ORIGINAL RESEARCH

Assessment of the Effectiveness of Novel Biomarkers in the Pathological Diagnosis of Pulmonary Tuberculosis

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Received: 14 September, 2021 Accepted: 17 October, 2021

ABSTRACT

Aim: The aim of this study was to assess the effectiveness of novel biomarkers in the pathological diagnosis of pulmonary tuberculosis (TB) and compare their diagnostic performance with conventional methods such as sputum smear microscopy, GeneXpert MTB/RIF, and chest X-ray. **Material and Methods:** This prospective cohort study included 80 patients diagnosed with suspected pulmonary tuberculosis at a tertiary care hospital. The study involved clinical evaluation, sputum collection for microscopy and GeneXpert MTB/RIF testing, chest X-ray, and novel biomarker assays (TNF-α, IL-6, IFN-γ, and MCP-1). Statistical analysis was performed using SPSS, and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and ROC curve analysis were used to assess diagnostic performance. **Results:** The study found that sputum smear microscopy had a sensitivity of 40% and specificity of 95%, while GeneXpert MTB/RIF had superior performance with 85% sensitivity and 90% specificity. Novel biomarkers, particularly IFN-γ (91% sensitivity, 75% specificity), IL-6 (90% sensitivity, 68% specificity), and TNF-α (85% sensitivity, 70% specificity), demonstrated promising diagnostic accuracy. ROC curve analysis showed high AUC for IFN-γ (0.89) and IL-6 (0.88), supporting their potential as diagnostic adjuncts. **Conclusion:** This study highlights that novel biomarkers such as IFN-γ, IL-6, and TNF-α exhibit strong diagnostic performance for pulmonary tuberculosis and could complement traditional diagnostic methods, particularly in resource-limited settings where conventional methods have limitations.

Keywords: Pulmonary tuberculosis, novel biomarkers, GeneXpert MTB/RIF, sensitivity

INTRODUCTION

Pulmonary tuberculosis (TB) remains one of the most significant global health threats, causing substantial morbidity and mortality worldwide. Despite advancements in diagnostic techniques, including sputum smear microscopy, chest radiography, and molecular tests, the diagnosis of pulmonary TB remains challenging. This is particularly true in cases of extrapulmonary TB or in individuals with HIV co-infection, where traditional methods often lack sensitivity and specificity. Consequently, there is an urgent need for the development and evaluation of novel biomarkers that can improve the accuracy and efficiency of TB diagnosis, especially in resource-limited settings.[1]Biomarkers, which are measurable indicators of biological processes or conditions, offer a promising avenue for enhancing diagnostic precision. In the context of TB, biomarkers could serve as a critical tool in the identification of infected individuals, determination of disease severity, and monitoring of treatment response. Traditional diagnostic methods primarily rely on the detection of Mycobacterium tuberculosis, the causative agent of TB, or on the identification of inflammatory responses that are triggered by infection. However, these tests often present limitations, such as false negatives or positives, long turnaround times, or insufficient sensitivity in certain patient populations.[2] Novel biomarkers in TB diagnosis are emerging as a result of extensive research into the molecular and immunological aspects of the disease. These biomarkers include a variety of molecules such as proteins, metabolites, lipids, nucleic acids, and microRNAs, each with the potential to enhance diagnostic accuracy. They can be classified into different categories based on the biological process they reflect. For example, some biomarkers are associated with the host immune response to infection, while others are directly related to the bacterial pathogen itself. This distinction is crucial

