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INTEGRATING MICROBIOLOGY AND CLINICAL RESEARCH: MOLECULAR MECHANISMS AND BIOMARKERS IN INFECTIOUS DISEASE DIAGNOSIS

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ABSTRACT

The problem of tuberculosis, as one of the major infectious disease issues, is frequently restricted by the accessibility and sensitivity. Host-Response transcriptomic biomarkers, especially plasma cell-free RNA, have a promising non-sputum-based method of infectious disease diagnosis. This study aimed to integrate clinical metadata and transcriptomic profiles to identify molecular mechanisms and candidate biomarkers associated with tuberculosis. A retrospective computational analysis was conducted using plasma cfRNA RNA-sequencing data from a validation cohort of 60 individuals classified as TB-positive or TB-negative. Gene expression data were filtered, log-normalized, and integrated with clinical variables. Differential expression analysis, gene ontology enrichment, and supervised logistic regression modeling were applied to identify candidate biomarkers, characterize biological pathways, and evaluate diagnostic performance. TB-positive individuals exhibited upregulation of immune-related genes, including *BATF2, GBP5, GBP1, IFITM3, IFIT3, MMP8, SERPING1,* and *STAT1.* Enrichment analysis revealed significant involvement of interferon-gamma response, type I interferon signalling, cytokine-mediated pathways, and host defense processes. The classification model demonstrated moderate diagnostic performance, achieving an area under the curve of approximately 0.78. These findings indicate that plasma cfRNA profiles capture a coordinated interferon-driven host-response signature associated with tuberculosis. The results support the potential of transcriptomic biomarkers as complementary tools for non-sputum-based infectious disease diagnosis, although validation in larger and independent cohorts is necessary for clinical translation.

KEYWORDS: Tuberculosis, cell-free RNA, transcriptomics, interferon signaling, diagnostic biomarkers

1. INTRODUCTION

Tuberculosis (TB) has remained a serious global health burden, being among the major causes of death due to infectious diseases in the world. As established by the world health reports, socioeconomic and healthcare disparities disproportionately contribute to the burden of TB, which is concentrated in high-incidence regions in disproportionate amounts (World Health Organization, 2021). The interactions among environmental, demographic, and biological factors shape the epidemiology of TB and influence the disease progression and outcomes (Khan et al., 2019). Even when cured of the disease, patients who have previously had TB are at higher risk of becoming long-term mortalities, which is indicative of the long-term effects of the disease on overall health (Romanowski et al., 2019). Along with its clinical implications, TB has a significant economic burden, and with decreased productivity and premature deaths, effective diagnostic and intervention strategies are also needed (Silva et al., 2021).

Proper diagnosis is the key to TB control, but the traditional diagnostic tools are still rather limited in terms of their applicability and effectiveness. Microscopy and culture methods of sputum are sometimes limited by the low sensitivity of these approaches and the delay in detection (Acharya et al., 2020). These restrictions have led to growing interest in the creation of alternative diagnostic methods that do not rely on sputum and could offer reliable and accessible alternatives (Sossen & Meintjes, 2023). The discovery of biomarkers has been enhanced by the development of high-throughput technologies based on biomarker discovery, allowing a comprehensive analysis of biological systems at the molecular level (Bharti et al., 2024). These methods have provided new avenues of determining host-derived signals that are indicative of the physiological response to infection.

Host transcriptomics has proved to be a highly promising approach towards the understanding of infectious disease, and also in the identification of diagnostic biomarkers. Transcriptomic analyses, by capturing changes in gene expression caused by infection, give an insight into the disease mechanisms as well as potential clinical indicators (Burel et al., 2019). RNA signature of the blood has been widely studied in TB, and it has been shown that the signature of specific immune-related expression patterns can distinguish the infected individuals of the study against healthy or symptomatic controls (Sivakumaran et al., 2021). Systematic assessments have also indicated that there are important genes and pathways that are active in response to active TB (Chen et al., 2021). Among them, mechanisms involving interferon have continuously been implicated, as it is a central mechanism of host defense against Mycobacterium tuberculosis (Shanmuganathan et al., 2022). More recently, the use of plasma-derived cell-free RNA has been investigated as a minimally invasive source of biomarkers, and studies have demonstrated its potential to capture systemic immune responses and improve diagnostic performance (Chang et al., 2024).

