Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Identification of Specific LncRNA Markers in Severe Tuberculosis for Early Diagnosis

Siyu Yao[1,*], Xiuxiu Ji[2,*], Ruize Wang[1], Liping Pan[2], Qiuyue Liu[1]

[1]Department of Critical Care Medicine, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, Beijing, People's Republic of China; [2]Department of Molecular Biology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Liping Pan, Department of Molecular Biology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, No.9 Beiguan Street, Tongzhou District, Beijing, 101149, People's Republic of China, Tel/Fax +86 1089509365, Email panliping2006@163.com; Qiuyue Liu, Department of intensive care unit, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, No.9 Beiguan Street, Tongzhou District, Beijing, 101149, People's Republic of China, Tel/Fax +86 13426108188, Email liuqiuyue@bjxkyy.cn

Background: The progression of tuberculosis to severe disease is the main cause of death of tuberculosis patients. Early identification of severe tuberculosis is very important. LncRNA can be used as a potential marker in the diagnosis of tuberculosis, but there is a lack of research on lncRNA in the field of severe tuberculosis.

Methods: Peripheral blood samples of severe pulmonary tuberculosis patients, mild pulmonary tuberculosis patients and healthy controls were collected to extract peripheral blood monocytes. The RNA was then extracted and sent to the SBC human ceRNA V1.0 analysis. The results were verified by qPCR and analyzed by KEGG and GO analyses. Differentially expressed lncRNAs were measured by ROC curves.

Results: Four lncRNAs exhibited statistically distinct expression patterns in STB versus MTB groups (NR_033909: p=0.0097; lnc-MYCBPAP-2:4: p=0.0053; lnc-PRDM7-1:2: p<0.0001; NR_033841: p=0.0279). The areas under the curve (AUC) value are respectively 0.7280(lnc-PRDM7-1:2), 0.7288(lnc-MYCBPAP-2:4), 0.6647(NR_033909) and 0.6615(NR_033841).

Conclusion: LncRNAs NR_033909, lnc-MYCBPAP-2:4, lnc-PRDM7-1:2 and NR_033841 may demonstrate diagnostic potential for differentiating severe from mild pulmonary tuberculosis cases. These results create a platform for monitoring TB progression and open new avenues for studying disease pathogenesis.

Keywords: severe tuberculosis, diagnosis, lncRNA, biomarker

Introduction

Tuberculosis (TB) is still one of the leading causes of death from a single infectious agent in the world.[1] As a contagious disease with severe social implications due to *Mycobacterium tuberculosis* (*M.tb*), TB remains a major public health burden in developing countries.[2,3] The COVID-19 pandemic has led to an increase in hospitalizations and fatalities related to tuberculosis, exacerbating the situation for public health systems worldwide.[4] There were approximately 10.8 million new TB cases and 1.25 million TB deaths worldwide in 2023, which means TB has surpassed COVID-19 to once again become the leading infectious disease killer worldwide. TB is the foremost cause of mortality among individuals living with HIV and a significant driver of deaths related to antimicrobial resistance. The tuberculosis control situation is still very serious worldwide.[5]

Advancing disease stage accounts for the majority of TB-associated deaths globally, and there has been persistently high mortality (more than 50%) in patients with severe pulmonary TB patients.[6–8] The addition of Bedaquiline to anti-TB treatment significantly reduces mortality and improves treatment success.[9–12] However, the treatment success of severe TB remains low globally, approximately between 50% and 60%.[13,14] Patients with severe tuberculosis, particularly those complicated by respiratory failure, demonstrated unfavorable clinical outcomes due to the lack of standardized assessment protocols. This critical condition, frequently encountered in the intensive care unit (ICU) settings, carries significant

Received: 15 May 2025
Accepted: 30 August 2025
Published: 16 September 2025

risks of mortality and adverse health consequences.[15] Severe pulmonary TB diagnosis integrates clinical, radiological, and pathological findings with microbiological confirmation (acid-fast bacilli culture) and oxygenation assessment (PaO_2).[8] The development of reliable diagnostic tools for severe tuberculosis represents an urgent unmet clinical need. Despite clinical urgency, the pathophysiological basis of TB advancement is incompletely understood, and reliable severity indicators remain scarce.[16]

While sputum smear microscopy and thoracic imaging remain conventional diagnostic modalities for tuberculosis, contemporary practice is increasingly adopting other molecular biological diagnostic methods as primary detection tools. The World Health Organization technical consultation endorsed next-generation, non-sputum biomarker assays as pivotal TB detection tools. Representative platforms like Xpert MTB/RIF enable microbiological confirmation within 120 minutes, demonstrating transformative diagnostic capabilities.[17] Despite diagnostic advancements, empirical TB diagnosis remains prevalent in practice due to resource constraints. In recent years, accumulating evidence demonstrates that long non-coding RNAs (lncRNAs) participate in diverse disease mechanisms through modulation of critical physiological and pathological pathways.[18] Dysregulated lncRNAs contribute to tuberculosis pathogenesis and disease advancement.[19,20] Meanwhile, studies have also shown that lncRNAs can serve as molecular biomarkers in the diagnosis in TB. For example, lncRNAs uc.48+ and NR 105053 may serve as new diagnostic markers in TB recovery;[21] Yang X et al suggested that lncRNAs MIR3945HG V1 and MIR3945HG V2 emerge as new TB diagnostic candidates.[22]

The development of novel vaccines is critical for global TB control. While Bacille Calmette-Guérin (BCG) provides effective protection against severe pediatric TB, new vaccines capable of preventing all stages of TB infection across all age groups are essential to achieving the WHO's goal of ending the TB epidemic.[23] The current landscape of vaccine development features several candidates, which are broadly categorized into four main types: live attenuated whole-cell vaccines, inactivated whole-cell vaccines, adjuvanted protein subunit vaccines, and viral-vectored vaccines.[24] Biomarkers are indispensable in vaccine development, as appropriate biomarkers enable timely assessment of progress and vaccine efficacy, thereby conserving financial, human, and material resources. LncRNAs hold promise as valuable biomarkers for predicting the clinical progression of TB, potentially accelerating the development of novel vaccines.[25,26]

Our previous studies demonstrated the potential value of proteins as biomarkers for the diagnosis of severe pulmonary TB and found that there were specific proteins in severe TB.[27,28] It indicated there might be specific lncRNAs in severer TB patients and suggested that lncRNAs may play a role in the pathological processes in severe TB patients.