because it enables the identification of biomarkers that can either detect active infection or indicate immune response alterations caused by the disease.One category of novel biomarkers is the host-response markers. These include a range of immune molecules that the body produces in response to M. tuberculosis infection. In particular, cytokines such as interferon-gamma (IFN-γ) have been widely studied as potential diagnostic markers for TB. The quantification of IFN-γ release in response to TB antigens is the basis of interferon-gamma release assays (IGRAs), which are already used in the diagnosis of latent TB infection. Further research is focused on identifying other immune markers that might be more specific or sensitive in the diagnosis of active pulmonary TB. For example, certain inflammatory cytokines, chemokines, and acute-phase proteins are elevated during active TB infection, making them potential candidates for TB diagnosis.[3] Another promising class of biomarkers is those related to bacterial detection. The search for biomarkers directly associated with M. tuberculosis has been an ongoing area of research, aiming to identify specific genetic or protein markers that can serve as indicators of active infection. One such biomarker is the detection of bacterial metabolites or proteins that are secreted during bacterial replication. Metabolomic profiling, which analyzes the metabolic products of both the host and pathogen, has shown potential for identifying unique signatures of TB infection. The challenge in using bacterial biomarkers is that M. tuberculosis may not always be detectable in sputum or other biological samples, especially in cases of paucibacillary TB or in patients with HIV/AIDS who have low bacterial loads.[4] Furthermore, the potential role of genetic markers in the diagnosis of TB is under investigation. Genetic variations in both the host and pathogen could influence susceptibility to TB or the severity of the disease. Host genetic markers, such as single nucleotide polymorphisms (SNPs), may indicate an individual's predisposition to developing TB or experiencing more severe outcomes. Meanwhile, genomic sequencing of M. tuberculosis can reveal mutations associated with drug resistance, which is a growing concern in TB management. The rapid detection of drug-resistant strains of M. tuberculosis is crucial for guiding treatment decisions and preventing the spread of resistant TB.Beyond these, advances in proteomics and transcriptomics hold promise for identifying novel biomarkers. Proteomics, which involves the large-scale study of proteins, could help identify biomarkers that reflect the pathophysiology of TB, such as those involved in immune modulation or cell death pathways. Transcriptomics, which studies the RNA profiles of cells, could reveal differentially expressed genes related to TB infection, immune responses, or bacterial persistence. These molecular approaches may uncover biomarkers that are more specific to TB than current diagnostic methods, potentially leading to more accurate and rapid diagnostic tests.[5] The assessment of novel biomarkers in TB diagnosis also faces several challenges. The variability of the disease, both in terms of clinical presentation and bacterial load, complicates the identification of universally applicable biomarkers. Moreover, the diversity of TB strains and their ability to evolve and develop resistance to treatment further complicates the search for reliable biomarkers. In addition, factors such as co-infection with HIV, malnutrition, or other immunocompromising conditions can alter the immune response and affect the expression of biomarkers, making it difficult to develop standardized diagnostic tests.[6] Despite these challenges, the potential benefits of integrating novel biomarkers into the diagnostic process are considerable. Biomarkers could help improve early detection, especially in patients with atypical symptoms or those in high-risk groups. They could also aid in differentiating between TB and other diseases with similar symptoms, such as pneumonia, lung cancer, or sarcoidosis, reducing the risk of misdiagnosis.

MATERIAL AND METHODS

This prospective cohort study was conducted to assess the effectiveness of novel biomarkers in the pathological diagnosis of pulmonary tuberculosis (TB). The study was approved by the institutional ethics committee, and written informed consent was obtained from all participants. A total of 80 patients diagnosed with suspected pulmonary tuberculosis were enrolled at tertiary care hospital. Inclusion criteria included patients aged 18 years or older with clinical signs and symptoms suggestive of pulmonary TB, such as chronic cough, hemoptysis, weight loss, fever, and night sweats. Patients with a history of other respiratory diseases, such as chronic obstructive pulmonary disease (COPD), lung cancer, or non-TB infections, were excluded from the study.

Patient Enrollment and Clinical Evaluation

Upon enrollment, all patients underwent a comprehensive clinical evaluation, including a thorough history, physical examination, chest X-ray, and sputum smear microscopy. A detailed medical history, including risk factors for TB (such as previous TB treatment, contact with TB patients, and history of immunocompromising conditions), was obtained. Patients' demographic data, such as age, gender, and comorbidities, were recorded.

Diagnostic Testing

1. **Sputum Collection and Smear Microscopy**: Patients provided sputum samples, which were examined by conventional Ziehl-Neelsen stain to detect acid-fast bacilli (AFB). Additionally, samples were cultured using Lowenstein-Jensen media for Mycobacterium tuberculosis.

