tuberculosis correlation classification

Biomarker	Detection kit	Sensitivity and specificity	Summary	References	
Correlates of vaccine-induced protection	Rv1566c-444	ELISpot kit T-SPOT.TB (QuanBio, Beijing, China)	44.44% and 84.09%	Rv1566c-444 has demonstrated strong immunogenicity as a potential TB vaccine antigen, although its diagnostic sensitivity is lower than that of commercial kits. It induced a robust Th1 immune response in immunized BALB/c mice, suggesting the need to combine it with other antigens for enhanced sensitivity.	(Luan <i>et al.</i> , 2022)
Correlates of TB disease	HMGB1	HMGB1 ELISA Kit II (SHINO-TEST Corporations, Kanagawa, Japan)	1.0 ng/mL and < 10%	CSF HMGB1 levels are a promising diagnostic biomarker for tuberculous meningitis (TBM).	(Chen <i>et al.</i> , 2016)
	IP-10, IFN- γ , Ferritin, and 25(OH)D	QuantiFERON-TB Gold In-Tube (QFT-GIT)	93.20% and 90.00%	A combination of IFN- γ , IP-10, and 25(OH)D has been identified as the optimal set of biomarkers for diagnosing active TB and latent TB infection (LTBI), achieving high sensitivity and specificity. This combination accurately classified cases of active TB and LTBI with high precision.	(Comella-del-Barrio <i>et al.</i> , 2019)
	IL-1ra, IL-1 β and GM-CSF	Bio-Plex Pro Human Cytokine Multi-Plex, Group I kit (Bio-Rad Laboratories Inc., Hercules, CA, USA)	IL-1ra (6.65 pg/mL and 6.21–34.949 pg/mL), IL-1 β (0.24 pg/mL and 0.29–4.672 pg/mL), and GM-CSF (0.19 pg/mL and 0.48–7.846 pg/mL)	In TB cases, levels of IL-1ra, IL-1 β , and GM-CSF were lower, while fibroblast growth factor-basic levels increased significantly. A model including IL-2, TNF- α , VEGF, and ALS demonstrated 82.20% sensitivity in diagnosing TB.	(Sariko <i>et al.</i> , 2019)
	Tpx-IgA, NCAM-1, Vitronectin, CFH, Ferritin, and α -2-macroglobulin	ELISA kit	95.00% and 88.50%	Combining antibodies with cytokines improved the accuracy of TB diagnosis. IgA levels combined with cytokines achieved 97% AUC for TB diagnosis. Antibodies against <i>M. tuberculosis</i> antigens enhanced diagnostic accuracy when used alongside cytokines.	(Jacobs <i>et al.</i> , 2022)
Fibronectin	Enzyme-linked immunosorbent assay (ELISA) kit (human fibronectin [FN]) (Catalogue No: CK-bio-11486, Shanghai Coon Koon Biotech Co., Ltd., Shanghai, China)	10.0 ng/mL and 50.0 ng/mL–1600.0 ng/mL	Fibronectin levels correlate with TB severity based on sputum analysis and X-rays, suggesting its potential as a biomarker for assessing TB severity. Larger clinical studies are needed to confirm these findings.	(Kulkarni <i>et al.</i> , 2024b)	

Continue of Table I. Potential biomarkers diagnosis kit based on tuberculosis correlation classification