This study utilized lncRNA microarray technology for biomarker discovery in the peripheral blood monocytes (PBMCs) of severe pulmonary TB patients. Our study contributes to improved TB early diagnosis and disease mechanism discovery.

Materials and Methods

Patients

Ethical clearance was granted by the Chinese Clinical Trial Registry (ChiCTR2000032719), and all subjects or their guardians provided written consent. This study has been approved by the Ethics Committee of Beijing Chest Hospital Affiliated to Capital Medical University. Clinical data of the patients were retrieved via a query process, with protection measures in place for patient privacy information throughout this procedure. This prospective cohort study enrolled participants at Beijing Chest Hospital, Capital Medical University (Beijing, China) from January 2022 to February 2024, adhering strictly to the Declaration of Helsinki guidelines. Eligible participants (≥18 years) provided written consent. Exclusion criteria included: immunomodulatory treatment, extrapulmonary TB, autoimmune/chronic diseases, malignancies, or HIV infection. TB diagnosis followed WHO integrated clinical criteria incorporating laboratory, molecular, and clinical-radiographic parameters.[29] Figure 1 presents the study design flowchart. The initial cluster comprised 207 participants. After excluding 24 cases due to incomplete data, final assessments were completed by 183 enrolled subjects. The 183 participants were evenly divided into three groups: 61 with severe pulmonary TB(STB), 61 with mild pulmonary TB(MTB), and 61 healthy controls (HC). Ten samples were meticulously selected from each group to undergo ceRNA analysis, among which abnormal data samples (4 samples of STB,1sample of HC) were excluded. The residual samples were designated for quantitative real-time PCR (qPCR) to confirm the microarray-derived results.

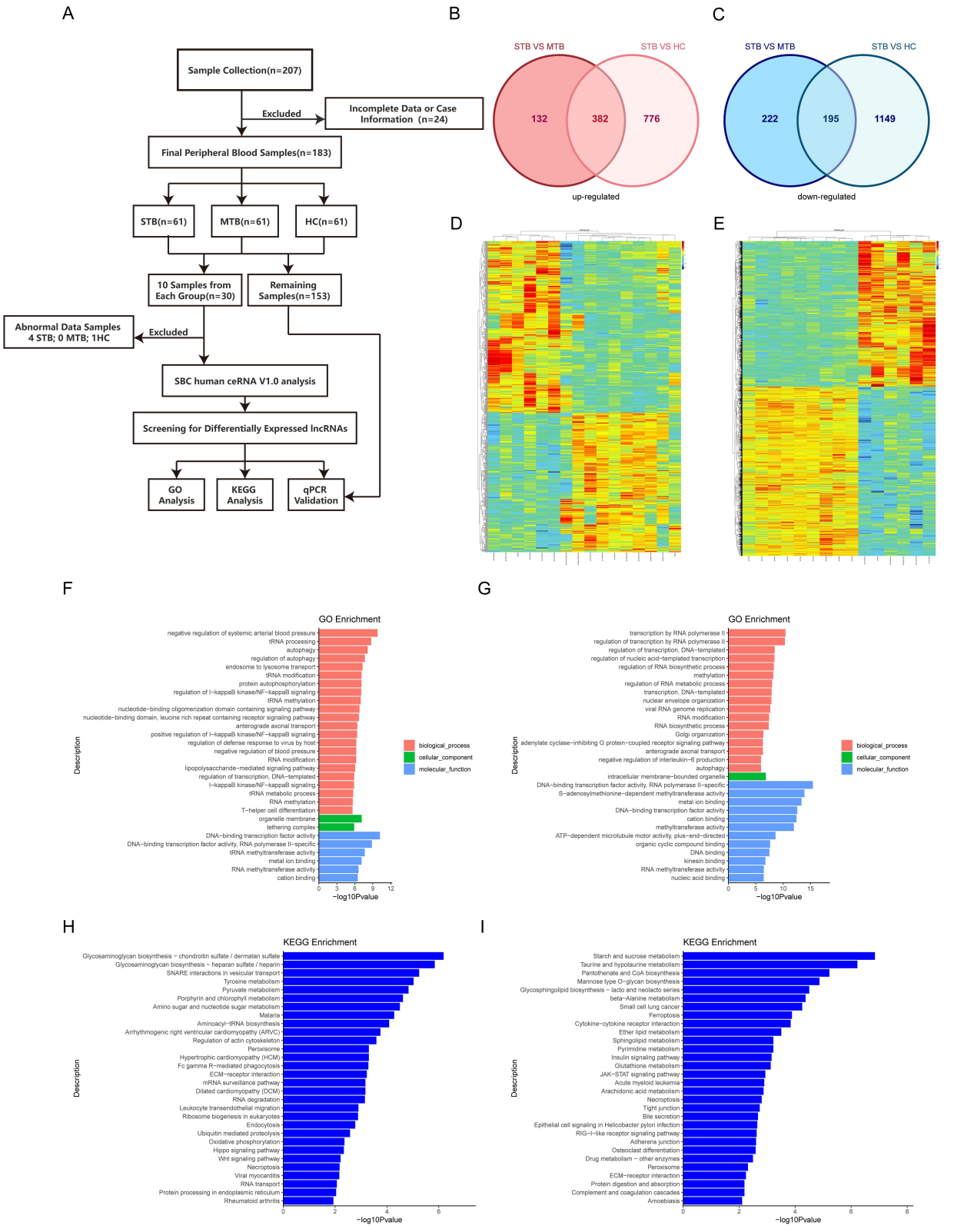

Figure 1 Differentially expressed lncRNAs between three groups. (**A**) Flowchart of the study. (**B**) Venn diagram of the up-regulated (The numbers in the picture are the numbers of differentially expressed lncRNAs). (**C**) Venn diagram of the down-regulated. (**D**) The heat map of differentially expressed lncRNAs between the STB group and the MTB group (Red-up regulated, blue-down regulated). (**E**) The heat map of differentially expressed lncRNAs between the STB group and the HC group. (**F**) GO analysis of upregulated lncRNAs (STB VS MTB). (**G**) GO analysis of downregulated lncRNAs (STB VS MTB). (**H**) The most enriched items of the KEGG pathway analysis of the down-regulated (STB VS MTB). (**I**) The most enriched items of the KEGG pathway analysis of the up-regulated (STB VS MTB).

https://doi.org/10.2147/IDR.S540446

The microarray data has been uploaded to the Gene Expression Omnibus (GEO) database. Microarray profiles are accessible through the Gene Expression Omnibus database (GSE290321). All data are included in the article and its Supplementary Materials.