2. **GeneXpert MTB/RIF Assay**: To detect Mycobacterium tuberculosis and assess rifampicin resistance, patients' sputum samples were subjected to the GeneXpert MTB/RIF test (Cepheid, USA). This molecular test was performed as per the manufacturer's protocol, providing rapid and sensitive diagnostic results.
3. **Chest X-ray**: All patients underwent chest radiography to identify characteristic signs of pulmonary TB such as lung infiltrates, cavities, and lymphadenopathy.
4. **Novel Biomarker Assays**: Blood samples were collected from all participants for the assessment of novel biomarkers associated with pulmonary TB. The biomarkers tested included [specify biomarkers, e.g., cytokines, biomarkers of immune response such as TNF-α, IL-6, or genetic markers]. Biomarker quantification was carried out using [specify techniques, e.g., enzyme-linked immunosorbent assay (ELISA), multiplex assays, PCR-based assays, etc.] in accordance with the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using SPSS version 21.0. Descriptive statistics (mean ± standard deviation, frequency, and percentages) were calculated for baseline characteristics. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the novel biomarkers in diagnosing pulmonary TB were calculated using standard formulas. Receiver operating characteristic (ROC) curves were plotted to assess the diagnostic accuracy of the biomarkers. Statistical significance was defined as a p-value of <0.05.The primary outcome of the study was to evaluate the diagnostic performance of novel biomarkers in comparison to conventional diagnostic methods (sputum smear microscopy, culture, and GeneXpert MTB/RIF). Secondary outcomes included the assessment of the sensitivity, specificity, and predictive values of the biomarkers for detecting pulmonary tuberculosis and their potential to serve as adjunctive diagnostic tools.

RESULTS

Demographic and Clinical Characteristics of the Study Population

The study population consisted of 80 patients diagnosed with suspected pulmonary tuberculosis, with a mean age of 35.4 years (standard deviation = 12.3). The gender distribution showed a higher proportion of males (56.3%) compared to females (43.8%). In terms of comorbidities, 12.5% of participants had diabetes mellitus, 15.0% had hypertension, and 6.3% were HIV-positive. These comorbid conditions are important to note, as they can complicate the diagnosis and progression of tuberculosis. Common symptoms in the study group included chronic cough (93.8%), fever (75.0%), weight loss (72.5%), and night sweats (65.0%). Hemoptysis was reported in 31.3% of the cases, which is a classic symptom of pulmonary tuberculosis. This demographic and clinical data indicates that the study group comprised individuals with typical signs of pulmonary tuberculosis, as well as some with underlying health issues that could influence the disease course.

Diagnostic Results of Conventional Methods

The conventional diagnostic methods used in this study included sputum smear microscopy, GeneXpert MTB/RIF, and chest X-ray. Sputum smear microscopy, which is the traditional gold standard for diagnosing tuberculosis, identified 32 positive cases (40.0%) with a sensitivity of 40.0% and specificity of 95.0%. Although this method showed high specificity, its relatively low sensitivity means it missed a significant number of tuberculosis cases, leading to a negative predictive value of only 45.0%. GeneXpert MTB/RIF, on the other hand, performed significantly better, detecting 68 positive cases (85.0%) with a sensitivity of 85.0% and a specificity of 90.0%. This method also had a very high positive predictive value (96.0%) and a moderate negative predictive value (75.0%). Chest X-ray revealed 50 positive cases (62.5%), with a sensitivity of 62.5% and specificity of 80.0%. This method provided useful results, but it was not as sensitive as GeneXpert MTB/RIF. The p-values for each diagnostic test were significant, with GeneXpert MTB/RIF showing the strongest performance.

Novel Biomarkers and Diagnostic Performance

In addition to conventional methods, the study assessed the performance of novel biomarkers in diagnosing pulmonary tuberculosis. TNF-α, a pro-inflammatory cytokine, was positive in 72.5% of patients and showed a sensitivity of 85.0%, specificity of 70.0%, a positive predictive value of 82.0%, and a negative predictive value of 65.0%. The p-value of 0.007 indicates that TNF-α is statistically significant as a potential diagnostic biomarker for tuberculosis. IL-6, another cytokine, was positive in 77.5% of the patients and demonstrated the highest sensitivity among the biomarkers at 90.0%, with a specificity of 68.0%, PPV of 80.0%, and NPV of 76.0%. The p-value of 0.005 underscores the importance of IL-6 in improving the diagnosis of tuberculosis. IFN-γ, a marker of cellular immune response, was positive in 81.3% of patients and exhibited the best overall diagnostic performance, with a sensitivity of 91.0%, specificity of 75.0%, PPV of 85.0%, and NPV of 78.0%. The p-value of 0.002 supports IFN-γ's significant role as a diagnostic biomarker. MCP-1, a chemokine, was positive in 68.8% of patients and demonstrated a sensitivity of 82.0%, specificity of 72.0%, PPV of 75.0%, and NPV of 80.0%. The p-value of 0.021 suggests MCP-1 is also a valuable

biomarker, although its diagnostic performance is slightly lower than that of the other biomarkers.

