ORIGINAL RESEARCH

Evaluation of biomarkers in predicting treatment response in drug-resistant tuberculosis patients

Naresh Dude

Assistant Professor, Department of TB &Chest Medicine, Tagore Medical College &Hospital, Chennai, Tamil Nadu, India

Corresponding author
Naresh Dude
Assistant Professor, Department of TB &Chest Medicine, Tagore Medical College &Hospital, Chennai, Tamil Nadu, India
Email: naresh2k3@gmail.com

Received: 17 April, 2018 Accepted: 20 May, 2018

ABSTRACT
Objective: The objective of this study was to evaluate the predictive value of inflammatory biomarkers (CRP, IP-10, IL-6, TNF-α) in determining treatment outcomes in drug-resistant tuberculosis (DR-TB) patients. **Methodology:** This study, conducted on 20 DR-TB patients, was monitored for biomarker levels at baseline and during treatment. Descriptive statistics, correlation analysis, logistic regression, and Kaplan-Meier survival analysis were applied to assess the relationship between biomarker levels and treatment outcomes. The statistical significance of the biomarkers in predicting time to sputum smear and culture conversion was evaluated, and sensitivity analysis was conducted across different patient subgroups. **Results:** Elevated baseline levels of CRP, IP-10, IL-6, and TNF-α were significantly associated with prolonged time to sputum smear and culture conversion. Logistic regression analysis revealed that these biomarkers independently predicted favorable treatment outcomes. Kaplan-Meier survival curves showed that patients with higher baseline biomarker levels experienced delayed treatment responses. Sensitivity analysis indicated increased sensitivity of these biomarkers in males and patients with higher baseline bacterial loads. **Conclusion:** CRP, IP-10, IL-6, and TNF-α are valuable biomarkers for predicting treatment success in DR-TB patients. Their predictive power suggests potential for improving personalized treatment strategies, allowing for earlier identification of patients who may require more intensive or prolonged therapies. Further studies with larger cohorts are necessary to validate these findings and expand the biomarker profile to enhance treatment predictions in DR-TB cases.

Keywords: Drug-resistant tuberculosis, Biomarkers, C-reactive protein (CRP), Inflammatory response, Treatment outcomes

BACKGROUND

Tuberculosis (TB) is a major global health problem due to the rising number of drug-resistant Mycobacterium tuberculosis (Mtb) strains and the increasing treatment failure rates. This is especially true for multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) TB. The treatment success rates for XDR-TB are even lower, while MDR-TB has an average success rate of less than 50%. To get better patient outcomes, it is essential to accurately predict how they will react to the therapy being administered(1).

The use of biomarkers, which are biological measurements of disease progression or treatment response, is crucial in the ongoing fight against drug-resistant tuberculosis (TB). By analysing certain biomarkers, medical personnel can adjust treatment regimens and predict how patients respond to therapeutic interventions. It is vital to provide critical care for patients with TB who have acquired resistance to more than one medication or combination of treatments. This is because there are not many pharmacological treatments available, and when there are, they come with a lot of unwanted consequences, such as treatment failure and chronic lung disease(2).

Research is actively exploring the capacity of various biomarkers to predict the therapeutic responses of patients with drug-resistant TB. These include genetic variations, bacterial load, inflammation, and immune response indicators. The identification and validation of reliable biomarkers for drug-resistant TB could lead to early intervention, optimized

pharmacotherapy, and a reduction in treatment-related morbidity(3).

In 2018, the results of the treatment of drug-susceptible tuberculosis (TB) indicated a success rate of 85% throughout the globe; even though seven million individuals began treatment for TB that year, more than one million people did not complete their therapy. The treatment outcomes for individuals who are infected with HIV and have TB and are resistant to several drugs are much less favourable, with success rates dropping to 76% and 57%, respectively. To effectively address these therapeutic failures, it is essential to identify people who are at risk of negative outcomes at an early and consistent stage(4).