Patients with pulmonary TB were stratified STB group and MTB group according to disease severity. STB diagnosis required: (1)≥3 affected lung lobes on chest computed tomography (CT) scan, and (2) concurrent respiratory failure/hypoxemia;[15,30] MTB diagnosis required: (1)<3 affected lung lobes on chest CT, and (2) normal blood oxygenation status. Healthy controls were selected based on: (1) no *M.tb* exposure history, (2) negative tuberculin skin test and T-SPOT TB results, (3) unremarkable chest imaging findings, and (4) absence of comorbid conditions. STB patients were admitted from the ICU and emergency medicine department, while MTB patients were admitted from the general tuberculosis department. There was no significant difference in age, sex ratio among patients in the STB, MTB and HC groups (all P> 0.05; Table 1).

Peripheral blood samples (10mL, ethics committee-approved) were subjected to Ficoll-based PBMCs isolation through differential centrifugation using density gradient using Lympholyte Cell Separation Media (HY2015, Tianjin Haoyang Biological Manufacture Co., Ltd., China). The isolated PBMCs were lysed using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and stored at −80°C to prevent RNA degradation.

RNA Extraction and Purification

Total RNA was extracted and purified using miRNeasy Mini Kit (Cat#217004, QIAGEN, GmBH, Germany) following the manufacturer's instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US).

RNA Amplification and Labeling

Total RNA was amplified and fluorescently labeled using the Agilent Low Input Quick Amp Labeling Kit (Cat. # 5190–2305), with subsequent purification performed with QIAGEN RNeasy columns (Cat. # 74106). For microarray hybridization, 1.65μg of Cy3-labeled cRNA was applied to each array using Agilent's Gene Expression Hybridization Kit (Cat. # 5188–5242) in a standardized 17-hour protocol, followed by stringent washing with Agilent buffers (Cat. # 5188–5327) in Thermo Shandon staining chambers (Cat. # 121). All procedures strictly followed the manufacturers' specifications.

Data Acquisition

Array scanning was performed using an Agilent G2565CA microarray scanner with standard parameters (Green channel, 3μm resolution, 100% PMT, 20-bit depth). Raw intensity data were acquired with Agilent Feature Extraction 10.7 software and subsequently normalized using the Quantile method via limma package in R.

GO and KEGG Pathway Analysis

Gene Ontology analysis provided functional categorization of genes along molecular, cellular and process dimensions, with Fisher's test identifying enriched terms (P≤0.05). KEGG pathway mapping revealed the differentially expressed lncRNA-associated biological pathways meeting the same statistical threshold.

Table 1 Characteristics of the Microarray Cohort

	Characteristics	HC	STB	MTB	P-Value
Microarray	Age, years, mean ± SD	56.67±12.35	55.33±21.21	55.50±16.06	0.983[a]
	Gender(male), n	9(4)	6(4)	10(6)	0.679[b]

Notes: Age is presented as mean ± SD; [a] One-way ANOVA; [b] Chi-square test.
Abbreviations: HC, healthy control group; STB, severe pulmonary TB group; MTB, mild pulmonary TB group.

RNA Reverse Transcription and qPCR

RNA concentration and purity were assessed using NanoDrop 2000 spectrophotometry (Thermo Scientific). Reverse transcription was performed with the ReverTra Ace qPCR RT kit (TOYOBO, FSQ-101), followed by quantitative PCR analysis using PowerUp SYBR Green Master Mix (Thermo Fisher, A25742) on a 7500 Real-Time PCR system (Thermo Fisher Scientific).

The qPCR program (50 °C for 2-min, 95°C for 10-min, 40-cycles of 95 °C for 15-sec and 60 °C for 1-min, with the melting curve stage as an end) was run by the 7500 Real-Time PCR Instrument (Thermo Fisher Scientific) according to the manufacture instructions. GAPDH RNA was used as internal control. Relative lncRNAs levels were calculated by the $2^{(-\Delta\Delta Ct)}$ method, and melt curve analysis was performed to confirm non-specific amplification.[31] The basic information of lncRNAs is listed in Table 2.

Statistical Analysis

All results were analyzed with SPSS 26.0 software or Graphpad Pism9.5 software. The data was statistically analyzed and represented as means±standard deviations (SD), and the P value <0.05 was considered as statistically significant. The One-way ANOVA was used for quantitative variables (age) analysis and the chi-square test was used for qualitative variables (gender) analysis between the STB, MTB and HC group. The non-parametric Kruskal–Wallis test was used to compare continuous variables such as the qPCR results, which were performed and presented as means ± standard errors (SE) with Graphpad Pism9.5 software. Receiver Operating Characteristic (ROC) analysis was used to evaluate the diagnostic values of the lncRNAs biomarkers and were also performed with Graphpad Pism9.5 software.

Result

LncRNA Microarray Analysis results

A total of 58539 lncRNAs were identified in the STB, MTB and HC group. LncRNAs with a fold change (FC) >2 or <0.5 and the P value <0.05 were deemed as differentially expressed. Compared with the HC group, STB group have 2502 differentially expressed lncRNAs (1158 upregulated and 1344 downregulated lncRNAs).

The STB group demonstrated 931 significant lncRNA expression changes compared to the MTB group, comprising 514 elevated and 417 reduced transcripts. Transcriptomic analysis identified 577 significantly dysregulated lncRNAs in STB samples, comprising 382 upregulated and 195 downregulated transcripts relative to MTB and healthy controls. The heat maps of differentially expressed lncRNAs are shown in Figure 1.

