Potential of microRNA as Biomarker for Syphilis: A Systematic Review

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ABSTRACT

Syphilis remains one of the world's most prevalent sexually transmitted infections, posing a serious public health concern due to its complex clinical presentation and latent phases. This systematic review investigates the potential of microRNAs as biomarkers for syphilis, a disease caused by *Treponema pallidum*. By adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, this study examines existing pertinent literature to evaluate the diagnostic utility of microRNAs in syphilis detection. A comprehensive search was conducted using databases such as PubMed and Google Scholar, with additional references from sources, including the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and the Department of Health (DOH). By synthesizing current evidence, this review aimed to explain the role of microRNA profiles in syphilis diagnosis, potentially contributing to the development of novel, more sensitive and specific diagnostic assays.

Key words: Syphilis, *Treponema pallidum*, microRNA, biomarker

With millions of cases affecting people globally and posing challenges for healthcare systems, sexually transmitted infections (STIs) remain a serious worldwide public health concern. In 2020, the World Health Organization (WHO) reported approximately 374 million cases of one of the four curable STIs, with 7.1 million of these cases attributed to syphilis, a chronic inflammatory disorder caused by the non-cultivable spirochete *Treponema pallidum*. According to the Centers for Disease Control and Prevention or CDC (2021), the standard laboratory tests used to diagnose syphilis comprise non-treponemal and treponemal tests. However, these tests generally have a high likelihood of producing false-positive outcomes, particularly in individuals with concurrent medical conditions or recent vaccinations and may also remain positive indefinitely after successful treatment due to persistent antibodies indicating past exposure rather than active infection. Despite the availability of diagnostic tests, there remains a deficiency in data concerning the prevalence of syphilis among affected individuals. This deficiency primarily stems from the disease's diverse clinical manifestations and the constraints of available diagnostic methods.

MicroRNAs (miRs or miRNAs) are minute, non-coding RNA molecules that modulate gene expression by binding to complementary sequences on messenger RNA (mRNA) and disrupting post-translational processes, thereby hindering translation or promoting mRNA degradation. MiRs are evolutionarily conserved and are critically involved in diverse biological processes, including growth, cell differentiation, apoptosis, and disease pathogenesis. The abnormal regulation of miR expression has been implicated in the development of numerous diseases, indicating their potential as biological markers. By targeting miR expression profiles associated with syphilis infection,

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novel diagnostic assays can be developed. This offers potential improvements in syphilis diagnosis, particularly in cases where conventional tests may lack sensitivity or specificity.

The study aimed to determine the capability of microRNAs to serve as biomarkers for detecting syphilis by identifying specific patterns of microRNAs involved in the pathogenesis of syphilis, by comparing their expression levels between syphilis patients in different stages of syphilis and healthy controls; and by presenting the sensitivity and specificity of microRNA-based assays in conjunction to conventional serological tests for detecting syphilis.

MATERIALS AND METHODS

Search Strategy

Studies related to the diagnosis of syphilis using microRNA and PCR, published from 2010 onward, were retrieved from Google Scholar, PubMed, Embase and Scopus. Search terms or keywords and phrases used within the inclusion criteria were as such: "Syphilis OR Venereal Syphilis", "Congenital Syphilis OR Neurosyphilis", '*Treponema pallidum subsp. pallidum NOT Treponema pallidum subsp. pertenue OR Treponema pallidum subsp. endemicum OR Treponema carateum*", "microRNA OR miRNA", "Blood OR CSF", and "PCR". Database searches were collated, sorted and assessed for duplicates, relevance and eligibility through full text reviews. Using the selection features in Pubmed, the studies were selected according to observational studies namely: "randomized control trial", "cohort", "case-control", and "cross- sectional" only. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines and a PICO statement for DTA (Diagnostic Test Accuracy Reviews) were used to guide the review.

Inclusion Criteria

Search included randomized controlled trials (RCTs) and non-randomized trials such as cohort, case-control and cross-sectional studies of populations of any age or gender, on any stage of Syphilis (*Treponema pallidum subsp. pallidum*). Studies were selected when there was use of microRNA and PCR as diagnosis for Syphilis with blood or CSF (neurosyphilis) as specimens. Comparative studies were also screened when current tests for Syphilis — the treponemal and nontreponemal tests for Syphilis — were the comparators against microRNA. To be included, studies should be published and peer-reviewed originally written in the English or Filipino languages. Considering that there is no current systematic review on microRNA in relation to diagnosing Syphilis, the limit to the date of publication was from 2010 to 2024.

In addition, studies were first accessed in October 2024 of which the updated list of the studies can be seen in Table 3. Studies were chosen from publication databases (Google Scholar, PubMed, Embase, and Scopus), WHO and CDC records, and reports from non-profit research organizations or academic institutions. They must be accessed in full-text or, if restricted, provide enough contextualized tabulated results with what is open access.

Exclusion Criteria

Animal studies were excluded, i.e, any studies that regard animals as subjects were not included. Other excluded studies were qualitative studies for the discussion of microRNA; in silico studies; and studies lacking diagrams, figures and tabulated results. Translated studies from its original text to English or Filipino were not considered. Research involving non-syphilitic conditions was omitted. Exclusion extended to studies focusing on Syphilitic infections other than the diseases caused by *T. pallidum subsp. pallidum* such as *T. pallidum subsp. pertenue* (yaws), *T. pallidum subsp. endemicum* (bejel or endemic syphilis), and *T. carateum* (pinta). Studies published prior to or after the specified date range were not included.

