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Serum Biomarkers for Diagnosis Drug Resistance Tuberculosis: A review

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Multidrug-resistant tuberculosis (MDR-TB) poses a significant challenge to global health. Current gold-standard diagnostic methods, such as culture, GeneXpert, and line probe assays, facing limitations including high costs, long processing times, and reliance on sputum samples. These challenges have driven the search for serum biomarkers for diagnostic approach. Serum biomarkers offer the potential for rapid and scalable diagnosis, especially for patients unable to produce sputum. This review synthesizes findings on key serum biomarkers, categorizing them into cytokines, oxidative stress markers, metabolic indicators, lipids, and genetic elements such as long non-coding RNAs and miRNAs. These biomarkers highlight the complex immune, metabolic, and molecular adaptations in MDR-TB, offering enhanced diagnostic precision and treatment monitoring capabilities. Future prospects include the development of cost-effective assays, large-scale validation studies, and the integration of multi-biomarker panels with advanced computational tools.
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1. Introduction

Multidrug-resistant tuberculosis (MDR-TB), defined as TB resistant to at least isoniazid and rifampicin—the two most potent first-line anti-TB drugs—poses a significant challenge to global TB control efforts (1,2). MDR-TB is associated with prolonged and more complex treatment regimens, which are often less effective, more toxic, and costly compared to those used for drug-sensitive TB (DS-TB) (3).

The early and accurate detection of MDR-TB is therefore critical, not only for ensuring that patients receive appropriate treatment but also for preventing the transmission of resistant strains within communities. However, traditional methods of drug susceptibility testing, such as culture-based tests, are time-consuming and require specialized laboratory infrastructure, limiting their use in resource-constrained settings (4).

Serum-based biomarkers are gaining attention as a potential solution for the rapid and non-invasive detection of MDR-TB (5–7). Unlike conventional testing methods, serum biomarkers can be detected through blood samples, which are easier to collect and analyze. Serum biomarkers encompass a range of molecules, including proteins, cytokines, and metabolites, that reflect the host immune response to MTB infection. Identifying unique serum biomarkers that differentiate MDR-TB from DS-TB could streamline diagnostic processes, enabling clinicians to identify resistant cases early and adjust treatment plans accordingly. These biomarkers may also offer insights into the underlying mechanisms of drug resistance and disease progression, potentially guiding future therapeutic strategies.

Recent research has identified several potential serum biomarkers that could be valuable in detecting MDR-TB. For instance, some studies have reported altered levels of specific cytokines and acute-phase proteins

in MDR-TB patients compared to those with DS-TB (8–10). These immune-modulating proteins are thought to reflect the heightened inflammatory response associated with drug-resistant infections. Additionally, molecular markers such as microRNAs (miRNAs) and other signaling molecules in serum have shown promise in differentiating MDR-TB from DS-TB cases. However, despite these advances, a definitive panel of serum biomarkers for MDR-TB diagnosis has not been established, and further research is needed to validate and standardize these findings.

This review will explore the current landscape of serum biomarkers for MDR-TB detection, summarizing recent advancements and evaluating the diagnostic potential of different biomarker candidates. By providing a comprehensive overview of these biomarkers, we aim to highlight the most promising options for clinical application and identify areas where further validation is required. Such insights are essential for advancing the field toward a practical, serum-based diagnostic tool that can facilitate early MDR-TB detection and improve patient management globally.

2. Result and Discussion

Current MDR-TB Diagnosis and Its Challenges

The diagnosis and monitoring of multidrug-resistant tuberculosis (MDR-TB) rely heavily on culture-based methods (11–13). Culture remains the gold standard for MDR-TB detection due to its ability to confirm *Mycobacterium tuberculosis* (Mtb) and evaluate drug susceptibility testing (DST) against both first- and second-line anti-TB drugs. Despite its accuracy, culture techniques are labor-intensive, time-consuming, and require specialized laboratory infrastructure, which limits their accessibility in resource-limited settings. Molecular methods, such as GeneXpert MTB/RIF, offer a faster alternative by detecting Mtb DNA and rifampicin resistance within hours. Line Probe Assay provide further drug-resistance profiling by targeting multiple genetic mutations, enabling precise detection of MDR-TB. While these methods enhance diagnostic efficiency, their availability is often restricted to centralized laboratories, delaying early diagnosis and treatment initiation for many patients.