Table 2 The Information of Top 14 Differentially Expressed lncRNAs Selected

Genes	p-Values	FC (STB/MTB)	Chromosome	Start	End	Relation	Associated Gene
NR_033841	0.0002796	0.0430297	Chr2	240954616	240967451	Exonic_antisense	CROCC2
Lnc-MRPL16-1:1	0.0027465	2.0383157	Chr11	59,794,110	59794716	Antisense lncRNA	STX3
Lnc-GBP3-2:1	0.0179420	2.1627879	Chr1	89058989	89060277	Sense_intronic ncRNA	GBP1
NR_038080	0.0312053	2.4920895	Chr17	81,302,823	81309248	Intergenic	TMEM105
ENST00000454631.1	0.0208038	2.6842020	Chr1	231522517	231528556	Antisense	TSNAX
NR_033909	0.0001466	3.7480859	Chr14	20,919,340	20920176	Exonic_sense	-
NR_029637	0.0014327	4.1523557	Chrx	66018869	66018979	Exonic_sense	VSIG4
NR_046173	0.0058821	5.2200778	Chr8	23084354	23103558	Intergenic	TNFRSF10C
NR_003087	0.0032647	5.4617903	Chr21	14,273,798	14301386	Exonic_sense	ABCC13
NR_003542	0.0038847	5.7127653	Chr4	184798295	184799046	Intronic_sense	SLED1
Lnc-PRDM7-1:2	0.0044455	6.3649484	Chr16	90,101,205	90106142	LincRNA	TUBB8P7
Lnc-MYCBPAP-2:4	0.0003417	6.4715091	Chr17	50,532,737	50536346	Sense_intronic ncRNA	EPN3
Lnc-ETS2-10:1	0.0040748	10.5681050	Chr21	38,273,475	38275519	Sense_intronic ncRNA	KCNJ15
Lnc-CHI3L1-2:3	0.0095542	18.3107425	Chr1	203212834	203218021	Sense_intronic ncRNA	CHIT1

https://doi.org/10.2147/IDR.S540446

GO and KEGG Analysis Results

The enrichment results of GO and KEGG analysis may precisely pinpoint the critical factors in the progression and exacerbation of tuberculosis. GO analysis showed that the upregulated lncRNAs were most significantly enriched in DNA−binding transcription factor activity, organelle membrane, and negative regulation of systemic arterial blood pressure in the STB group, in contrast to the MTB group, which may be linked to the aggravate of the disease. It is noteworthy that the GO analysis of upregulated lncRNAs is predominantly associated with autophagy, involves the NF-kappaB pathway (regulation of I-kappaB kinase/NF-kappaB signaling) and implicates immune responses (T-helper cell differentiation). The downregulated lncRNAs were most significantly enriched in the transcription by RNA polymerase II, intracellular membrane−bounded organelle, and DNA−binding transcription factor activity, RNA polymerase II-specific. Down-regulated lncRNAs are associated with autophagy and inflammatory pathways (negative regulation of interleukin−6 production). The GO analysis results are shown in Figure 1. The KEGG pathways terms associated with up-regulated lncRNAs in STB patients were Starch and sucrose metabolism, Taurine and hypotaurine metabolism, Pantothenate and CoA biosynthesis, and Mannose type O-glycan biosynthesis. The KEGG pathway terms associated with down-regulated lncRNAs in STB patients were Glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate, Glycosaminoglycan biosynthesis-heparan sulfate/heparin, and SNARE interactions in vesicular transport. The KEGG Pathway results are shown in Figure 1.

qPCR Verification Results

First, we selected differentially expressed lncRNAs using the thresholds of $p < 0.01$ and $|\log_2 FC| > 1$ in both the STB vs HC and STB vs MTB comparisons. Common differentially expressed lncRNAs shared between these two comparison sets were subjected to qPCR primer design. Ultimately, 14 lncRNAs that satisfied all the above criteria and could be successfully assayed with qualified primers were obtained for further qPCR validation. Compared with the MTB group, the expression levels of lncRNAs lnc-MYCBPAP-2:4 and NR_033841 were downregulated in the STB group ($p < 0.01$, $p = 0.0279$); the expression levels of lncRNAs NR_033909 and lnc-PRDM7-1:2 were upregulated in the STB group ($p < 0.01$, $p < 0.0001$).

Compared with the HC group, the expression levels of lncRNAs lnc-MYCBPAP-2:4 and NR_033841 were down-regulated in the STB group ($p < 0.0001$, $p< 0.0001$); the expression levels of lncRNAs NR_033909 and lnc-PRDM7-1:2 were upregulated in the STB group ($p < 0.0001$, $p < 0.0001$), as shown in Figure 2.

ROC Curve Analysis

ROC was utilized to assess the performance of lncRNAs in differentiating between various groups. The diagnostic model was constructed by 4 differentially expressed lncRNAs, which could distinguish between the STB group and the MTB group; the areas under the curve (AUC) value are respectively 0.7280(lnc-PRDM7-1:2), 0.7288(lnc-MYCBPAP-2:4), 0.6647(NR_033909) and 0.6615(NR_033841). Moreover, ROC curves showed that each lncRNA diagnostic model had a good ability to screen STB from healthy people distinctly.

Discussion

TB continues to be a major cause of morbidity and mortality globally despite being curable and preventable. Over the past 20 years, the rise of drug-resistant tuberculosis, particularly multidrug-resistant tuberculosis (MDR-TB; resistant to isoniazid and rifampin resistance) and extensively drug-resistant tuberculosis (XDR-TB; resistant to isoniazid and rifampin resistance, along with fluoroquinolones and ≥1 second-line injectable agent [eg, amikacin, kanamycin, or capreomycin]), has become evident. These forms are more challenging to treat than drug-susceptible disease.[32,33] MDR-TB and XDR-TB often lead to severe consequences such as acute respiratory distress syndrome (ARDS) and other complications due to difficulties in diagnosis and treatment.[33,34] Missing early recognition of TB's progression to severe disease is dangerous. A hasty but accurate diagnosis is necessary when it comes to TB infections.[34,35]

The World Health Organization also recognizes the importance of early and rapid identification of TB for intervention. The diagnosis of TB evolves from the sputum-smear microscopy to Xpert MTB/RIF assay, to Xpert MTB/RIF Ultra assay, to the moderate complexity automated NAATs class of tests, to the Truenat MTB, MTB Plus and MTB-RIF Dx assays, reducing detection time frames and expanding disease information step by step, such as drug resistance.[36]