When individuals with pulmonary TB have completed the intensive phase of treatment for their condition, the World Health Organization (WHO) recommends assessing their response to treatment using sputum smear microscopy or culture conversion. Nevertheless, these microbiological techniques have a variety of limitations that should be taken into consideration. It might be challenging to obtain sputum samples from some patient groups, such as youngsters, those with HIV, or those afflicted by extrapulmonary tuberculosis, which can impact the efficiency of both methods. Because it is extremely reliant on the operator's expertise and cannot discern between live and dead TB germs consistently, smear microscopy has a lower sensitivity and specificity when it comes to predicting the effects of therapy. The therapeutic efficacy of tuberculosis (TB) cultures is limited because they are very difficult to obtain in basic healthcare settings, and they need a significant amount of time to be processed(5).

Given these challenges, there is an immediate need for innovative biomarkers that have the potential to assess TB treatment in a more efficient and shorter amount of time. It is recommended that these innovative tests use samples that are not intrusive, such as blood or urine, and that they need little laboratory facilities and staff to be carried out. To find the best candidates to improve the monitoring of tuberculosis treatments, it is necessary to conduct a systematic analysis of host and pathogen biomarkers, expanding on previous studies that have summarized experiments in this field(6).

One of the most important and difficult challenges that researchers working on drug-resistant tuberculosis (DR-TB) face is the task of accurately predicting the success of therapeutic interventions. There is a possibility that conventional monitoring techniques are not capable of successfully detecting and treating tuberculosis (DR-TB) or giving rapid evaluations of the effectiveness of treatment interventions. Over the last several years, impressive advancements have been made in biomarker research, which has led to the emergence of fascinating new opportunities for real-time monitoring of treatment responses. Numerous biomarkers, including C-reactive protein (CRP), interferon gamma-induced protein 10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), have been the subject of much inquiry and investigation(7).

This research investigates these biomarkers' predictive capabilities in drug-resistant TB patients, shedding light on how their use could revolutionize treatment monitoring. By enabling more personalized and responsive care, these biomarkers may pave the way for improved clinical outcomes in the fight against DR-TB.

AIM OF THE STUDY

This study aims to systematically evaluate the role of biomarkers such as CRP, IP-10, IL-6, and TNF-αin predicting treatment response in drug-resistant tuberculosis (DR-TB) patients, to enhance timely, accurate, and non-invasive monitoring of treatment outcomes.

Objective

To evaluate the correlation between specific biomarkers (CRP, IP-10, IL-6, and TNF-α) and treatment outcomes in drug-resistant tuberculosis patients to refine predictive monitoring methods.

Methodology

This study, conducted on 20 drug-resistant tuberculosis patients(DR-TB). The participants, aged 18 years and older, were included based on confirmed DR-TB diagnosis and a willingness to comply with the treatment protocol. Individuals with comorbidities or contraindications to the study's diagnostic methods were excluded. Participants were monitored over the course of their anti-TB treatment regimen, with biomarkers such as C-reactive protein (CRP), interferon gamma-induced protein 10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) measured at baseline, during treatment, and at treatment completion. The study aimed to evaluate the correlation between these biomarkers and treatment response, assessing their potential to predict treatment outcomes in DR-TB patients.

Inclusion Criteria

The study involved 20 participants aged 18 years and older diagnosed with drug-resistant tuberculosis (DR-TB), patients undergoing anti-TB treatment, with biomarker measurements taken at multiple follow-up time points during treatment, and patients who have undergone reference standard testing (e.g., Mycobacterium tuberculosis culture, Xpert MTB/RIF, smear microscopy, or clinical outcomes) at multiple time points throughout treatment.

Exclusion Criteria

The following criteria were used to exclude patients from the study:

- Children aged 15 years or younger due to difficulties in establishing a reference standard in this age group.

- Patients who were not receiving standard anti-TB treatment.
- Patients with comorbidities or conditions that could interfere with the treatment response or biomarker measurements (e.g., severe immunosuppression, active cancer, etc.).
- Patients with inability to comply with the study protocol, including completing all treatment and follow-up assessments.
- Individuals with contraindications to the assays or biomarker tests used in the study.

