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Circular RNA as Diagnostic Biomarker for Active Tuberculosis: A Systematic Review

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Abstract

Circular RNAs (circRNAs) have emerged as promising candidates in various diagnostic applications due to their stability and specificity. Their potential role in diagnosing active pulmonary tuberculosis (APTB) warrants systematic evaluation. This systematic review aims to assess the potential of circRNAs as diagnostic biomarkers for APTB. A comprehensive literature search was conducted using PubMed and Scopus databases. Articles were screened based on relevance and quality, including ten studies for final analysis. The reviewed studies indicate that specific circRNAs are differentially expressed in APTB patients compared to healthy controls. These circRNAs were found to be dysregulated in APTB patients and might correlate with disease severity. The diagnostic accuracy was high, with area under the receiver operating characteristic curve (AUC) values ranging from 0.773 to 0.974. These findings collectively suggest that circRNAs have the potential to serve as reliable diagnostic markers for tuberculosis. Their high diagnostic accuracy highlights their promise in improving early detection and patient management of APTB. Further studies are recommended to validate these findings and explore their practical applications in clinical settings.

Keywords: biomarker, circRNA, molecular diagnostic techniques, tuberculosis

INTRODUCTION

Tuberculosis (TB) continues to be a prominent worldwide health issue, with an estimated 10 million new cases and 2 million fatalities reported each year, establishing it as the most lethal infectious disease globally.[1] High-burden countries, predominantly in low- and middle-income regions with low socio-demographic indices, face significant challenges in TB control, including socio-economic factors that worsen the transmission of TB and the emergence of drug-resistant strains such as multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB).[1] Patients often encounter barriers to timely diagnosis and treatment, including long distances to healthcare facilities, high costs, stigma, and operational challenges in resource-limited settings, which hinder effective TB control efforts.[2]

Effective diagnostics are essential for timely treatment and control of TB, yet current methods such as sputum microscopy, tuberculin skin tests (TST), and molecular tests like GeneXpert have significant limitations.[3] Sputum microscopy has a sensitivity of only around 35-70%, particularly in patients with low-bacterial-loads, and requires specialized laboratory infrastructure. Tuberculin skin tests cannot distinguish between latent and active TB, and cross-reactivity with the Bacillus Calmette-Guérin (BCG) vaccine leads to false positives.[3,4]

Although GeneXpert provides rapid results with a sensitivity of 90.91%, its reliance on sputum samples presents a significant limitation, particularly in patients who struggle to produce sputum, such as children, or those with low bacterial loads. Sputum is the standard specimen for both conventional and molecular diagnostics, yet it is often difficult or impossible to obtain, leading to diagnostic delays and increased mortality. These challenges highlight the urgent need for non-sputum-based biomarkers that are more accessible and reliable across diverse patient populations.[3,4] One promising alternative is the identification of blood-based biomarkers, such as host gene expression signatures, which could enable non-sputum-based testing.

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Circular RNAs (circRNAs) are considered a highly promising group of biomarkers because of their distinctive covalently closed-loop structure. This structure renders them exceptionally robust and resistant to degradation, surpassing linear RNAs.[5] These non-coding RNAs exhibit diverse biological functions, including acting as microRNA sponges, binding proteins, regulating transcription, and even producing proteins through cap-independent translation.[6] Recent studies have shown that circRNAs exhibit altered expression in TB patients and play roles in disease pathogenesis by influencing host immune responses and interacting with microRNAs.[7–9] For instance, circAGFG1 and circZNF277, are expressed in macrophages infected with *Mycobacterium tuberculosis* (Mtb), playing a role in disease pathogenesis by influencing autophagy and apoptosis pathways.[10,11] Their stability, specificity, and presence in various bodily fluids offer significant advantages over traditional biomarkers, presenting opportunities for developing circRNA-based diagnostic tools that are rapid, non-invasive, and highly sensitive.[5]

Despite the availability of conventional diagnostic tools, current approaches still face several limitations and challenges in clinical practice. These limitations hinder early detection and effective disease management, contributing to ongoing transmission and poor patient outcomes. In this context, the exploration of novel molecular biomarkers such as circular RNAs (circRNAs) offers a promising alternative. The integration of circRNAs into TB diagnostic algorithms could revolutionize TB management by addressing current diagnostic gaps and enabling more precise, timely, and accessible detection strategies.[10]

This systematic review aims to identify and analyse existing studies on the role of circRNAs as diagnostic biomarkers for active TB, evaluate their potential in enhancing TB detection, and suggest future research directions. By providing robust evidence for the clinical utility of circRNAs, this study seeks to influence health policies and clinical practices, ultimately contributing to improved TB management and control efforts globally.