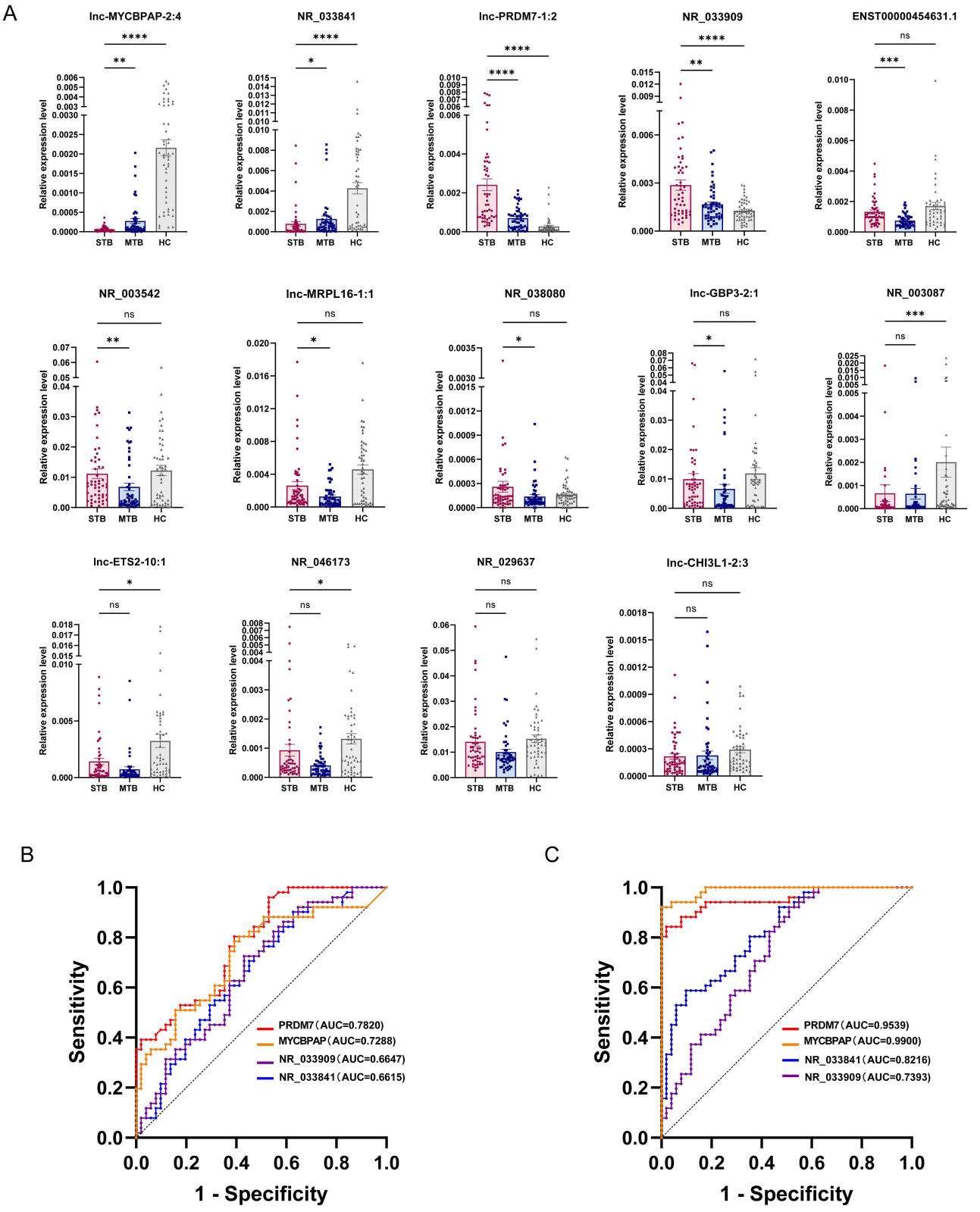

Figure 2 Validation of the differentially expressed lncRNAs. (**A**) Validation results by qPCR (*p<0.05,**p<0.01, ***p<0.001, ****p<0.0001). (**B**) The ROC curve discriminating STB from MTB (lncRNAs lnc-MYCBPAP-2:4, NR_033841, NR_033909 and lnc-PRDM7-1:2). (**C**) The ROC curve discriminating STB from HC.

https://doi.org/10.2147/IDR.S540446

However, the diagnosis of TB rarely involves the identification of severe degree. There is a lack of research in the field of severe tuberculosis. Our ceRNA Microarray study identified potential lncRNAs in severe TB patients, which may aid in developing diagnostic biomarkers.

Using ceRNA microarray platforms, we systematically compared PBMC-derived lncRNA profiles among STB patients, MTB patients, and HCs. We identified a total of 58539 lncRNAs in the STB group. Analysis of differentially expressed lncRNAs GO and KEGG enrichment pathways may be associated with TB disease progression. Finally, lncRNAs NR_033909, lnc-MYCBPAP-2:4, lnc-PRDM7-1:2 and NR_033841 distinctively expressed in STB patients and were selected for diagnosis model. The AUC peaked at 0.7820(lnc-PRDM7-1:2). The four selected lncRNAs have not been previously reported in the existing literature. However, GO analysis and KEGG analysis indicate that TB-associated lncRNAs could modulate critical disease mechanisms (inflammation/immunity/metabolism/autophagy), suggesting their functional involvement in tuberculosis progression through distinct molecular pathways. The GO analysis of upregulated lncRNAs involves the NF-kappaB pathway. NF-κB signaling activation constitutes an essential host defense mechanism for pathogen clearance. Research indicates that lincRNA-Cox2 and lincRNA-AK170409 are regulators of the NF-kappaB pathway.[37,38] The dysregulated lncRNAs in this study are associated with immunity and Yi Wang's team discovered through single-cell RNA sequencing the immune response in patients that the immune response in STB patients significantly differs from others. Severe cases exhibited increased frequencies of pro-inflammatory monocytes alongside reduced lymphocyte populations (particularly NK and γδT cells), indicating potential lymphopenia as a characteristic immunopathological feature. Significant activation of cell apoptosis pathways and cell migration pathways was observed, leading to reduced lymphocyte abundance. The immune microenvironment in severe presentations demonstrated exhausted Th1, CD8+T, and NK cells, along with high cytotoxicity in CD8+T and NK cells.[39] Our study found that both upregulated and down-regulated lncRNA GO analysis were associated with autophagy, which was consistent with the results of existing studies. Mingying Li and colleagues' research indicates that LncRNA PCED1B-AS1 regulates macrophage apoptosis and autophagy through miR-155-mediated signaling pathways in active TB.[40]

Currently, there is limited research on the specific 14 lncRNAs selected in our study. Dong-liang Chen et al revealed that NR_033841(lncRNA UICLM) drives colorectal cancer liver metastasis by competitively sponging miR-215 to upregulate ZEB2 expression. NR_033841 exerts a strong pro-tumorigenic effect, significantly promoting the invasive and metastatic capabilities of colorectal cancer cells.[41] Yanming Lin et al demonstrated that silencing NR_038080 (LINC00482) was found to sensitize non-small cell lung cancer (NSCLC) cells to cisplatin chemotherapy via the E2F1/CLASRP signaling axis.[42] Wenwen Xu et al identified NR_038080 (LINC00482) as a key mediator in the brain metastasis of NSCLC, which reprograms microglia towards a tumor-promoting M2 phenotype.[43] NR_029637(miR-223) has been confirmed that it can regulate key immune processes (including neutrophil infiltration, macrophage function, dendritic cell maturation, and inflammasome activation) and its dysregulation is implicated in infectious diseases such as viral hepatitis, HIV-1, and tuberculosis.[44,45] Yu Zhang et al concluded that a set of four lncRNA-mRNA pairs, such as NR_046173 (LOC254896)-TNF receptor superfamily member 10c, have a pivotal regulatory role in governing the process of bone metastasis in breast cancer.[46] Xie Hong-Yuan et al implicated differentially expressed lncRNAs in ankylosing spondylitis development, among which NR_003542 shows particular promise as a potential indicator of disease activity.[47] We find most lncRNAs are well-established key regulators in cancer biology, where they modulate cell proliferation, apoptosis, and immune evasion. Notably, these very same processes-apoptosis, immune response, and metabolic reprogramming, are also central to the host-pathogen interaction during infectious diseases.