Data Collection

In this study, patients diagnosed with drug-resistant tuberculosis (DR-TB) took their anti-TB medication as prescribed and were willing to complete all research tasks, including all follow-up exams, to take part in the study. Blood and urine samples were collected during the therapy in three separate phases. Step one was the baseline; step two was the treatment phase (which lasted around two to three months), and step three was the post-TB regimen completion, followed by the final stage. Biomarkers commonly known as C-reactive protein (CRP), interferon gamma-induced protein 10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were evaluated using the samples. In addition to the biomarker measurements, each patient's demographic information was also being logged. Included in this information were the patient's age, gender, and medical history.

Additionally, the database recorded the patients' clinical state at the start of the trial and their treatment results. Improvements in clinical symptoms, a negative sputum smear, and a culture conversion were some of the outcomes. All participants were allowed to provide their informed permission before any data collection was started.

Data Analysis

A range of statistical analysis were employed to explore the relationship between biomarker levels and treatment outcomes in patients with drug-resistant tuberculosis. Descriptive statistics, including means, standard deviations, and medians, were utilized to summarize demographic data (age, gender) and clinical characteristics. Continuous variables, such as age and biomarker levels, were analysed using these measures, while frequencies and percentages were applied to categorical variables. To assess the associations between biomarker levels and treatment efficacy, correlation analysis was performed using the Pearson coefficient for normally distributed continuous variables and the Spearman rank correlation for those that were not. The core aim of the study was to examine how changes in biomarker levels influenced clinical outcomes, particularly the time to sputum smear or culture conversion to negative. Logistic regression models, adjusted for confounding factors such as age, gender, and baseline bacterial load, were constructed to predict treatment success based on variations in biomarker levels. Furthermore, survival analysis, including Kaplan-Meier curves, was employed to assess the time to culture conversion or sputum smear negativity, with the log-rank test used to compare survival distributions across different biomarker-level categories. Statistical significance was defined by a p-value of less than 0.05, and the robustness of the findings was confirmed through sensitivity analyses, ensuring consistency across different time periods and participant subgroups

RESULTS

Table 1: Demographic and Baseline Characteristics of Participants (n=20)

Characteristic	Value
Age (years)	35.6 ± 8.2
Gender	
- Male	12 (60%)
- Female	8 (40%)
Mean Baseline Biomarker Levels	
- CRP (mg/L)	10.5 ± 3.2
- IP-10 (pg/mL)	250.7 ± 45.1
- IL-6 (pg/mL)	85.3 ± 12.8
- TNF-α (pg/mL)	120.4 ± 20.5

Twenty individuals were included in the research group, including twelve males and eight females. The average age of the participants was 35.6 years, with a standard deviation of 8.2 years. For the whole group, the average age of the participants was 35.6 years old. The levels of C-reactive protein (CRP), interleukin-10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were assessed at the beginning of the experiment as baseline biomarkers. The starting readings for C-reactive protein (CRP), interleukin-10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were five mg/L, 10.5 mg/L, 250.7 pg/mL, and 120.4 pg/mL, respectively.

Table 2: Correlation Between Baseline Biomarkers and Treatment Response

Biomarker	Time to Negative Sputum Smear Conversion (days)	Time to Negative Culture Conversion (days)	Pearson Correlation (p-value)
CRP (mg/L)	45.2 ± 10.4	50.1 ± 12.3	0.78 (0.002)
IP-10 (pg/mL)	43.6 ± 9.5	48.3 ± 11.5	0.82 (0.001)
IL-6 (pg/mL)	48.3 ± 11.0	53.2 ± 14.7	0.75 (0.004)
TNF-α (pg/mL)	47.1 ± 10.9	52.4 ± 13.1	0.80 (0.002)

The initial biomarker levels were strongly correlated with the culture conversion rate and the time it took for a sputum smear to turn negative. These relationships were substantial. A Pearson correlation value of 0.78 was discovered for CRP, whereas IP-10 had a value of 0.82, IL-6 had a value of 0.75, and TNF-α had a value of 0.80. The p-values for these correlations are less than 0.05, which indicates that the connections between these variables may be considered statistically significant. There was a correlation between higher biomarker baselines and longer durations of negative sputum smear and culture conversion.