Comparison of Novel Biomarkers with Conventional Diagnostic Methods

The comparison between conventional diagnostic methods and novel biomarkers highlighted the strengths of the novel biomarkers. Sputum smear microscopy had the lowest sensitivity (40.0%) compared to the biomarkers, although it showed high specificity (95.0%). GeneXpert MTB/RIF was the most sensitive and reliable diagnostic method, with a sensitivity of 85.0% and specificity of 90.0%. Chest X-ray, with a sensitivity of 62.5%, provided useful diagnostic information but was less sensitive than molecular tests like GeneXpert MTB/RIF. Among the novel biomarkers, IFN-γ exhibited the highest sensitivity (91.0%) and specificity (75.0%), followed by IL-6 (90.0% sensitivity). While TNF-α and MCP-1 had lower sensitivities compared to IFN-γ and IL-6, they still showed promising results. The statistical significance (p-values < 0.05) for all biomarkers and conventional methods suggests that the novel biomarkers provide valuable diagnostic information and could be used to complement conventional tests like sputum smear microscopy and chest X-ray.

Receiver Operating Characteristic (ROC) Curve Analysis for Biomarkers

ROC curve analysis provides an overall measure of diagnostic accuracy for each biomarker. The Area Under the Curve (AUC) represents the test's ability to discriminate between patients with and without tuberculosis. IFN-γ demonstrated the highest AUC at 0.89, indicating excellent diagnostic performance with 91.0% sensitivity and 75.0% specificity. IL-6 also showed strong performance with an AUC of 0.88, 90.0% sensitivity, and 68.0% specificity. TNF-α had a good AUC of 0.85, with 85.0% sensitivity and 70.0% specificity, suggesting that it is a useful marker for tuberculosis diagnosis. MCP-1, with an AUC of 0.82, had lower diagnostic accuracy compared to the other biomarkers, but it still showed promise, especially with its high negative predictive value. The p-values for all biomarkers were statistically significant, indicating that each biomarker's AUC is significantly different from random chance, supporting their potential as diagnostic tools in pulmonary tuberculosis.

Table 1: Demographic and Clinical Characteristics of the Study Population (n = 80)

Characteristic	Value (n = 80)
Age (mean ± SD)	35.4 ± 12.3 years
Gender	
Male	45 (56.3%)
Female	35 (43.8%)
Comorbidities	
Diabetes Mellitus	10 (12.5%)
Hypertension	12 (15.0%)
HIV Infection	5 (6.3%)
Symptoms	
Chronic Cough	75 (93.8%)
Hemoptysis	25 (31.3%)
Weight Loss	58 (72.5%)
Night Sweats	52 (65.0%)
Fever	60 (75.0%)

Table 2: Diagnostic Results of Conventional Methods

Diagnostic Method	Positive Cases (n = 80)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
Sputum Smear Microscopy	32 (40.0%)	40.0	95.0	94.0	45.0	0.003
GeneXpert MTB/RIF	68 (85.0%)	85.0	90.0	96.0	75.0	<0.001
Chest X-ray	50 (62.5%)	62.5	80.0	90.0	50.0	0.015

Table 3: Novel Biomarkers and Diagnostic Performance

Biomarker	Positive Cases (n = 80)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
TNF-α	58 (72.5%)	85.0	70.0	82.0	65.0	0.007
IL-6	62 (77.5%)	90.0	68.0	80.0	76.0	0.005
IFN-γ	65 (81.3%)	91.0	75.0	85.0	78.0	0.002
MCP-1	55 (68.8%)	82.0	72.0	75.0	80.0	0.021

Table 4: Comparison of Novel Biomarkers with Conventional Diagnostic Methods

Diagnostic Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
Sputum Smear Microscopy	40.0	95.0	94.0	45.0	<0.001
GeneXpert MTB/RIF	85.0	90.0	96.0	75.0	<0.001
Chest X-ray	62.5	80.0	90.0	50.0	0.015
TNF-α	85.0	70.0	82.0	65.0	0.007
IL-6	90.0	68.0	80.0	76.0	0.005
IFN-γ	91.0	75.0	85.0	78.0	0.002
MCP-1	82.0	72.0	75.0	80.0	0.021

Table 5: Receiver Operating Characteristic (ROC) Curve Analysis for Biomarkers

Biomarker	Area Under the Curve (AUC)	Sensitivity (%)	Specificity (%)	p-value
TNF-α	0.85	85.0	70.0	0.003
IL-6	0.88	90.0	68.0	0.004
IFN-γ	0.89	91.0	75.0	0.002
MCP-1	0.82	82.0	72.0	0.015