With these developments, there are a number of challenges related to converting transcriptomic data into clinically useful diagnostic instruments. Numerous studies have focused on predictive accuracy but have not provided much information on the biological mechanisms that underlie the observed gene signatures. Also, this variability of gene expression patterns between populations and sample types makes the reproducibility and generalizability of proposed biomarkers difficult. Integrative analytical methods in which differential expression analysis is combined with pathway-level interpretation and machine learning-based classification in a unified framework are required as well. Moreover, to ensure the strength and relevance of host-response biomarkers in different clinical settings, it is necessary to have an independent analysis of the current datasets.

The current research paper fills the mentioned gaps by combining transcriptomic and clinical data based on the plasma cell-free RNA profiles to investigate host-response biomarkers related to tuberculosis. The analysis will determine genes that are differentially expressed between individuals who are TB-positive and TB-negative, characterize the biological pathways underlying these changes, and evaluate the diagnostic potential of gene expression signatures using a supervised classification model. It is through this combined effort that the study aims to provide a mechanistic understanding, as well as a practical significance of biomarker-based diagnosis of infectious diseases.

2. METHODOLOGY

2.1 Research Design

In this study, a retrospective computational framework that incorporated clinical metadata with transcriptomic profiles was used to investigate host-response biomarkers related to tuberculosis (TB). The analysis design aimed at determining differentially expressed genes, defining underlying molecular processes, and assessing their diagnostic utility. The links between gene expression patterns and clinical disease status were established using statistical analysis, functional enrichment and supervised machine learning approaches.

2.2 Data Source

The data applied in the current study were derived from Chang et al. (2024), who evaluated circulating cell-free RNA (cfRNA) as a host-response biomarker to detect tuberculosis. The data is a sample of RNA sequencing-based gene expression profiles obtained on plasma samples of individuals who present with a cough of at least two weeks to outpatient TB clinics in Uganda, Vietnam and the Philippines. Sequencing of samples was carried out on the Illumina NextSeq 500 platform, which provided transcript-level count data. Clinical TB-positive and TB-negative patients were clinically categorized, and related metadata included demographic variables and HIV status. In the present analysis, the validation cohort of 60 samples was used to study the host transcriptional responses in relation to disease status.

2.3 Data Preprocessing

The data on the number of genes were processed so that the analytical consistency could be ensured and technical variability could be reduced. The filtered genes with lowly expressed genes were filtered to eliminate those features that contribute minimally to the downstream analysis. The resultant count table was normalized by applying the log2 transformation in order to stabilize the variance between the samples. Ensembl IDs were changed to gene symbols to enable biological interpretation and compatibility with annotation resources.

2.4 Clinical Data Integration

The curation and alignment of clinical metadata and gene expression data were done using unique sample identifiers. The variables were removed and standardized: TB status, age, sex, HIV status, and geographic site. TB status was coded as a binary variable to facilitate classification modelling. Only samples where there was complete agreement between clinical and transcriptomic data were kept for further analyses.

2.5 Differential Expression Analysis

The analysis of differentially expressed genes was conducted to reveal the genes that were differentially expressed between TB-positive and TB-negative groups. Mean levels of expression of each gene were compared across groups, and expression changes represented the log2 fold change. Hypothesis testing on normalized data was used to determine the statistical significance. The nominal p-values in combination with the effect size were used to rank genes, which represents an exploratory method for identifying candidate biomarkers.

2.6 Functional Enrichment Analysis

Functional enrichment analysis was performed on the identified genes to determine their biological relevance using gene ontology (GO) biological process annotations. The overrepresentation of biological pathways among candidate genes compared to a reference background was analyzed. To correct the effect of multiple testing, adjusted p-values were used to determine the statistical significance and combined scores were used to rank the pathways.

2.7 Machine Learning Classification

A supervised -classification model was constructed that could assess the diagnostic potential of the identified gene signatures. Input features were the values of gene expression, and the outcome variable was TB status. Stratified sampling was used to divide the dataset into training and testing subsets in order to balance the classes. Before model training, feature scaling was performed. A logistic regression model was estimated using the training data, and receiver operating characteristic (ROC) analysis and area under the curve (AUC) measures were used to assess the predictive performance on the test set.