	Biomarker	Detection kit	Sensitivity and specificity	Summary	References
	<i>M. tuberculosis</i> β -lactamase	Reporter enzyme fluorescence (REF) TB assay	89.00% and 82.00%	The REFtb assay demonstrates high sensitivity and specificity for diagnosing TB, making it suitable for use as a triage test at the point of care. There is also a strong correlation between BlaC concentration and fluorescence in sputum samples.	(Sule <i>et al.</i> , 2019)
Correlates of TB risk	IFN- γ	QuantiFERON-TB Gold Plus	98.90% and 98.10%	The combined use of IFN- γ release assays, advanced imaging, and molecular diagnostics for early TB detection and management offers a promising pathway for developing a comprehensive diagnostic kit.	(Kim <i>et al.</i> , 2024)
Correlates of TB infection	CC16	ELISA kit	100% and 95.00%	The CC16 kit is effective for early detection of silicosis among workers, aiding in minimizing further exposure and improving clinical management by providing a screening tool with high sensitivity and specificity.	(Nandi <i>et al.</i> , 2021)
	TIMP-1	ELISA kits for TIMP-1 (RayBiotech, Inc., Norcross, GA, USA, cat. no. ELH TIMP1)	91.80% and 91.41%	Plasma TIMP-1 shows high levels and is considered a potential biomarker for TB. The expression of TIMP-1 mRNA is increased by mycobacteria, supporting its potential for use in diagnostics.	(Chen <i>et al.</i> , 2017)
	IFN- γ	QuantiFERON-TB Gold Plus	98.90% and 98.10%	The IFN- γ response can be influenced by an individual's immune status, particularly in those with immune-mediated inflammatory diseases (IMIDs) who may exhibit altered IFN- γ responses due to their condition or immunosuppressive therapy. Despite these challenges, the specificity and relevance of IFN- γ in TB immune response make it a promising candidate for inclusion in diagnostic kits, aiding in early detection, differentiation between active and latent TB, and monitoring of treatment response.	(Petruccioli <i>et al.</i> , 2021)

Continue of Table I. Potential biomarkers diagnosis kit based on tuberculosis correlation classification

Biomarker	Detection kit	Sensitivity and specificity	Summary	References
IP-10/CCL19	Human XL Cytokine Magnetic Luminex Performance Assay 45-plex	100% and 100%	Soluble TB-specific biomarkers, particularly the IP-10/CCL19 ratio, are effective in predicting short-term risk for TB progression, making them promising predictive markers for active TB.	(Daniel <i>et al.</i> , 2023)
Ag85B	ELISA kit	0.6 µg/mL µg/mL and 2.5 µg/mL	The study evaluated an immunosensor for detecting Ag85B—a biomarker for <i>M. tuberculosis</i> . The sensor was constructed using gold nanoparticles, self-assembled monolayers, and antibodies specific to Ag85B, demonstrating successful detection through changes in electrochemical signals. The sensor exhibited high sensitivity and specificity, with significant binding indicated by changes in oxidation and reduction currents.	(Murphy <i>et al.</i> , 2020)
IL-1β	ELISA EIA-BEST kits (Vector-Best, RF)	1.0pg/mL and 0–250 pg/mL	IL-1β is identified as a potential biomarker for distinguishing HIV/TB patients with TB recurrence.	(Nosik <i>et al.</i> , 2024)
IP-10	Bead-based multiplex assays HCYTOMAG-60K (Merck Millipore, Merck Chemicals B.V., Amsterdam, the Netherlands)	8.60 pg/mL and 87%–107%	IP-10 levels can effectively identify TB patients responding to treatment, whereas antibody kinetics are not reliable for accurate TB diagnosis.	(Hertog <i>et al.</i> , 2015b)
suPAR	R&D Systems Human uPAR Quantikine ELISA Kit	33.0 pg/mL and natural and recombinant human uPAR	suPAR levels decrease in pulmonary tuberculosis (PTB) patients after treatment, serving as an indicator of efficacy and predicting prognosis and treatment outcomes within two months.	(Indumati <i>et al.</i> , 2017)

Petruccioli *et al.* (2021) investigated various immune biomarkers associated with *M. tuberculosis* infection, focusing on specific T-cell responses. The study utilized the QuantiFERON-TB Plus (QFT-Plus) assay to evaluate T-cell responses to Mtb peptides in tuberculosis infection (TBI) patients with immune-mediated inflammatory diseases (IMID) (Mugenyi *et al.* 2024). The findings revealed that TBI subjects exhibited a higher proportion of Mtb-specific CD45RA-CD27+CD4+ T

cells, as identified by Corrêa *et al.* (2019), indicating an immune response capable of containing the infection. Conversely, active TB patients exhibited a higher proportion of Mtb-specific CD45RA-CD27-CD4+ T cells, indicating a distinctive immunological profile associated with active infection. Notably, the study found that TB therapy in TBI-IMID patients did not significantly alter the functional status or phenotype of Mtb-specific CD4 T cells. However, there was a

reduction in triple functional CD4 T cells in both TBI and active-TB groups. These findings suggest that these immune markers can help distinguish different stages of TB infection and evaluate the effectiveness of TB therapy. Consequently, the differentiation of Mtb-specific T-cell subsets and their response to TB therapy could be valuable tools for monitoring disease progression and treatment efficacy (Corrêa *et al.*, 2019).