METHODS

Protocol and reporting standards

The present systematic review was carried out in accordance with the recommendations specified in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2015.[11] The protocol was prepared to ensure transparency, reproducibility, and a systematic approach in evaluating the role of circRNAs as diagnostic biomarkers for active pulmonary tuberculosis (APTB).

Inclusion and exclusion criteria

The review's inclusion criteria were: (1) studies that analyzed circRNAs in patients with APTB; (2) studies that compared APTB samples with control samples; (3) studies that used circRNA expression arrays, polymerase chain reaction (PCR), or RNA-sequencing techniques; and (4) studies that provided sample sizes. The evaluation specifically omitted entries with the following categories: reviews, letters, editorials, case reports, and case series studies.

Participants

This review included studies involving patients diagnosed with active pulmonary tuberculosis through various diagnostic methods, including sputum analysis, radiological assessment, PCR, or clinical TB diagnosis.

Data sources and search strategy

A comprehensive search was performed in the PubMed and Scopus databases to locate pertinent original papers published in the English language until August 10, 2024. There was no lower date limit set for the search. The search syntax used was a combination of the following terms: a) "circular RNA" OR "circRNA"; and b) "Tuberculosis" OR "*Mycobacterium tuberculosis*" OR "Koch Disease".

Screening of studies

Titles and abstracts retrieved from the electronic search were screened, and duplicates were removed.

Studies deemed irrelevant based on the title and abstract review were excluded. Full-text screening was then conducted on the remaining articles to ensure that they met the inclusion criteria.

Data extraction

Detailed information was obtained from each eligible study, encompassing: (1) study attributes such as authorship, publication year, country, specimen type, sample size, number of cases and controls, age, and sex; and (2) clinical data including circRNA targets, expression patterns, sensitivity, specificity, area under the curve (AUC), and assumed functions.

Risk of bias assessment

The studies included in the analysis were evaluated for quality and risk of bias using the QUADAS-2 instrument. This technique assesses the methodological correctness and reliability of diagnostic accuracy studies.

RESULTS

From the initial search, 151 articles were identified (Figure 1). Thirty-four articles were duplicates hence removed. There were 104 articles fallen into exclusion criteria such as reviews and editorial. Two articles were removed because of wrong study designs and one article was removed because of unavailable full-text. All ten included studies were conducted in China and utilised various specimen types, including plasma and peripheral blood mononuclear cells (PBMCs). The study participants were predominantly young adults, and detailed characteristics of each study are presented in Table 1.

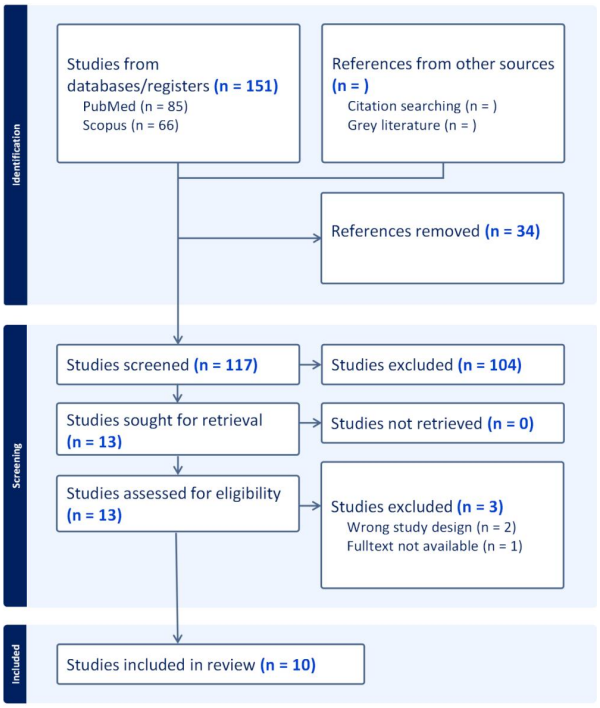
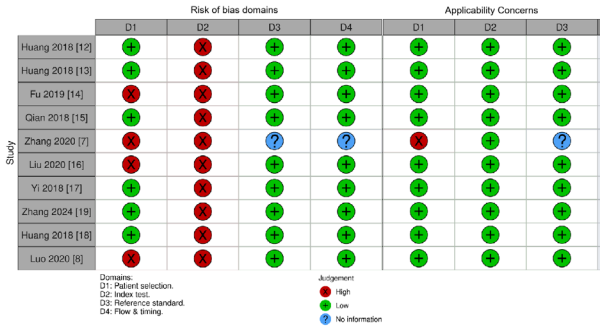