3. RESULTS

3.1 Clinical Characteristics of the Study Cohort

The participants of the study cohort were 60 people presenting with a prolonged cough, of whom 35 were TB-positive, and 25 were TB-negative. The age of the subjects was 33.32 years (mean), 31 (median). There was a male dominance in the cohort (41 males, 19 females). Ten people had HIV co-infection, and 50 respondents were HIV-negative. These demographic and clinical characteristics represent a representative population in TB-endemic settings and offer an adequate basis on which downstream molecular analyses may be conducted (Table 1).

Table 1. Clinical Characteristics of the Study Cohort

Variable	Value
Total samples	60
TB positive	35
TB negative	25
Mean age	33.32
Median age	31
Male	41
Female	19
HIV positive	10
HIV negative	50

3.2 Identification of Candidate Biomarker Genes

A comparative transcriptomic analysis of TB-positive and TB-negative groups showed that a number of genes were highly expressed in infected persons. Despite statistical significance not being achieved due to multiple testing correction, a number of genes showed consistent upregulation with significant fold changes and small nominal p-values, and these genes are candidates to be biomarkers.

The major upregulated genes were *BATF2, GBP5, GBP1, IFITM3, IFIT3* and *STAT1*, which have been known to be involved in interferon signaling, innate immune responses. Other genes like *SERPING1, FCGR3B,* and *MMP8* indicate that the complement pathways, recruitment of immune cells, and inflammatory processes have been activated (Table 2).

Table 2. Top Candidate Biomarker Genes Associated with TB

Gene Symbol	Ensembl ID	Log2 Fold Change	P-value
BATF2	ENSG00000168062.10	1.575	0.000029
GBP5	ENSG00000154451.14	1.747	0.000217
GBP1	ENSG00000117228.11	1.498	0.000868
SERPING1	ENSG00000149131.17	1.513	0.001702
FCGR3B	ENSG00000162747.12	1.145	0.002790
LINC01572	ENSG00000261008.7	1.224	0.002842
GBP4	ENSG00000162654.9	1.241	0.002921
IFITM3	ENSG00000142089.17	1.417	0.006439
C1QA	ENSG00000173372.17	1.145	0.010415
ZSCAN16-AS1	ENSG00000269293.3	1.345	0.010898
IFIT3	ENSG00000119917.15	1.119	0.012555
MTCO1P40	ENSG00000262902.1	1.973	0.013479
MMP8	ENSG00000118113.12	1.208	0.014362
STAT1	ENSG00000115415.20	1.041	0.016278
ENSG00000254786	ENSG00000254786.1	1.085	0.018591

3.3 Functional Enrichment of Candidate Genes

Pathway enrichment analysis was carried out to explain the biological meaning of the identified genes. The findings showed that the immune-related pathways were strongly enriched, in particular interferon signaling and host defense responses. Response to interferon-gamma, type I interferon signaling, and cytokine-mediated signaling pathways were the most noticeable processes. These pathways were always triggered by genes like STAT1, IFIT3, IFITM3, IFI6, and GBP family members, indicating a coordinated activation of innate immune responses during TB infection (Table 3).

Table 3. Top Enriched Biological Processes

Biological Process	**Adjusted P-value**	**Combined Score**	**Associated Genes**
Response to interferon-gamma	0.000138	641.21	*IFITM3, GBP5, STAT1, GBP1, GBP4*
Cellular response to type I interferon	0.000826	493.68	*IFITM3, STAT1, IFI6, IFIT3*
Type I interferon signaling pathway	0.000826	493.68	*IFITM3, STAT1, IFI6, IFIT3*
Cellular response to interferon-gamma	0.006371	204.55	*GBP5, STAT1, GBP1, GBP4*
Defense response to symbiont	0.006371	197.43	*IFITM3, STAT1, IFI6, IFIT3*
Defense response to the virus	0.006969	178.35	*IFITM3, STAT1, IFI6, IFIT3*
Regulation of mitochondrial depolarization	0.010976	1130.91	*SRC, IFI6*
Defense response to protozoan	0.015836	799.14	*BATF2, GBP4*
Interleukin-6-mediated signaling pathway	0.023560	561.46	*SRC, STAT1*
Cytokine-mediated signaling pathway	0.034489	45.11	*IFITM3, SRC, STAT1, IFI6, GBP1, IFIT3*

3.4 Global Gene Expression Patterns Distinguish TB Status

A hierarchical clustering of normalized gene expression profiles showed a strict division between TB-positive and TB-negative samples (Figure 1). The upregulation of interferon-stimulated genes was consistently upregulated in TB-positive samples, but remained relatively lower in TB-negative samples. This clear clustering trend underscores the existence of a strong transcriptional signature in relation to TB infection.