Tissue inhibitor of metalloproteinases 1 (TIMP-1) has emerged as a promising biomarker for TB diagnosis, demonstrating a significant correlation with TB infection. In a study published in *Molecular Medicine Reports* by Chen *et al.* (2017), plasma levels of TIMP-1 were found to be significantly higher in TB patients compared to both pneumonia patients and healthy controls. Specifically, the median TIMP-1 concentrations were 1201.0 ng/mL in TB patients, 1140.0 ng/mL in pneumonia patients, and 415.4 ng/mL in healthy controls. These findings highlight the potential utility of TIMP-1 in distinguishing TB from other respiratory conditions and healthy states (Chen *et al.*, 2017).

To assess the diagnostic performance of TIMP-1, researchers utilized an enzyme-linked immunosorbent assay (ELISA). The results indicated that the ELISA test for plasma TIMP-1 achieved a sensitivity of 91.80% (95% CI = 85.44–96.00) and a specificity of 91.41% (95% CI = 85.14–95.63) in distinguishing TB patients from healthy controls. Receiver operating characteristic (ROC) curve analysis further confirmed the diagnostic potential of TIMP-1, establishing a cut-off value of 727.0 ng/mL to optimize discrimination between positive and negative TB cases. At this threshold, 91.80% of TB patients were correctly identified as test-positive, while only 8.59% of healthy controls were incorrectly classified as positive (Chen *et al.*, 2017).

The study also investigated the mRNA expression levels of TIMP-1 in a THP-1 human monocytic cell model following infection with *M. bovis* and Bacillus Calmette-Guérin (BCG). TIMP-1 mRNA expression showed significant upregulation in a time-dependent manner post-infection. At 24 hours post-infection, the expression levels were markedly increased, with a *p*-value of 0.006 for BCG and 3.2×10^{-7} for *M. bovis* (Chen *et al.*, 2017).

A separate study by Daniel *et al.* (2023) examined the potential of various biomarkers to predict the progression from latent to active tuberculosis (TB). The study focused on household contacts (HHCs) of TB patients, with 14 individuals

progressing to active TB (progressors) and 20 individuals who did not (non-progressors). Using the Luminex Multiplex Array kit, they measured levels of 45 cytokines, chemokines, and growth factors in QuantiFERON supernatants. Significant differences in several analytes between the two groups underscored the relevance of these biomarkers in predicting tuberculosis progression (Daniel *et al.*, 2023).

Key findings from the study by García-bacteria *et al.* (2018) highlighted interferon- γ inducible protein (IP)-10 and chemokine ligand (CCL)19 as highly predictive biomarkers. Progressors showed significantly elevated levels of IP-10 (median = 725.9 pg/mL) compared to non-progressors (median = 160.1 pg/mL), whereas CCL19 levels were notably lower in progressors (median = 268.5 pg/mL) compared to non-progressors (median = 1159.1 pg/mL). These biomarkers—along with interferon (IFN)- γ , interleukin (IL)-1ra, CCL3, and granulocyte-macrophage colony-stimulating factor (GM-CSF)—demonstrated robust potential as predictors of TB progression. Notably, the IP-10/CCL19 ratio exhibited 100% sensitivity and specificity in predicting TB progression, with an optimal cut-off value of 0.24 determined through Classification and Regression Tree (CART) analysis (García-bacteria *et al.*, 2018).

The study further validated the performance of these biomarkers using receiver operating characteristic (ROC) analysis. IP-10, CCL19, IFN- γ , IL-1ra, CCL3, and GM-CSF each exhibited an area under the curve (AUC) of 90 or above, indicating high diagnostic accuracy. Particularly, IP-10 and CCL19 achieved perfect discrimination between progressors and non-progressors with an AUC of 100 (95% CI = 92–100). Principal component analysis (PCA) and dominance analysis affirmed the robustness of (these biomarkers in distinguishing between the two groups (García-bacteria *et al.* 2018). In another study, Namuganga *et al.* (2017) observed significant differences in the levels of GM-CSF and VEGF between saliva and serum. Saliva notably displayed higher levels of both GM-CSF and VEGF. Additionally, research on proinflammatory factors IL-1 β , TNF- α , and IL-6 in Mtb-infected macrophages highlighted that upregulation of miR-618 significantly reduced their levels, while downregulation led to a marked increase (Sun *et al.*, 2022).