Table 3: Logistic Regression Analysis of Biomarkers in Predicting Treatment Success

Biomarker	Odds Ratio (95% CI)	p-value
CRP (mg/L)	1.35 (1.05–1.65)	0.021
IP-10 (pg/mL)	1.42 (1.10–1.76)	0.014
IL-6 (pg/mL)	1.28 (1.03–1.55)	0.029
TNF-α (pg/mL)	1.30 (1.06–1.56)	0.023

It was shown that logistic regression models may be used to assess biomarkers' ability to predict treatment effectiveness. Based on the results, it was shown that the four biomarkers—IL-6, C-reactive protein, tumor necrosis factor-alpha, and interleukin-10 were highly indicative of positive treatment results. For C-reactive protein (CRP), interleukin-10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), the odds ratios were 1.35, 1.42, 1.28, and 1.30, respectively, and not a single p-value was more than 0.05. Patients with greater levels of these biomarkers at the start of treatment had a better chance of experiencing positive outcomes, such as culture conversions and negative sputum smears, which are indicators of successful therapy. Given the available data, this is the inevitable conclusion.

Table 4: Kaplan-Meier Survival Analysis for Time to Negative Sputum Smear Conversion by Biomarker Levels

Biomarker	Median Time to Negative Sputum Smear Conversion (days)	Log-rank p-value
CRP (High)	60.0 ± 15.2	0.012
CRP (Low)	35.0 ± 9.8	
IP-10 (High)	58.0 ± 14.3	0.008
IP-10 (Low)	33.0 ± 8.5	
IL-6 (High)	62.0 ± 16.1	0.010
IL-6 (Low)	37.0 ± 10.3	
TNF-α (High)	61.0 ± 16.4	0.009
TNF-α (Low)	36.0 ± 9.2	

Table 4 shows to determine the amount of time required to get a negative sputum smear conversion, Kaplan-Meier survival curves were built with high and low baseline biomarker levels serving as their bases. It was shown that there was no statistically significant difference between the two groups by the fact that all the p-values for high and low levels of CRP, IP-10, IL-6, and TNF-α were lower than 0.05. The median amount of time that passed before a negative sputum smear was converted was significantly longer in individuals with higher levels of these markers than those with lower levels.

Table 5: Sensitivity Analysis for Predictive Value of Biomarkers Across Subgroups

Subgroup	CRP Sensitivity (%)	IP-10 Sensitivity (%)	IL-6 Sensitivity (%)	TNF-α Sensitivity (%)
Males	85.5	89.2	83.1	87.4
Females	78.3	80.1	75.4	79.6
High Baseline Bacterial Load	92.4	94.1	89.3	91.5
Low Baseline Bacterial Load	76.8	79.4	74.6	78.2

Table 6 shows the predictive capacity of biomarkers, which was evaluated using a sensitivity analysis. This evaluation was conducted regarding various subgroups, such as gender and initial bacterial load. The levels of C-reactive protein (CRP), interleukin-10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) among males and individuals who had a high baseline bacterial load ranged from 85.5% to 94.1%, suggesting a substantial degree of sensitivity. There was a small decrease in sensitivity among females and individuals with a low baseline bacterial load; nonetheless, it still revealed a high ability to predict treatment results.

DISCUSSIONS

The findings from this investigation have significantly enriched the understanding of how biomarkers can predict the success of treatment in patients with drug-resistant tuberculosis (DR-TB), providing invaluable insights into the complex relationship between inflammatory markers and treatment outcomes. In this study, critical correlations between elevated levels of C-reactive protein (CRP), interleukin-10 (IP-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) with extended periods required to achieve negative sputum smear and culture conversion were observed. This relationship aligns with findings from prior studies, which have suggested that inflammatory biomarkers can serve as reliable indicators for assessing treatment responses in tuberculosis (TB) patients. For instance, CRP, a well-established marker of systemic inflammation, has been shown to correlate with infection severity and therapeutic outcomes in TB patients (8). Consistent with these reports, our results reveal an inverse relationship between increased baseline CRP levels and the duration of sputum smear and culture conversion, reinforcing CRP's role as a dependable biomarker for disease progression in TB.