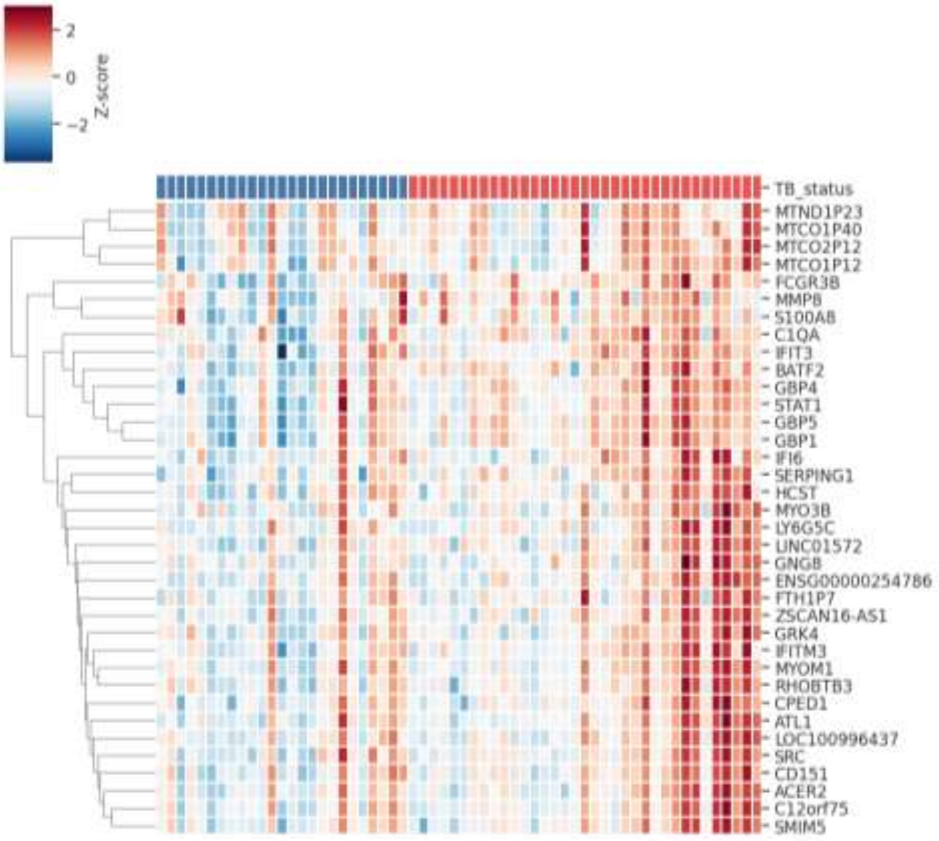

Figure 1. Heatmap of Differentially Expressed Genes Distinguishing TB-Positive and TB-Negative Samples

3.5 Diagnostic Performance of Gene-Based Classification Model

A logistic regression model was developed in order to determine the clinical utility of the identified gene signature. The model demonstrated an area under the receiver operating characteristic curve (AUC) of around 0.78, which showed moderate discriminatory performance of TB-positive versus TB-negative individuals. The model does not hold up well as an independent diagnostic tool, but it does reveal the promise of host-response biomarkers in assisting in TB detection (Figure 2).