Another focus of biomarker research is the antigen 85 complex, which plays a crucial role in the pathogenesis and survival of *M. tuberculosis* by

facilitating cell wall synthesis. Murphy and Dempsey (2020) conducted a study aimed at developing a label-free screening electrochemical sensor for the detection of Ag85B—a significant component of the Antigen 85 complex and a major secretory protein of *M. tuberculosis* (Karbalaei *et al.*, 2017; Goins *et al.*, 2018). This protein serves as a critical biomarker for TB disease (Ernst *et al.*, 2019). The researchers optimized an indirect ELISA Ag85B assay, determining capture antibody and antigen levels through a checkerboard titration at 0.6 µg/mL and 2.5 µg/mL, respectively. Subsequently, they crosslinked the bioreceptor anti-Ag85B into electrochemically deposited gold nanoparticle (AuNP)-modified carbon electrodes. Ag85B binding was then successfully evaluated electrochemically using cyclic voltammetry. The modification steps were monitored by measuring ΔE_p of a redox probe, with results indicating that the GCE/AuNP/anti-Ag85B electrochemical transducers are suitable for Ag85B detection, capable of measuring antigen levels below 2.5 µg/mL. Ag85B remains a key antigen; its detection via electrochemical immunosensor showed high accuracy and low detection thresholds, further confirming its diagnostic potential (Murphy & Dempsey, 2020). These key biomarkers, their biological sources, and associated diagnostic platforms are summarized (Table I) to support a clearer understanding of their clinical relevance.

Biomarkers that correlate of responses to tuberculosis treatment

In their study, Nosik *et al.* (2024) investigated the correlation between cytokine levels and TB recurrence in individuals co-infected with HIV. The research involved 211 participants categorized into several distinct groups: 47 patients with HIV/TB co-infection, 15 with recurrent TB and HIV co-infection, 52 with HIV mono-infection, 52 with TB mono-infection, and 45 healthy controls. Using ELISA, the researchers quantified plasma levels of IFN- γ , TNF- α , IL-10, and IL-1 β . The results revealed significantly reduced levels of IFN- γ (Suprapti *et al.*, 2018), TNF- α (Carvalho *et al.*, 2018), and IL-10 (Flores-Gonzalez *et al.*, 2023) in individuals with HIV/TB co-infection compared to those with HIV or TB mono-infection. Furthermore, IL-1 β levels were approximately four times lower in the recurrent TB group compared to the HIV/TB co-infected group ($p=0.0001$), suggesting its potential as a biomarker for TB recurrence (Nosik *et al.*, 2024).

The study's comprehensive cytokine analysis revealed significant differences in IFN- γ production among tuberculosis mono-infection patients compared to those with HIV mono-infection and HIV/TB co-infection. Specifically, IFN- γ levels were 2.5 times lower in HIV/TB patients compared to HIV patients and 4.5 times lower compared to tuberculosis patients ($p < 0.0001$). Similarly, TNF- α levels were markedly reduced in HIV/TB co-infected patients, exhibiting a 3.1-fold decrease compared to HIV mono-infection and a 3.4-fold decrease compared to tuberculosis mono-infection (both with $p < 0.0001$). IL-10 levels followed a similar pattern, decreasing by 11.1 times compared to HIV mono-infection and 14.8 times compared to tuberculosis mono-infection (both with $p < 0.0001$). Notably, IL-1 β exhibited the most pronounced decrease in the recurrent tuberculosis group, highlighting its potential as a predictive biomarker (Nosik *et al.*, 2024; Carvalho *et al.*, 2018; Guo *et al.*, 2014)).