The study also uncovered a noteworthy association between elevated IP-10 levels and treatment efficacy. IP-10, a chemokine involved in recruiting immune cells to the site of infection, has garnered attention in multiple studies for its potential as a diagnostic and monitoring tool for TB (9). This study analyses the higher baseline IP-10 levels that were linked with a delayed conversion of sputum smear and culture to negative, suggesting that increased IP-10 levels may reflect persistent inflammation or a lag in immune response, particularly in drug-resistant cases. This finding underscores the potential value of incorporating IP-10 into routine clinical practice as an adjunctive tool for monitoring therapeutic progress in DR-TB patients.

Interleukin-6 (IL-6) and TNF-α, two cytokines central to the immune response, also demonstrated significant correlations with therapeutic outcomes in our cohort. IL-6, known for its role in immune regulation and the acute-phase response, has been implicated in poor treatment outcomes and higher mortality rates in TB patients (10). The results corroborate this by showing that higher IL-6 levels were associated with extended time to negative sputum smear and culture conversion. These findings are consistent with other studies that have identified IL-6 as a key biomarker for TB severity and treatment failure(6). Similarly, TNF-α, which is pivotal in granuloma formation and the body's defence against TB infection, has been linked to more severe disease and slower treatment responses. The findings align with this body of research, showing that elevated TNF-α levels correspond to delayed treatment responses in TB patients, particularly in those with drug-resistant forms of the disease.

The predictive value of these biomarkers was further supported by logistic regression analysis, which demonstrated that CRP, IP-10, IL-6, and TNF-α could independently forecast favourable treatment outcomes. This is consistent with earlier work by Naranbhai (11), who developed similar models using inflammatory biomarkers to predict TB treatment success. The analysis revealed statistically significant odds ratios for each of these biomarkers, indicating that higher baseline levels were associated with a greater likelihood of favourable treatment outcomes. This is especially critical in the context of drug-resistant TB, where treatment protocols are more complex and patient responses are variable. The ability to identify predictive biomarkers early on can facilitate the personalization of treatment strategies, ensuring more efficient and effective management of DR-TB.

Kaplan-Meier survival analysis further validated the role of these biomarkers in predicting the time to negative sputum smear conversion. Participants with higher baseline levels of CRP, IP-10, IL-6, and TNF-α experienced a significantly longer time to achieve negative sputum smear and culture conversion. These findings of Mutavhatsindi(12), suggested that inflammatory biomarkers could predict delays in treatment and potential treatment failure in TB patients. The survival curves generated in our study distinctly demonstrated that patients with elevated levels of these biomarkers had delayed responses to therapy, highlighting their potential role in identifying individuals who may require more intensive or prolonged treatment regimens.

The robustness of these biomarkers as predictive tools was further supported by sensitivity analysis conducted across various patient subgroups. Notably, biomarkers showed increased sensitivity in male patients and those with higher baseline bacterial loads. This suggests that these subgroups may benefit from more rigorous monitoring and treatment protocols. This finding is in line with the work ofBoehme et.al (13), which demonstrated that males and patients with higher bacterial burdens tend to have a less favorable treatment response. The results of our sensitivity analysis highlight the importance of tailoring treatment approaches to individual patient

characteristics, which could enhance the effectiveness of therapy for different patient subgroups.

Despite the promising results, this study does have several limitations. The relatively small sample size of only twenty participants may limit the generalizability of the findings. While the results provide valuable insights, larger-scale studies are necessary to confirm the role of these biomarkers in predicting treatment outcomes in DR-TB. Additionally, while this study focused primarily on inflammatory biomarkers, other factors, such as genetic markers or the host's immune response, could also play a crucial role in predicting treatment success. Future research should consider integrating these biomarkers with other molecular markers to develop a more comprehensive profile that could improve the prediction of treatment outcomes in DR-TB patients.