Figure 1. A flow diagram demonstrating the study selection process

Table 1. Characteristics of human circRNA expression studies

Study	Location	Sampel Size (Case/ Control)	Specimen	Age (years)		Gender (Male/ Female)	
				Case	Control	Case	Control
Huang 2018[12]	China	170/150	plasma	44.5 ± 15.8	44.5 ± 15.8	115/55	98/52
Huang 2018[13]	China	155/130	PBMCs	43.7 ± 14.5	43.7 ± 14.5	108/47	85/55
Fu 2019[14]	China	31/30	PBMCs	38.9±15.7	38.9±15.7	17/14	16/14
Qian 2018[15]	China	11/10	PBMCs	32.7±14.2	32.7±14.2	7/4	7/3
Zhang 2020[7]	China	20/20	PBMCs	-	-	-	-
Liu 2020[16]	China	128/50	serum	43.5 ± 19.84	43.5 ± 19.84	87/41	29/21
Yi 2018[17]	China	32/29	plasma	36.1±13.4	36.1±13.4	17/15	15/14
Zhang 2024[19]	China	30/32	PBMCs	41.88 ± 19.57	41.88 ± 19.57	16/14	14/18
Huang 2018[18]	China	145/120	plasma	40.6±13.9	40.6±13.9	95/50	78/42
Luo 2020[8]	China	32/31	PBMCs	36.03±13.96	36.03±13.96	22/10	20/11

The risk of bias within the studies was assessed using the QUADAS-2 tool, revealing specific levels of bias in patient selection, index tests, reference standards, as well as flow and timing, as summarised in Figure 2.



Gambar 2. Results of quality assessment according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)

Table 2 provides an overview of the circRNA biomarkers identified as potential diagnostic tools for APTB, highlighting their associated functions. Several studies have identified specific circRNAs with altered expression in tuberculosis patients, many showing strong diagnostic performance with AUC values ranging from 0.773 to 0.974. Some circRNAs also demonstrate potential functional roles in TB pathogenesis, such as modulating immune responses or influencing bacterial proliferation. The functional descriptions listed in the table were derived directly from the same studies cited for each circRNA, based on the authors' bioinformatic analyses and experimental findings. These findings support the promise of circRNAs as non-sputum-based biomarkers for TB diagnosis.

Table 2. Significantly dysregulated circRNAs in APTB

Study	CircRNA	Pattern	Sensitivity	Specificity	AUC (Lower-Upper)	Function
Huang 2018[12]	hsa_circ_0001953	↗	69.17%	89.00%	0.826 (0.770-0.881)	N/A
Huang 2018[12]	hsa_circ_0009024	↗	60.00%	86.00%	0.777 (0.716-0.838)	hsa_circ_0009024 has the ability to interact with miR-6844, miR-1248, miR-141-5p, miR-545-5p, and miR-95-5p.
Huang 2018[13]	hsa_circ_001937	↗	72.20%	90.00%	0.850 (0.796-0.903)	hsa_circRNA_001937 may target miR-22-5p, miR-26b-3p, miR-10b-3p, miR-376a-5p, and miR-597-3p, through targeting PTEN, MiR-26b modulates the NF-kB pathway and hence contributes to the inflammatory response.
Fu 2019[14]	hsa_circ_103017	↗	N/A	N/A	0.87 (0.779-0.961)	N/A
Fu 2019[14]	hsa_circ_059914	↗	N/A	N/A	0.821 (0.714-0.928)	N/A
Fu 2019[14]	hsa_circ_101128	↗	N/A	N/A	0.817 (0.712-0.922)	hsa_circRNA_101128 may have a role in the development of active tuberculosis by inhibiting let-7a. By modulating the NF-kB pathway, Let-7 can govern the immunological response to Mtb infection and restrict bacterial proliferation.

Qian 2018[15]	7-circRNA signature (hsa_circ_0000414, hsa_circ_0000681, hsa_circ_0002113, hsa_circ_0002362, hsa_circ_0002908, hsa_circ_0008797, hsa_circ_0063179)	↗	N/A	N/A	0.946	N/A
Zhang 2020[7]	hsa_circ_0028883	↗	N/A	N/A	0.773 (0.630-0.920)	hsa_circ_0028883 may contribute to infection and TB pathogenesis by sponging miR-409. miR-409 plays a role in the generation of inflammatory cytokines triggered by IL-17 and in diseases caused by Kaposi's sarcoma-associated herpesvirus (KSHV).
Liu 2020[16]	hsa_circ_051239	↗	N/A	N/A	0.974 (0.958-0.989)	circRNA_051239 may act as a sponge for miR-320a, therefore releasing the target genes of miR-320a and perhaps contributing significantly to the development of treatment resistance in tuberculosis.
Liu 2020[16]	hsa_circ_029965	↗	N/A	N/A	0.944 (0.917-0.972)	N/A
Liu 2020[16]	hsa_circ_404022	↗	N/A	N/A	0.968 (0.949-0.987)	N/A
Yi 2018[17]	hsa_circ_103571	↙	N/A	N/A	0.838 (0.734-0.941)	Bioinformatics investigation identified hsa_circRNA_103571 as mainly implicated in the ras signalling pathway, actin cytoskeleton modulation, and T- and B-cell receptor signalling networks.