The study utilized ROC analysis to assess IL-1 β 's efficacy in distinguishing between HIV/TB and recurrent HIV/TB groups. IL-1 β exhibited a high AUC of 0.927 ($p < 0.0001$), indicating robust discriminatory capability. Multiple linear regression analysis further validated the significant association of decreased IL-1 β levels with tuberculosis recurrence, suggesting its potential as a reliable biomarker for identifying high-risk individuals among HIV-infected patients. The consistent findings across various analyses underscore IL-1 β 's clinical relevance in predicting and managing tuberculosis recurrence in this vulnerable population (Pai *et al.*, 2023). Indumati *et al.* (2017) explored soluble plasminogen activator receptor (suPAR) as a biomarker for monitoring TB treatment effectiveness. The research involved 60 pulmonary tuberculosis patients categorized into three groups: newly diagnosed patients before treatment initiation, patients 2–3 months into Directly Observed Therapy Short-course (DOTS), and patients who completed 6 months of DOTS. Additionally, 12 healthy controls were included. Serum suPAR levels were quantified using a quantitative sandwich enzyme immunoassay. The findings indicated that suPAR levels were markedly elevated in TB patients before treatment initiation and decreased progressively with successful treatment (Indumati *et al.*, 2017).

In newly diagnosed tuberculosis patients (Group I), the mean suPAR level was 3.3 ± 2.1 ng/mL, which decreased significantly to 2.2 ± 1.2

ng/mL after 2-3 months of treatment (Group II) and further to 1.5 ± 0.9 ng/mL after completing 6 months of treatment (Group III). These reductions in suPAR levels were statistically significant, with p -values of 0.0483 for the comparison between Group I and Group II, and 0.0514 for the comparison between Group II and Group III. Additionally, hematological findings supported these results, showing a significant decrease in erythrocyte sedimentation rate (ESR) and improvements in hemoglobin levels consistent with the decline in suPAR levels, indicating effective tuberculosis treatment (Indumati *et al.*, 2017). The study's findings highlight suPAR as a reliable biomarker for assessing tuberculosis (TB) treatment efficacy. The early decrease in suPAR levels observed within the first two months of therapy serves as an early predictor of treatment response, potentially guiding timely adjustments in treatment protocols. Elevated suPAR levels before treatment initiation, followed by significant reductions during and after treatment, underscore suPAR's utility in guiding clinical decisions and improving patient care and treatment outcomes. This research emphasizes suPAR's promise as a valuable tool in the clinical monitoring of TB treatment, thereby enhancing overall disease management (Indumati *et al.*, 2017; Rehan *et al.*, 2023).

The study by Hertog *et al.* (2015) investigated the serum kinetics of several cytokines, including IP-10, IFN- γ , IL-6, MIG, TNF α , and MCP-1, in patients with presumptive tuberculosis (TB) before and during the initial two weeks of treatment. The research observed significant changes in cytokine levels, particularly in IP-10, which showed a notable decrease in serum concentrations after seven days of therapy in most bacteriologically confirmed cases. Specifically, 75% of both smear-positive and smear-negative patients exhibited a reduction of more than 300.0 pg/mL between baseline and day 7, indicating that this trend is consistent regardless of sputum bacilli count. These findings suggest that IP-10 could potentially serve as an early marker for monitoring TB treatment response, aiding in the identification of patients responding to appropriate therapy (Hertog *et al.*, 2015; Fisher *et al.*, 2022; Muchsin *et al.*, 2022).

Furthermore, the study found that HIV-TB co-infected patients had significantly higher baseline IP-10 levels compared to HIV-negative patients. Despite this initial disparity, TB therapy resulted in reductions of IP-10 in both groups,

suggesting that IP-10 could serve as a valuable biomarker regardless of HIV status. Receiver operating characteristic (ROC) curves illustrated that cytokine dynamics, particularly the decrease in IP-10 from day 0 to day 7, could effectively distinguish patients responding to appropriate treatment. AUC analysis showed a value of 0.927, indicating strong potential for relapse prediction. This approach accurately classified 75% of culture-positive patients, including 75% of smear-negative culture-positive patients, as responders based on a threshold of a 300.0 pg/mL decrease in IP-10 levels (Hertog *et al.*, 2015).