Many studies have already shown that lncRNAs are emerging as promising molecular biomarkers and therapeutic targets for tuberculosis. Jing Dong et al discovered that lncRNAs ABHD17B and ENST00000607464.1 were efficient in distinguishing TB patients from HCs.[48] Guoli Li et al identified eight specific lncRNAs in TB patients and latent infections as potential markers for active pulmonary tuberculosis.[49] Taosheng Ye et al found that the expression of lncRNA CCAT1 was associated with the survival of TB patients.[16] Wenna Sun et al confirmed that lncRNA NORAD is a potential diagnostic biomarker for PTB and is involved in *M.tb*-infected macrophage activity and inflammation by targeting miR-618.[50] Xuejiao Hu et al combined lncRNAs and corresponding predictive models to diagnose clinically diagnosed pulmonary tuberculosis patients and achieved good results.[51]

STB patients often have complex conditions, making treatment extremely challenging. ARDS and acute hypoxemic respiratory failure emerged as the predominant complications necessitating intensive care unit transfer. High mortality rates have recently been reported in patients with acute respiratory failure arising from TB.[52] The literature indicates that the mortality rate among severe pulmonary tuberculosis patients admitted to the ICU is high, with an average of 52.9% across all studies and some reports showing rates exceeding 90%.[53] Delays in diagnosis and treatment led to disease progression into severe stages, which in turn resulted in worse patient outcomes. What's more, the severity of TB varies, and the disease is complex, involving nutritional status, oxygenation, complexity of infection, pregnancy and other aspects.[54–57] The introduction of novel therapies and precision ventilation in ICUs has not overcome two fundamental barriers: the diverse spectrum of STB presentations and persistent diagnostic limitations.[33,58,59] These complexities underscore the need for continued research and innovative diagnostic tools to improve patient outcomes.

Limitations

We analyze tuberculosis cases meeting severity criteria based on life-threatening clinical manifestations and requirement for escalated care protocols. Different definitions of the severity of tuberculosis may affect the comparability of results across various studies. In addition, this study is a single-center study, which may introduce selection bias. Furthermore, the sample size is relatively small, and the functional studies of verified lncRNAs need further validation through basic experiments.

Conclusions

Treatment of STB remains a significant challenge in the field of TB prevention and control, due to the complexity of patient conditions and the associated difficulties in treatment. Patients with STB tend to develop MDR-TB or XDR-TB, suffer from severe infections, and often necessitate respiratory support. The current research lacks a unified criterion for diagnosing severe pulmonary tuberculosis and seldom involves its molecular immunology aspects. This study takes the newly emerged lncRNA as the breakthrough point to investigate the expression of specific lncRNA in patients with severe pulmonary tuberculosis. In summary, this study identified four potential lncRNA biomarkers for early recognizing STB from MTB patients and HCs, which may serve as potential biomarkers to assess TB disease progression and provide the basis for the target treatment of TB.

Abbreviations

TB, Tuberculosis; *M.tb*, Mycobacterium tuberculosis; TB, tuberculosis; ICU, intensive care unit; lncRNAs, long non-coding RNAs; PBMC, peripheral blood monocyte; STB, severe pulmonary TB; MTB, mild pulmonary TB; HC, healthy controls; qPCR, quantitative real-time PCR; GEO, Gene Expression Omnibus; CT, chest computed tomography; SD, means ± standard deviations; SE, means ± standard errors; ROC, Receiver Operating Characteristic; FC, fold change; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; AUC, areas under the curve; ARDS, Acute Respiratory Distress Syndrome; NSCLC, non-small cell lung cancer.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials. The qPCR data can be found in the Supplementary Materials. The microarray data has been uploaded to the Gene Expression Omnibus (GEO) database. GEO Series accession number is GSE290321.

Ethics Approval and Consent to Participate

The project was authorized by the Ethical Committees of the Chinese Clinical Trial Registry, the number of the approval: ChiCTR2000032719 and written informed consents were obtained from all participants and/or their legal guardians.

Consent for Publication

This manuscript is approved by all authors for publication.

Acknowledgments

The authors of this study extend their gratitude to the Beijing Chest Hospital Affiliated with Capital Medical University for their support and to all the participants for their involvement.

Funding

The research was funded by the National Natural Science Foundation of China (82100011).

Disclosure

The authors declare that they have no competing interests in this work.