Zhang 2024[19]	hsa_circ_0002371	↗	N/A	N/A	0.806 (0.691-0.922)	hsa_circ_0002371 enhances the proliferation of BCG within cells while suppressing the process of autophagy in macrophages infected with BCG and suppressed the expression of hsa-miR-502-5p on ATG16L1 to control the proliferation of BCG and autophagy in macrophages.
Huang 2018[18]	hsa_circ_0001204	↙	73.10%	92.50%	0.871 (0.827-0.916)	hsa_circ_0001204 targets several miRNAs, including miR-612, miR657, miR-362-3p, miR-377-3p, and miR-136-5p.
Huang 2018[18]	hsa_circ_0001747	↙	71.03%	82.50%	0.830 (0.780-0.880)	hsa_circ_0001747 targets several miRNAs including miR-616-5p, miR-30d-3p, miR-320b, miR-320a, and miR-302c-5p.
Luo 2020[8]	hsa_circ_0001380	↙	93.75%	87.5%	0.950 (0.888-1.000)	hsa_circ_0001380 targets several miRNAs including hsa-miR-622 and hsa-miR-136-5p.

Arrows indicate expression pattern: upregulation (↗); downregulation (↙). N/A: No information available from the paper.

DISCUSSION

The findings demonstrated significant diversity in the discovered circRNAs, with multiple transcripts showing differential expression in APTB patients relative to healthy controls. This diversity in findings is partly attributable to the heterogeneity of circRNA exploration algorithms and analytical pipelines used across studies, which complicates cross-comparison and highlights the need for methodological standardisation in future research. These circRNAs showed dysregulation correlated with radiological severity scores, with diagnostic performance measured by AUC values ranging from 0.773 to 0.974, suggesting strong diagnostic potential.

In comparison, while sputum microscopy is widely used due to its simplicity, its sensitivity remains limited (47–70%) and depends heavily on sputum quality, which is often difficult to obtain. GeneXpert offers higher sensitivity (>90%) and detects drug resistance, but still relies on sputum samples.[3,4] CircRNA testing offers a promising alternative because it does not depend on sputum samples, making it especially valuable for patients who are unable to produce sputum or have low bacterial loads.

CircRNAs hold significant potential as diagnostic biomarkers for APTB due to their unique stability and specific expression patterns that set them apart from traditional biomarkers. Their covalently closed circular structure renders them resistant to exonuclease-mediated degradation, resulting in a longer half-life compared to linear RNAs. This inherent stability enhances their reliability in clinical samples, making them well-suited for diagnostic applications. Integrating circRNAs with existing biomarkers could enhance diagnostic sensitivity and specificity, offering a more comprehensive approach that supports earlier detection and better differentiation within TB cases.[10]

In addition, their ability to modulate immune responses and influence cellular processes such as apoptosis and autophagy, key factors in TB pathogenesis, provides additional insight into disease mechanism.[10] Certain circRNAs have been associated with altered macrophage activity, impacting the

intracellular survival of *Mycobacterium tuberculosis*, further highlighting their role in TB biology.[20,21] These findings not only support the diagnostic potential of circRNAs but also open avenues for their development as therapeutic targets.

However, several limitations must be acknowledged before circRNAs can be translated into routine clinical diagnostics. The lack of standardised bioinformatic pipelines contributes to inconsistencies in circRNA identification and reported performance metrics. This methodological variability hinders reproducibility and the establishment of universally accepted biomarker panels. Additionally, most existing studies are constrained by small cohorts, limited longitudinal data, and insufficient validation across diverse populations, which restricts generalisability. Future research should focus on harmonised bioinformatic pipelines and validation frameworks for circRNA discovery. Candidate circRNAs must be tested in large, multicentre trials spanning TB subtypes and demographic groups to ensure repeatability.

CONCLUSION

In conclusion, circRNAs have the potential to serve as reliable diagnostic markers for tuberculosis, with high diagnostic accuracy that could improve early detection and patient management of APTB. Future research should focus on validating these findings in clinical settings, exploring the interaction of circRNAs with other molecular pathways, and standardizing detection methods. Expanding studies to larger, diverse populations will be crucial for integrating circRNAs into TB diagnostic algorithms, ultimately enhancing global efforts in TB control and management.

Conflicts of Interest

The corresponding author is a member of the editorial team of the Journal of Medical Science (Jurnal Ilmu Kedokteran), but was not involved in the peer review process or editorial decisions related to this manuscript. The other authors declare no conflicts of interest.

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