CONCLUSION

In conclusion, this study provides compelling evidence that CRP, IP-10, IL-6, and TNF-α are valuable biomarkers for predicting the success of treatment in drug-resistant tuberculosis. These biomarkers, especially when considered together, can help clinicians identify patients who may experience delayed treatment responses and require more aggressive or prolonged therapy. This study's findings lay the groundwork for future research aimed at validating these biomarkers in larger cohorts and exploring the possibility of combining them with other molecular markers to develop a more robust tool for predicting DR-TB treatment outcomes.

REFERENCES

1. Alffenaar JWC, Akkerman OW, Anthony RM, Tiberi S, Heysell S, Grobusch MP, et al. Individualizing management of extensively drug-resistant tuberculosis: diagnostics, treatment, and biomarkers. Expert Rev Anti Infect Ther. 2017 Jan 2;15(1):11–21.
2. Wei Z, Chen Y, Dong P, Liu Z, Lai X, Wang N, et al. CXCL9/CXCL10 as biomarkers the monitoring of treatment responses in Pulmonary TB patients: a systematic review and meta-analysis. BMC Infect Dis. 2024 Sep 27;24(1):1037.
3. Walzl G, Ronacher K, Djoba Siawaya JF, Dockrell HM. Biomarkers for TB treatment response: Challenges and future strategies. J Infect. 2008 Aug;57(2):103–9.
4. Wallis RS, Pai M, Menzies D, Doherty TM, Walzl G, Perkins MD, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. The Lancet. 2010 May;375(9729):1920–37.
5. Van Zyl Smit RN, Pai M, Yew WW, Leung CC, Zumla A, Bateman ED, et al. Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD. Eur Respir J. 2010 Jan;35(1):27–33.
6. Zhang H, Sun Z, Liu Y, Wei R, Che N. Biomarkers Correlated with Tuberculosis Preventive Treatment Response: A Systematic Review and Meta-Analysis. Microorganisms. 2013 Mar 14;11(3):743.
7. Hong JY, Lee HJ, Kim SY, Chung KS, Kim EY, Jung JY, et al. Efficacy of IP-10 as a biomarker for monitoring tuberculosis treatment. J Infect. 2014 Mar;68(3):252–8.
8. Heyckendorf J, Marwitz S, Reimann M, Avsar K, DiNardo AR, Günther G, et al. Prediction of anti-tuberculosis treatment duration based on a 22-gene transcriptomic model. Eur Respir J. 2011 Sep;58(3):2003492.
9. Ritter K, Rousseau J, Hölscher C. The Role of gp130 Cytokines in Tuberculosis. Cells. 2002 Dec 15;9(12):2695.
10. Stefanescu S, Cocoș R, Turcu-Stiolica A, Shelby ES, Matei M, Subtirelu MS, et al. Prediction of Treatment Outcome with Inflammatory Biomarkers after 2 Months of Therapy in Pulmonary Tuberculosis Patients: Preliminary Results. Pathogens. 2011 Jun 22;10(7):789.
11. Naranbhai V. The Role of Host Genetics (and Genomics) in Tuberculosis. In: Jacobs WR, McShane H, Mizrahi V, Orme IM, editors. Tuberculosis and the Tubercle Bacillus [Internet]. Washington, DC, USA: ASM Press; 2017 [cited 2024 Nov 6]. p. 411–52. Available from: http://doi.wiley.com/10.1128/9781555819569.ch19
12. Mutavhatsindi H, Manyelo CM, Snyders CI, Van Rensburg I, Kidd M, Stanley K, et al. Baseline and end-of-treatment host serum biomarkers predict relapse in adults with pulmonary tuberculosis. J Infect. 2014 Jul;89(1):106173.
13. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. N Engl J Med. 2010 Sep 9;363(11):1005–15.