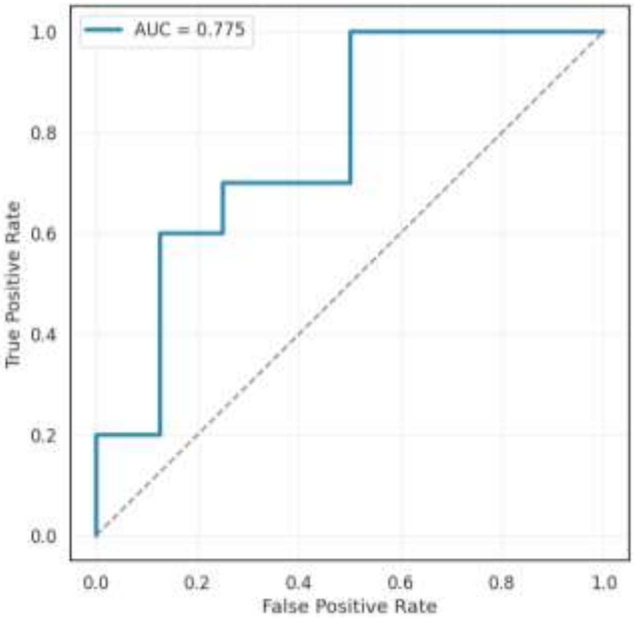

Figure 2. Receiver Operating Characteristic (ROC) Curve Demonstrating Diagnostic Performance of the Gene-Based Classifier

3.6 Interferon-Driven Immune Signature in TB

A detailed study of the interferon-related genes showed that there was a steadfast pattern of increased expression among the TB-positive individuals as compared to TB-negative controls (Figure 3). Strong upregulation was observed in genes such as *STAT1, IFITM3, GBP1, GBP4* and *GBP5*, which indicates that it has activated interferon-mediated immune pathways. The concerted appearance of this expression pattern implies a pathway-wide reaction instead of individual gene actions.

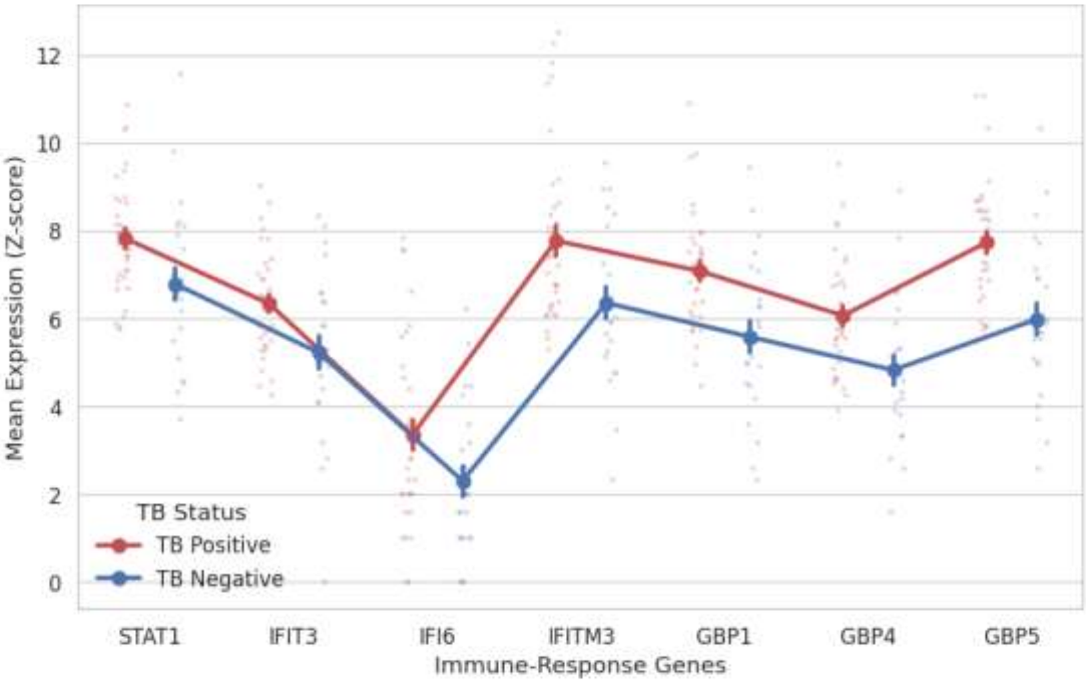

Figure 3. Gene-Wise Expression Profiles of Interferon-Related Biomarkers Across TB Status

3.7 Integrated Visualization of Molecular Mechanisms and Biomarkers

To combine gene-level and pathway-level results, enrichment results were represented in a visualization in conjunction with expression data. The mechanistic nature of the observed patterns of gene expression is further supported by the bar plot of enriched biological processes (Figure 4). The combination of these findings with the heatmap indicates that there is a consistent relationship between the activation of host immunity and the biomarker signatures that can be measured.