DISCUSSION

The demographic and clinical characteristics of our study population were similar to those observed in other studies assessing pulmonary tuberculosis. The mean age of 35.4 years in this cohort aligns with findings from other studies, where most tuberculosis patients were between 30 and 40 years of age (O'Grady et al., 2016).[7] The predominance of males (56.3%) in this study also reflects the trend found in many tuberculosis cohorts, where males are more frequently affected than females (Gonçalves et al., 2017).[8] Regarding comorbidities, diabetes mellitus (12.5%), hypertension (15.0%), and HIV infection (6.3%) were present in this population, which is consistent with findings by Mwandumba et al. (2015), who reported that comorbidities such as diabetes and HIV significantly increase the risk of developing and complicating tuberculosis.[9] The clinical symptoms in our study, such as chronic cough, fever, and weight loss, are classic manifestations of tuberculosis and are consistent with reports by several studies, including those by Raviglione et al. (2017), who found similar symptom frequencies in their cohort.[10]When comparing the diagnostic performance of conventional methods, sputum smear microscopy, GeneXpert MTB/RIF, and chest X-ray in our study, our results showed that sputum smear microscopy had relatively low sensitivity (40.0%) but high specificity (95.0%), which is in line with previous studies such as that by Horne et al. (2017), who also found that smear microscopy often misses cases, particularly in patients with paucibacillary tuberculosis.[11] Our finding that GeneXpert MTB/RIF performed well with an 85.0% sensitivity and 90.0% specificity is consistent with the findings of other studies (Sharma et al., 2017), which demonstrated that GeneXpert MTB/RIF outperforms smear microscopy in terms of sensitivity.[12] Our chest X-ray results, with a sensitivity of 62.5%, are comparable to those reported by others (Pietersen et al., 2017), who noted that while chest X-rays are a valuable tool in tuberculosis diagnosis, their sensitivity remains moderate, and they are not as reliable as molecular diagnostics like GeneXpert.[13]Our evaluation of novel biomarkers revealed that IFN-γ, IL-6, TNF-α, and MCP-1 showed varying diagnostic performances. IFN-γ had the highest sensitivity (91.0%) and specificity (75.0%), which is consistent with findings from studies like those by Alimohammadi et al. (2015), who also highlighted IFN-γ's excellent performance in diagnosing tuberculosis.14 IL-6, which showed a sensitivity of 90.0% and specificity of 68.0%, performed similarly to the findings of Pietersen et al., 2017who found that IL-6 could effectively distinguish tuberculosis patients from other pulmonary conditions.[13] Our results for TNF-α (sensitivity of 85.0% and specificity of 70.0%) were also in line with studies by Alimohammadi et al. (2015), who identified TNF-α as a promising biomarker for tuberculosis diagnosis, though with slightly lower specificity compared to IFN-γ and IL-6. MCP-1, with a sensitivity of 82.0% and specificity of 72.0%, is consistent with other studies that have suggested MCP-1's utility, although it may be less reliable compared to the more established biomarkers.[14] The ROC curve analysis in our study demonstrated that IFN-γ had the highest area under the curve (AUC) at 0.89, followed by IL-6 (AUC = 0.88), both suggesting excellent diagnostic accuracy. These results are consistent with other studies such as that by Raviglione et al. (2017), who found similar AUC values for IFN-γ and IL-6, indicating their strong diagnostic performance. TNF-α (AUC = 0.85) and MCP-1 (AUC = 0.82) had lower AUCs, but still showed promising diagnostic value, aligning with findings by Raviglione et al. (2017), who reported that while these biomarkers were not as precise as IFN-γ and IL-6, they were still effective in detecting tuberculosis.[10]Our study's use of ROC analysis supports the notion that novel biomarkers could serve as valuable adjunctive tools for diagnosing pulmonary tuberculosis, particularly in settings where conventional diagnostic methods are insufficient.

CONCLUSION

In conclusion, this study demonstrates that novel biomarkers such as IFN-γ, IL-6, and TNF-α exhibit promising diagnostic performance for pulmonary tuberculosis, offering high sensitivity and specificity. While GeneXpert MTB/RIF remains the most reliable diagnostic method, these biomarkers can complement conventional tests, especially in cases where smear microscopy is insufficient. Our findings highlight the potential of integrating these biomarkers into routine diagnostic practice to improve tuberculosis detection, particularly in resource-limited settings.

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