In addition, the study investigated serum antibody levels against HSP-16 and ESAT-6 at day 0 and day 14 of therapy. The results revealed significant changes in specific patient groups, particularly showing a notable difference ($p=0.039$) between day 0 and day 14 for smear-positive, culture/GeneXpert-positive patients regarding antibodies against HSP-16 and ESAT-6. These findings highlight the potential of these markers in monitoring TB infection and treatment response, laying the groundwork for further research to establish more precise criteria for treatment response or non-response in larger cohorts, including individuals with latent TB infection and uninfected suspects. This suggests the potential development of targeted and effective TB treatment strategies (Hertog *et al.*, 2015).

The biomarkers identified across various tuberculosis correlates contribute to a comprehensive framework for tuberculosis diagnosis, prevention, and treatment monitoring. For example, IFN- γ and T-cell subsets play a critical role in vaccine-induced protection, while CRP and PCT are essential for diagnosing active tuberculosis disease. Genetic markers and IL-10 levels assist in predicting tuberculosis risk, and IGRAs along with tuberculosis antigens such as ESAT-6 and CFP-10 are vital for detecting tuberculosis infection (Musvosvi *et al.* 2023). The WHO's TPP sets a minimum requirement of 90% for diagnostic sensitivity and 70% for specificity. Biomarkers like IP-10, IFN- γ , suPAR, TIMP-1, and IP-10/CCL19 meet these standards and demonstrate potential for both diagnosis and monitoring treatment. On the other hand, while Rv1566c-444 shows promise in triggering immune responses, it does not reach the required sensitivity threshold and thus needs to be combined with other markers to improve its performance. Finally, sputum culture conversion and CRP levels serve as key indicators of treatment response (Nyoman *et al.*, 2019) (Table I).

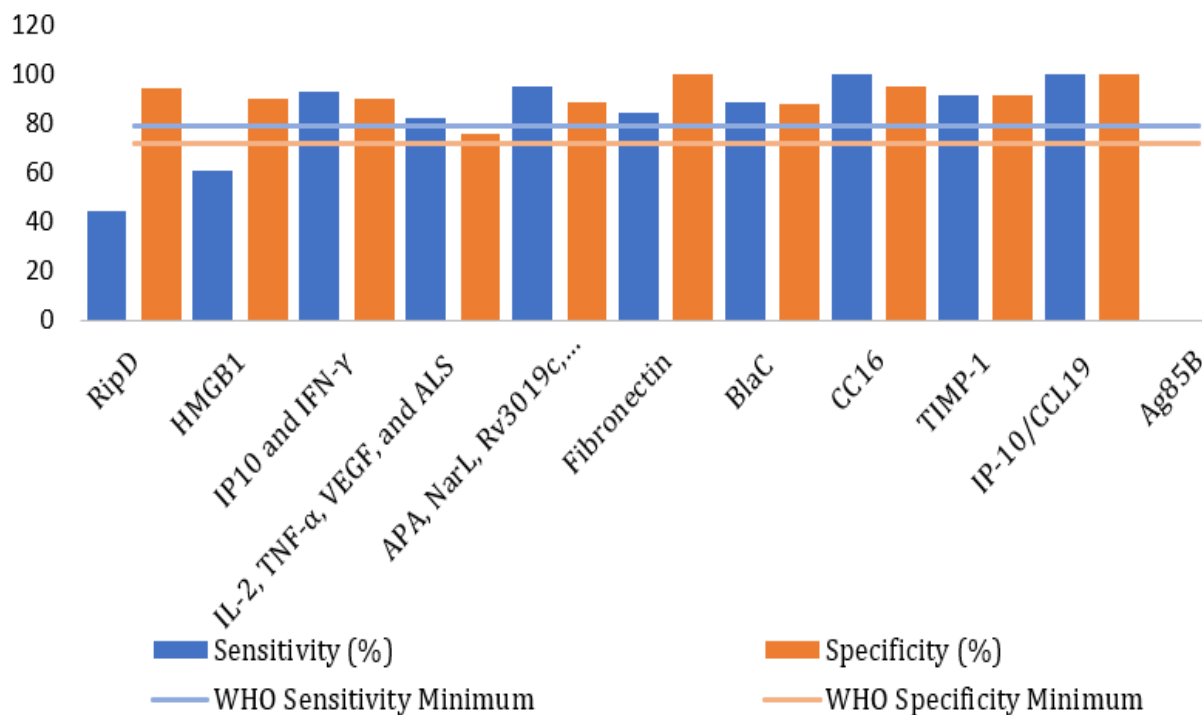


Figure 3. Sensitivity and specificity comparison of biomarkers for TB diagnosis.