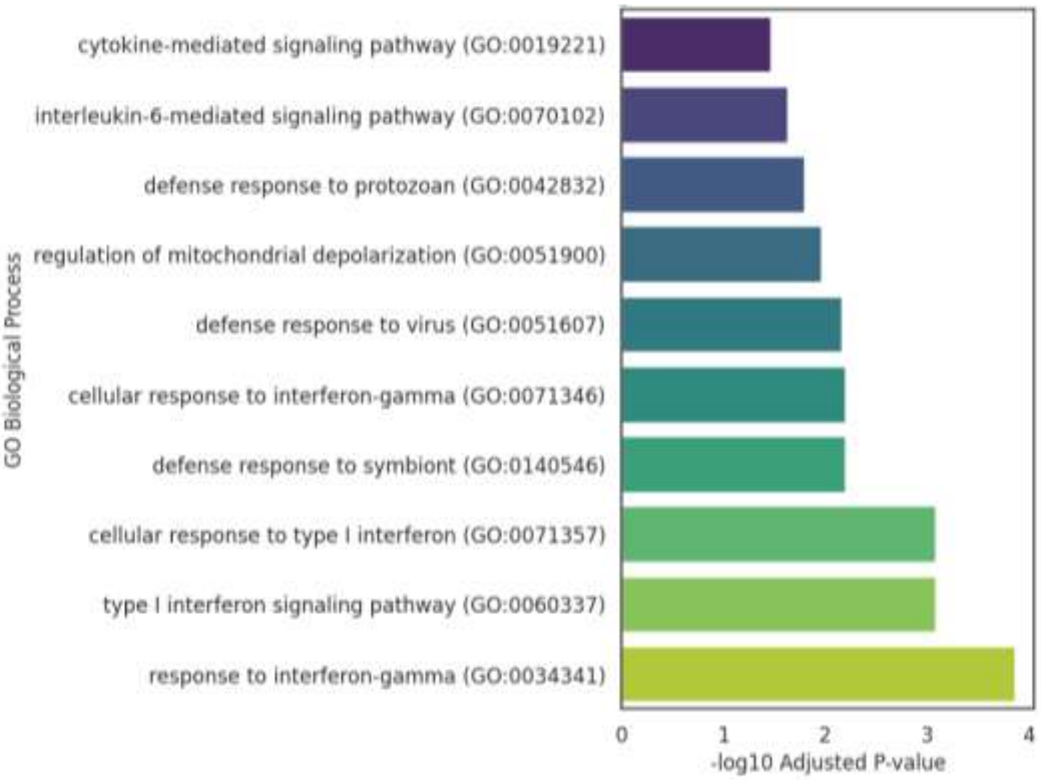

Figure 4. Enriched Biological Processes Associated with Candidate TB Biomarker Genes

4. Discussion

The findings suggest that tuberculosis can be identified by a unique host-response transcriptional pattern that can be detected in plasma-derived RNA profiles. Candidate biomarker genes were largely up-regulated in TB-positive individuals with notable up-regulation of *BATF2, GBP5, GBP1, IFITM3, IFIT3, MMP8, SERPING1* and *STAT1*. Whereas these findings on an immune-related upregulation on a gene-level were exploratory in nature, the consistency of the immune-related upregulation across different analyses of the gene sample suggests that the signal under observation is a biologically coordinated response and not random variation in the expression of the gene sample. The gradient of expression that was observed in the heatmap indicated that there was a clear expression gradient between and including many TB-positive and TB-negative samples, which supported the presence of a disease-associated molecular profile. This interpretation was reinforced by functional enrichment that demonstrated that the candidate genes were clustered around interferon-gamma response, type I interferon signaling, cytokine-mediated signaling and host defense processes. These findings indicate that the detection of TB by using the cfRNA is not only founded on the presence of individual biomarkers but on the overall immune activation that is related to the host-pathogen interaction. The diagnostic model had a moderate level of discriminatory performance with an AUC of about 0.78. This implies the selected gene signature has captured a useful diagnostic signal, but the performance is not adequate to be used on its own in clinical practice. The targeted interferon-related expression plot further demonstrated a coordinated up-regulation of a number of immune-response genes in TB-positive samples, which further supports the relevance of interferon-associated pathways as both mechanistic and diagnostic biomarkers.