References

1. World Health Organization. World health statistics 2024: monitoring health for the SDGs, sustainable development goals. Available from: https://www.who.int/publications/i/item/9789240094703. Accessed September 8, 2025. 2024.
2. Liu Y, Pang Y, Du J, et al. An Overview of Tuberculosis-Designated Hospitals in China, 2009-2015: a Longitudinal Analysis of National Survey Data. *BioMed Res Int*. 2019;2019:1–8. doi:10.1155/2019/9310917
3. Barberis NL, Bragazzi L, Galluzzo M, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. *J Prev Med Hyg*. 2017;58(1):E9–E12.
4. Louie JK, Agraz-Lara R, Romo L, Crespin F, Chen L, Graves S. Tuberculosis-Associated Hospitalizations and Deaths after COVID-19 Shelter-In-Place, San Francisco, California, USA. *Emerg Infect Dis*. 2021;27(8):2227–2229. doi:10.3201/eid2708.210670
5. World Health Organization. Global Tuberculosis Report 2024. 2024. Available from: https://www.who.int/publications/i/item/9789240101531. Accessed Sept 08, 2025.
6. Waitt CJ, Squire SB. A systematic review of risk factors for death in adults during and after tuberculosis treatment [Review article]. *Int J Tuberc Lung Dis*. 2011;15(7):871–885. doi:10.5588/ijtld.10.0352
7. Rp D, Figueiredo Dias P, Ferreira AA, et al. Severe Tuberculosis Requiring Intensive Care: a Descriptive Analysis. *Crit Care Res Pract*. 2017;2017:1–9. doi:10.1155/2017/9535463
8. Kim S, Kim H, Kim WJ, et al. Mortality and predictors in pulmonary tuberculosis with respiratory failure requiring mechanical ventilation. *Int J Tuberc Lung Dis*. 2016;20(4):524–529. doi:10.5588/ijtld.15.0690
9. Nahid P, Mase SR, Migliori GB, et al. Treatment of Drug-Resistant Tuberculosis. An Official ATS/CDC/ERS/IDSA Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2019;200(10):e93–e142. doi:10.1164/rccm.201909-1874ST
10. Schnippel K, Ndjeka N, Maartens G, et al. Effect of bedaquiline on mortality in South African patients with drug-resistant tuberculosis: a retrospective cohort study. *Lancet Respir Med*. 2018;6(9):699–706. doi:10.1016/s2213-2600(18)30235-2
11. Borisov SE, Dheda K, Enwerem M, et al. Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR- and XDR-TB: a multicentre study. *Eur Respir J*. 2017;49(5):1700387. doi:10.1183/13993003.00387-2017
12. Koirala S, Borisov S, Danila E, et al. Outcome of treatment of MDR-TB or drug-resistant patients treated with bedaquiline and delamanid: results from a large global cohort. *Pulmonology*. 2021;27(5):403–412. doi:10.1016/j.pulmoe.2021.02.006
13. World Health Organization. WHO Global Tuberculosis Report. 2023, Available from: https://www.who.int/publications/i/item/9789240083851. Accessed Sept 08, 2025.
14. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment, 2022 update. 2022. Available from: https://www.who.int/publications/i/item/9789240063129. Accessed Sept 08, 2025.
15. Wang L, He Y, Wang P, Lou H, Liu H, Sha W. Single-cell transcriptome sequencing reveals altered peripheral blood immune cells in patients with severe tuberculosis. *Eur J Med Res*. 2024;29(1):2. doi:10.1186/s40001-024-01991-5
16. Ye T, Zhang J, Zeng X, Xu Y, Li J. LncRNA CCAT1 is overexpressed in tuberculosis patients and predicts their survival. *Immun Inflamm Dis*. 2021;10(2):218–224. doi:10.1002/iid3.565
17. Denkinger CM, Kik SV, Cirillo DM, et al. Defining the Needs for Next Generation Assays for Tuberculosis. *J Infect Dis*. 2015;211(Suppl 2):S29–S38. doi:10.1093/infdis/jiu821
18. Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: from biomarkers to therapeutic targets. *Int J Cancer*. 2016;140(9):1955–1967. doi:10.1002/ijc.30546
19. Xia J, Liu Y, Ma Y, et al. Advances of Long Non-Coding RNAs as Potential Biomarkers for Tuberculosis: new Hope for Diagnosis? *Pharmaceutics*. 2023;15(8):2096. doi:10.3390/pharmaceutics15082096
20. Zhang X, Liang Z, Zhang Y, et al. Comprehensive analysis of long non-coding RNAs expression pattern in the pathogenesis of pulmonary tuberculosis. *Genomics*. 2020;112(2):1970–1977. doi:10.1016/j.ygeno.2019.11.009
21. Li Z, Han Y, Wei L, et al. Screening and identification of plasma lncRNAs uc.48+ and NR_105053 as potential novel biomarkers for cured pulmonary tuberculosis. *Int J Infect Dis*. 2020;92:141–150. doi:10.1016/j.ijid.2020.01.005
22. Yang X, Yang J, Wang J, et al. Microarray analysis of long noncoding RNA and mRNA expression profiles in human macrophages infected with Mycobacterium tuberculosis. *Sci Rep*. 2016;6(1):38963. doi:10.1038/srep38963
23. Colditz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA*. 1994;271(9):698–702. doi:10.1001/jama.1994.03510330076038
24. Gopalaswamy R, Subbian S. An Update on Tuberculosis Vaccines, Vaccine Design. *Methods in Molecular Biology (Clifton, N.J.)*. 2022;2410:387–409. doi:10.1007/978-1-0716-1884-4_20
25. Kaufmann SHE. Fact and fiction in tuberculosis vaccine research: 10 years later. *Lancet Infect Dis*. 2011;11(8):633–640. doi:10.1016/s1473-3099(11)70146-3