Despite advancements in MDR-TB diagnostic tools, significant limitations persist. Culture methods require up to eight weeks for complete DST results, which delays clinical decision-making and increases the risk of disease transmission. Molecular assays, while rapid, are limited by their inability to detect resistance to drugs beyond those included in their panels, and false negatives may occur in specimens with low bacillary loads (14–16). Additionally, GeneXpert's dependence on sputum samples poses challenges in populations with extrapulmonary TB or low sputum production, such as children and immunocompromised patients. Infrastructure constraints, high costs, and the need for technical expertise further hinder widespread implementation, particularly in low-resource regions where the TB burden is highest. Furthermore, molecular assays cannot distinguish live from dead bacilli, complicating the monitoring of treatment efficacy and relapse risk.

Recognizing the limitations of sputum-based diagnostics, the World Health Organization (WHO) has encouraged the exploration of non-sputum biomarkers, including serum-based biomarkers, as adjunctive tools for MDR-TB diagnosis and monitoring (17–19). Serum biomarkers, such as cytokines (e.g., TNF-α, IFN-γ), acute-phase proteins, and metabolites, hold promise due to their ability to reflect systemic immune responses and disease activity. These biomarkers can be particularly beneficial in extrapulmonary TB cases and patients unable to produce adequate sputum samples. WHO emphasizes the need for rigorous validation of serum biomarkers to establish their diagnostic accuracy, reproducibility, and clinical utility. Furthermore, integrating serum biomarker-based tests with existing molecular platforms could enhance diagnostic workflows, reduce delays, and improve patient outcomes in high-burden settings.

Serum Biomarkers for MDR-TB Diagnosis and Monitoring

Several studies have identified serum biomarkers (Table 1) that demonstrate significant potential in differentiating multidrug-resistant tuberculosis (MDR-TB) from drug-sensitive tuberculosis (DS-TB). These biomarkers encompass a wide range of molecular and cellular components, including cytokines, oxidative stress markers, metabolic enzymes, and non-coding RNAs, each reflecting distinct pathophysiological processes involved in MDR-TB.

Cytokines as Biomarkers for MDR-TB

Cytokines are integral to the immune response in tuberculosis (TB) and have been extensively studied as biomarkers to differentiate MDR-TB from DS-TB. Elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) are a consistent finding. TNF-α, a key regulator of granuloma formation, showed significantly higher plasma levels in MDR-TB patients (19.74 ± 3.62 pg/mL) compared to DS-TB patients (17.02 ± 1.84 pg/mL) (8). This suggests that MDR-TB induces a more robust inflammatory response, likely due to prolonged bacterial persistence and immune stimulation. IFN-γ, essential for macrophage activation, exhibited decreased levels in some MDR-TB cohorts compared to DS-TB, indicating immune exhaustion in chronic disease states (8,20). Additionally, the anti-inflammatory cytokine IL-10 was markedly elevated in MDR-TB (18.35 ± 3.56 pg/mL) versus DS-TB (15.90 ± 2.34 pg/mL) (20). This may represent a compensatory mechanism to control excessive inflammation but could also contribute to immunosuppression and disease progression. These findings underscore the utility of cytokines in understanding immune dysregulation in MDR-TB, while also highlighting their diagnostic and prognostic potential.

Oxidative Stress and Related Markers

Oxidative stress biomarkers provide critical insights into host-pathogen dynamics in TB. Inducible nitric oxide synthase (iNOS) and 3-nitrotyrosine (3-NT) are particularly notable in differentiating MDR-TB from DS-TB. iNOS, an enzyme responsible for nitric oxide production in activated macrophages, exhibited significantly higher activity in MDR-TB patients. This reflects heightened immune activation and the host's attempt to control the infection through reactive nitrogen species (21). Interestingly, 3-NT, a stable end-product of oxidative stress, showed an opposite trend: it was higher in DS-TB (25.06 ± 2.15 ng/mL) compared to MDR-TB (20.27 ± 1.80 ng/mL) (21). This discrepancy suggests distinct oxidative stress mechanisms operating in MDR-TB and DS-TB, with MDR-TB potentially developing mechanisms to evade or modulate oxidative damage. Elevated 3-NT in DS-TB could also indicate a more effective initial immune response, which might be compromised in MDR-TB due to prolonged immune engagement. Together, these markers illuminate the oxidative stress pathways in TB pathogenesis and offer avenues for targeted therapeutic strategies to mitigate immune-mediated tissue damage.