The moderate diagnostic performance here is in line with the rest of the host-response TB diagnostics field, where gene signatures often have promise but diverge in their performance across cohorts, platforms, and populations. The systematic comparison of several TB host-response signatures revealed that the diagnostic accuracy strongly depended on the composition of the cohort and the context of the validation (Warsinske et al., 2019). The overexpression of the processes associated with type I interferon within the current analysis is congruent with the mechanistic evidence that type I interferon signaling can affect the susceptibility and immune regulation in the context of TB infection (Kotov et al., 2023). Likewise, studies based on macrophages have demonstrated that the infection of mycobacteria triggers specific programs of interferon stimulation, thus supporting the biological plausibility of the interferon-related genes found in this study (Zhou et al., 2019).

The evidence that type I interferons and Mycobacterium tuberculosis antigenic stimulation can cause distinct transcriptional responses during infection is also correlated (Mutua et al., 2023). Also in line with the past biomarker studies is the identification of GBP family genes. One of the most upregulated *GBP5*s in the current analysis is reported to the protein level in active TB, indicating that its diagnostic utility may not be as limited to transcript abundance as it may have been previously (Yao et al., 2022). The presence of *SERPING1* and complement-associated markers is also in line with more recent machine learning studies that have found complement-related markers to be part of the diagnostic models of tuberculosis infection (Li et al., 2024).

Detection of *MMP8* amongst candidate genes has a biological meaning since an increase in plasma concentration of matrix metalloproteinase-8 has been linked with microbiological disease activity in pulmonary TB (Walker et al., 2022). Moreover, more recent blood-based gene-score assays have highlighted the possibilities of small transcriptional signatures to monitor and provide diagnostic support in pulmonary TB (Zhang et al., 2024). On a bigger scale, biomarker-oriented reviews point to the necessity to combine diagnostic, prognostic, and preventive uses of TB biomarkers, which is also in line with the translational nature of the current analysis (Matuku-Kisaumbi, 2024).

These results suggest the usefulness of combining clinical metadata and host transcriptomic profiles to diagnose infectious diseases. Biologically, the findings support the interferon-mediated immune activation as the key molecular characteristic of TB. Diagnostically, the model performance indicates that the possibility of using the gene signatures derived using the cfRNA may be utilized in non-sputum-based screening or triage programs, particularly in situations where the collection of sputum is challenging or when the traditional testing is delayed. The findings also indicate that the diagnostic models cannot be based solely on statistical feature selection but have to be supported by biological coherence. The genes in common pathways of immune responses may offer more consistent and interpretable diagnostic information compared to single markers. This especially applies to the clinical translation, where interpretability and reproducibility are vital.

It should be noted that there are a few limitations. To limit the statistical power of the analysis, first, the validation cohort (60 samples) was used, which can explain why candidate genes did not reach the significance threshold after multiple testing correction. Second, the model was constructed on one dataset; it needs external validation before one can conclude that the model is relevant to clinical practice. Third, the analysis did not cover the pathogen-level microbiological variables of bacterial burden, culture status, or strain information. Future research must consider the determined biomarkers in larger independent cohorts and in various clinical populations. Combining the use of cfRNA with protein biomarkers, radiological findings, and microbiological measurements could enhance the accuracy of the diagnosis and biology. Future research should also optimize small sets of genes to be used in reproducible clinical tests and determine whether interferon-related cfRNA signatures can be used to differentiate active TB and latent infection, other respiratory infections, or inflammatory lung diseases.

5. CONCLUSION

Plasma-derived cfRNA profiles were able to capture a biologically consistent host-response signature linked to tuberculosis. The upregulation of candidate genes, including *BATF2, GBP5, GBP1, IFITM3, IFIT3, MMP8, SERPING1,* and *STAT1,* was predominantly upregulated in TB-positive individuals and was linked to interferon signaling, cytokine-mediated responses, and host defense pathways. These results suggest that TB-related molecular alterations are not specific to individual biomarkers but rather indicative of orchestrated immune responses. The gene-based classification model demonstrated moderate diagnostic performance, which supports the potential value of transcriptomic biomarkers as complementary tools to detect TB using non-sputum-based methods. The results are, however, to be understood in the context of the validation cohort size and the lack of pathogen-level microbiological variables. To further refine biomarker panels, enhance diagnostic accuracy and enhance clinical translation, larger independent cohorts and integrated multi-modal datasets will be needed. The discussion justifies the combination of molecular profiling and clinical data as a good strategy to enhance the diagnosis of infectious diseases and the knowledge of the interaction between the host and the pathogen.

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