26. McShane H. Insights and challenges in tuberculosis vaccine development. *Lancet Respir Med*. 2019;7(9):810–819. doi:10.1016/s2213-2600(19)30274-7
27. Liu Q, Pan L, Han F, et al. Proteomic profiling for plasma biomarkers of tuberculosis progression. *Mol Med Rep*. 2018;2018:9134. doi:10.3892/mmr.2018.9134
28. Liu Q, Li R, Li Q, Luo B, Lin J, Lyu L. High levels of plasma S100A9 at admission indicate an increased risk of death in severe tuberculosis patients. *J Clin Tuberc Other Mycobact Dis*. 2021;25. doi:10.1016/j.jctube.2021.100270.
29. World Health Organization. International Standards for Tuberculosis Care (ISTC). 2006. Available from: https://www.who.int/publications/m/item/international-standards-for-tuberculosis-care-istc. Accessed Sept 08, 2025.
30. Lichtenstein DA, Mezière GA. Relevance of Lung Ultrasound in the Diagnosis of Acute Respiratory Failure*: the BLUE Protocol. *Chest*. 2008;134(1):117–125. doi:10.1378/chest.07-2800
31. Fang Y, Zhao J, Wang X, et al. Identification of differentially expressed lncRNAs as potential plasma biomarkers for active tuberculosis. *Tuberculosis*. 2021;128:102065. doi:10.1016/j.tube.2021.102065
32. Lewinsohn DM, Leonard MK, Lo Bue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of Tuberculosis in Adults and Children. *Clin Infect Dis*. 2017;64(2):e1–e33. doi:10.1093/cid/ciw694
33. Tiberi S, Torrico MM, Rahman A, et al. Managing severe tuberculosis and its sequelae: from intensive care to surgery and rehabilitation. *J Bras Pneumol*. 2019;45(2). doi:10.1590/1806-3713/e20180324
34. Shukla P, Lubrin S, Subramanian R, Joshi H. Severe Ards and Immune Reconstitution Syndrome in a Rare Case of Multidrug-Resistant Tuberculosis: a Case Report and Review. *Chest*. 2023;164(4):A1514. doi:10.1016/j.chest.2023.07.1041
35. Lee J, Nam HW, Choi SH, et al. Comparison of Early and Late Tuberculosis Deaths in Korea. *J Korean Med Sci*. 2017;32(4):3. doi:10.3346/jkms.2017.32.4.700
36. World Health Organization. *WHO Operational handbook on Tuberculosis. Module 3: Diagnosis - Rapid Diagnostics for Tuberculosis Detention*. 2021.
37. Covarrubias S, Robinson EK, Shapleigh B, et al. CRISPR/Cas-based screening of long non-coding RNAs (lncRNAs) in macrophages with an NF-κB reporter. *J Biol Chem*. 2017;292(51):20911–20920. doi:10.1074/jbc.M117.799155
38. Kundu M, Basu J. The Role of microRNAs and Long Non-Coding RNAs in the Regulation of the Immune Response to Mycobacterium tuberculosis Infection. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.687962.
39. Wang Y, Sun Q, Zhang Y, et al. Systemic immune dysregulation in severe tuberculosis patients revealed by a single-cell transcriptome atlas. *J Infect*. 2023;86(5):421–438. doi:10.1016/j.jinf.2023.03.020
40. Li M, Cui J, Niu W, et al. Long non-coding PCED1B-AS1 regulates macrophage apoptosis and autophagy by sponging miR-155 in active tuberculosis. *Biochem Biophys Res Commun*. 2019;509(3):803–809. doi:10.1016/j.bbrc.2019.01.005
41. Chen D-L, Lu Y-X, Zhang J-X, et al. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression. *Theranostics*. 2017;7(19):4836–4849. doi:10.7150/thno.20942
42. Lin Y, Li J, Li S, et al. Long noncoding RNA LINC00482 silencing sensitizes non-small cell lung cancer cells to cisplatin by downregulating CLASRP via E2F1. *Funct Integrat Genomics*. 2023;23(4). doi:10.1007/s10142-023-01260-4
43. Xu W, Patel N, Deng Y, Ding S, Wang T, Zhang H. Extracellular vesicle-derived LINC00482 induces microglial M2 polarization to facilitate brain metastasis of NSCLC. *Cancer Lett*. 2023;561:216146. doi:10.1016/j.canlet.2023.216146
44. Yuan S, Wu Q, Wang Z, et al. miR-223: an Immune Regulator in Infectious Disorders. *Front Immunol*. 2021;12. doi:10.3389/fimmu.2021.781815
45. Jiao P, Wang X-P, Luoreng Z-M, et al. miR-223: an Effective Regulator of Immune Cell Differentiation and Inflammation. *Int J Biol Sci*. 2021;17(9):2308–2322. doi:10.7150/ijbs.59876
46. Zhang Y, Huang X, Liu J, et al. New insight into long non-coding RNAs associated with bone metastasis of breast cancer based on an integrated analysis. *Cancer Cell Int*. 2021;21(1):1. doi:10.1186/s12935-021-02068-7
47. Hong-Yuan X, Yi-Ping T, Ting Y, et al. Study on the Expression and Potential Function of LncRNA in Peripheral Blood of Patients with Ankylosing Spondylitis. *Curr Rheumatol Rev*. 2024;20(5):544–554. doi:10.2174/0115733971283982240118045203
48. Dong J, Song R, Shang X, et al. Identification of important modules and biomarkers in tuberculosis based on WGCNA. *Front Microbiol*. 2024;15:1354190. doi:10.3389/fmicb.2024.1354190
49. Li G, Feng Z, Song H, Wang Y, Zhu L, Li Y. Long non-coding RNA expression in PBMCs of patients with active pulmonary tuberculosis. *Front Microbiol*. 2023;14:1257267. doi:10.3389/fmicb.2023.1257267
50. Sun W, He X, Zhang X, et al. Diagnostic value of lncRNA NORAD in pulmonary tuberculosis and its regulatory role in Mycobacterium tuberculosis infection of macrophages. *Microbiol Immunol*. 2022;66(9):433–441. doi:10.1111/1348-0421.12986
51. Hu X, Liao S, Bai H, Gupta S. Long Noncoding RNA and Predictive Model To Improve Diagnosis of Clinically Diagnosed Pulmonary Tuberculosis. *J Clin Microbiol*. 2020;58(7). doi:10.1128/JCM
52. Kim YJ, Pack KM, Jeong E, et al. Pulmonary tuberculosis with acute respiratory failure. *Eur Respir J*. 2008;32(6):1625–1630. doi:10.1183/09031936.00070907
53. Galvin J, Tiberi S, Akkerman O, et al. Pulmonary tuberculosis in intensive care setting, with a focus on the use of severity scores, a multinational collaborative systematic review. *Pulmonology*. 2022;28(4):297–309. doi:10.1016/j.pulmoe.2022.01.016
54. Sabelli RG, Chediack V, Cunto MS, et al. Severe HIV-associated pulmonary Tuberculosis: 2006-2016. *Int J Infect Dis*. 2018;73:351. doi:10.1016/j.ijid.2018.04.4210
55. Vonasek BJ, Radtke KK, Vaz P, et al. Tuberculosis in children with severe acute malnutrition. *Expert Rev Respir Med*. 2022;16(3):273–284. doi:10.1080/17476348.2022.2043747
56. Orazulike N, Sharma JB, Sharma S, Umeora OUJ. Tuberculosis (TB) in pregnancy – a review. *Eur J Obstet Gynecol Reprod Biol*. 2021;259:167–177. doi:10.1016/j.ejogrb.2021.02.016
57. Atalell KA, Haile RN, Techane MA. Magnitude of tuberculosis and its associated factors among under-five children admitted with severe acute malnutrition to public hospitals in the city of Dire Dawa, Eastern Ethiopia, 2021: multi-center cross-sectional study. *IJID Regions*. 2022;3:256–260. doi:10.1016/j.ijregi.2022.04.008
58. Murthy PR, K AKA, N N, G KVV. Adolescent tuberculosis in the ICU. *Indian J Tuberc*. 2023;70:S24–S8. doi:10.1016/j.ijtb.2023.06.020
59. Chaudhry D, Tyagi D. Tuberculosis in Intensive Care Unit. *Indian J Crit Care Med*. 2021;25(S2):S150–S4. doi:10.5005/jp-journals-10071-23872