Using the WHO's Target Product Profile (TPP) as a guide, Figure 3 compares how well different TB biomarkers perform in terms of sensitivity and specificity, based on various studies. The chart shows the WHO minimum sensitivity goal of 79% with a blue line, and the minimum specificity goal of 72% with an orange line. While the RipD biomarker has a high specificity of 94.40%, it falls short in sensitivity at just 44.44%. This indicates that RipD on its own isn't quite up to WHO standards for sensitivity to be a reliable diagnostic marker. In contrast, HMGB1 performs well, meeting WHO criteria with a sensitivity of about 79.49% and a specificity of 94.52%.

When combining IP-10 and IFN-γ, the sensitivity reaches 93.20%, and specificity is close to 90%, making this a very promising option. Other combinations, like APA, NarL, Rv3019c panel, IL-2, TNF-α, and VEGF, show sensitivities around 82.20% with specificities near 95.00%, and 76.20% with specificities around 88.50%, respectively. These biomarkers are quite promising when it comes to telling apart latent from active TB. The fibronectin biomarker, depending on the cutoff point used, has an estimated sensitivity and specificity between 75% and 80%, just slightly above WHO thresholds.

The BlaC biomarker, used in the REFtb test, meets WHO criteria with sensitivity in the 88–89% range and a specificity of 82%, making it suitable for healthcare settings with fewer resources. CC16, a biomarker linked to both silicosis and TB risk, performs exceptionally well with sensitivity at 100% and specificity at 95%. Similarly, TIMP-1 shows good performance with sensitivity of 91.80% and specificity of 91.41%. The IP-10/CCL19 ratio outperforms others, offering 100% sensitivity and specificity, making it the most promising candidate for predicting TB progression. Lastly, Ag85B shows around 80% sensitivity and 92% specificity, which meets WHO standards but still needs more validation before wider clinical use, as it's still in early research stages.

When we looked at different antigens, Ag85B stood out because it strikes a good balance between triggering an immune response and working well for those trying to diagnose TB. Its ability to detect TB is pretty close to the levels recommended by the WHO Target Product Profile, with sensitivity between 79% and 96%, and specificity from 72% to 89%. Compared to other markers like ESAT-6 and CFP-10, which are very specific but often need to be combined with other tests to pick up cases especially outside the lungs

or in smear-negative patients, Ag85B performs quite well on its own.

While LAM-based tests can be helpful for people living with HIV, they aren't as useful for wider TB screening because their sensitivity can vary quite a bit. Importantly, Ag85B was reliably found in serum samples whether the person had active TB or latent TB, with less background reactivity seen in people without TB. This makes it easier to tell TB apart from other conditions. Unlike some antigens that can react with environmental mycobacteria, like PPD or MPB64, Ag85B is more specific to the *M. tuberculosis* complex, which makes it more promising for quick, on-site testing

Another good thing about Ag85B is that it elicits a strong T-cell response, which means it could be useful not just for blood tests but also for other types of immune-based diagnostics. Although no single biomarker is perfect yet, our results suggest that Ag85B has a lot of potential, especially when used in combination with other markers or in new devices designed for use in resource-limited settings.

CONCLUSION

This review highlights the importance of integrating host- and pathogen-derived biomarkers to strengthen tuberculosis (TB) diagnosis and treatment monitoring, particularly in resource-limited settings. Host biomarkers such as IP-10, IFN- γ , HMGB1, CC16, TIMP-1, and IL-1 β show strong potential for differentiating active from latent TB, predicting progression risk, and evaluating therapeutic response. Among pathogen-derived candidates, Ag85B stands out due to its favorable sensitivity, specificity, and immunogenicity. Although no single biomarker fully satisfies all WHO Target Product Profile criteria, multiplex strategies combining Ag85B with selected host markers may substantially enhance diagnostic accuracy and feasibility for point-of-care applications. Further large-scale validation and standardization across diverse populations remain essential to translate these findings into affordable and accessible TB diagnostic solutions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this review.

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