Metabolic Biomarkers and Enzymatic Indicators

Metabolic adaptations in MDR-TB provide a rich source of diagnostic biomarkers. Indoleamine 2,3-dioxygenase (IDO), a key enzyme in tryptophan metabolism, was significantly elevated in MDR-TB patients (90.61 ± 49.09 μM/mM) compared to DS-TB (43.84 ± 19.53 μM/mM) (22). IDO-mediated tryptophan depletion suppresses T-cell proliferation and shifts immune responses toward tolerance, facilitating chronic infection. This enzymatic activity also correlated with disease severity, as higher IDO levels were associated with cavitary lung lesions, a hallmark of advanced TB. Complementary proteomic analyses identified elevated levels of prothrombin (F2) and complement receptor type 2 (CR2), emphasizing the role of coagulation and complement pathways in TB pathogenesis (23).

Chemokines and Immune Cell Dynamics

Chemokines, critical for immune cell recruitment, are markedly altered in MDR-TB, reflecting the host's attempts to control the infection. Elevated levels of monocyte chemoattractant protein-1 (MCP-1), CCL-7, and interferon gamma-induced protein 10 (IP-10) were observed in MDR-TB patients, with sustained elevation post-treatment indicative of unresolved inflammation (20). This prolonged immune activation may underlie the chronic nature of MDR-TB and its association with poorer clinical outcomes. Altered chemokine receptor expression further differentiates MDR-TB from DS-TB. For example, increased CXCR1+ and CXCR3+ T cells were identified in MDR-TB patients, suggesting dysregulated T-cell trafficking (20). These findings not only improve our understanding of immune cell dynamics in MDR-TB but also offer potential targets for immunomodulatory therapies aimed at restoring balanced immune responses.

Long Non-Coding RNAs (lncRNAs) as Biomarkers

Long non-coding RNAs (lncRNAs) are emerging as critical players in TB pathogenesis and diagnostics. Differentially expressed lncRNA n335659 was significantly upregulated in MDR-TB compared to DS-TB and healthy controls, with functional analyses linking it to the Notch signaling pathway, which regulates immune responses (24). This finding highlights the role of lncRNAs in modulating immune and inflammatory pathways during TB.

Integrated Biomarker Approaches and Their Clinical Potential

Combining multiple biomarker classes—cytokines, chemokines, metabolic markers, and lncRNAs—provides a robust framework for diagnosing and monitoring MDR-TB. For example, combining MCP-1, IP-10, sIL-2Rα, SAA, CRP, and baseline smear status demonstrated enhanced prognostic accuracy, reflecting the multifaceted nature of immune and inflammatory responses in TB (25). miRNA-based markers, such as miR-let-7e-5p, add another layer of diagnostic capability, particularly in tracking treatment responses and predicting relapse (26). These integrated approaches highlight the potential of precision diagnostics in TB, offering solutions for diverse patient populations.

3. Conclusion

In conclusion, the integration of serum biomarkers into the diagnostic and monitoring framework for MDR-TB offers a promising avenue for improving the accuracy and timeliness of disease management. The diverse biomarkers summarized, including cytokines, oxidative stress markers, metabolites, and non-coding RNAs, highlight the complex immune and metabolic adaptations in MDR-TB. These findings underscore the potential of combining multiple biomarker classes to enhance diagnostic precision, facilitate treatment monitoring, and identify high-risk patients. Future research on serum biomarkers for MDR-TB detection should focus on large-scale validation studies to confirm the diagnostic accuracy and reproducibility of promising biomarker candidates across diverse populations and clinical settings. Additionally, integrating multi-biomarker panels could enhance the sensitivity and specificity of MDR-TB diagnostics. Advances in omics technologies, including proteomics and transcriptomics, may also uncover novel biomarkers and deepen our understanding of host-pathogen interactions in drug-resistant TB. Finally, translating these biomarkers into point-of-care testing platforms that are affordable, rapid, and robust in low-resource settings will be crucial for maximizing their clinical utility and supporting global efforts to control the spread of MDR-TB.

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