Risk of Bias

Risk of bias was assessed with ROBIS, an assessment tool for systematic reviews rather than primary studies. The review authors evaluated risk of bias using the ROBIS 3-phase guidelines for DTA (Diagnostic Test Accuracy) reviews. Independent assessment involved 3 review authors and final conclusions were made by discussion. Lastly, transparency was appraised using PRISMA 2020 Checklist, completed by one review author.

Data Extraction and Collection

After approval from the Ethics Committee, data extraction was initially done manually and

individually by all review authors. The authors screened the full-text of the studies accessed for inclusion using the Screening Eligibility Form based on the PICO statement (Table 1). In the case of disagreements, consensus was reached by discussion of the entire group. Grading of the quality of the studies' findings were based on the journal's "indexed status" in the Science Citation Index Expanded or SCIE of the Institute for Scientific Information (ISI) Web of Science. At least 2 review authors were responsible for proofreading and editing the initial draft of data and table of Summary of Findings. The PRISMA 2020 flow diagram was filled in by each author using their own copy, which would be collated into one by the end of the process.

Table 1. PICO Statement

Population	Patients (from newborns or teenagers to the elderly, any gender, any ethnicity) diagnosed with syphilis in all its stages; primary, secondary, latent, and tertiary syphilis caused by T. pallidum *subsp. pallidum* No age group specifics as long as they have been diagnosed with syphilis or are afflicted with the disease
Intervention	PCR and MicroRNA biomarkers using blood or CSF (neurosyphilis) as specimen
Comparison	VDRL, RPR, TP-PA, and other nontreponemal or treponemal immunologic tests that are being used by the CDC or Philippines NRLs and that do not use MicroRNA
Outcome	Enhanced diagnosis of syphilis using MicroRNA

Using a Data Extraction Form formulated from Cochrane and Covidence, the following data were extracted from each study: author, date or year of publication, purpose of the study, stage of Syphilis studied, type of population, specimen collected, microRNA detected to have up or down regulated, type of PCR and comparative immunological tests or direct microscopy (if any) used among others. After data extraction, data collection was generally organized into 2 topics: the overall utility of microRNA in molecular diagnostics within each syphilis stage, and its potential role in diagnosing different stages of Syphilis.

RESULTS

Search Results

A total of 47 journals were identified during the initial search, 29 from Google Scholar and 18 from PubMed as shown in Figure 1. Before screening, 21 duplicates were identified and removed from the pool which yielded a sum of 26 journals. However, upon the retrieval of the said journals, two studies were excluded due to not being able to access their full texts. The remaining 24 articles were evaluated for eligibility, wherein 6 journals were excluded on the basis of not meeting the study's inclusion criteria derived from the PICO statement. Studies were excluded for reasons such as the journals' findings did not focus on syphilis, there were no mentions of any miRNA in the study, enhanced diagnosis of syphilis using miRNAs were not correlated to their outcome, and the comparison of treponemal and nontreponemal tests were not discussed. Following the screening of the studies for eligibility, only 18 journals were included. Of the included journals, 14 were indexed in the SCIE of the ISI Web of Science (Tables 2 and 3).

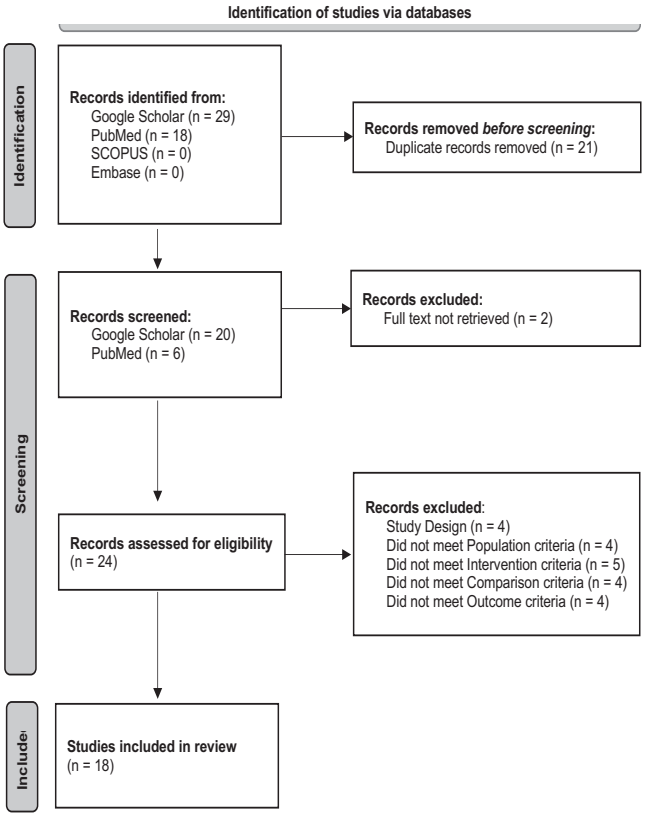

Figure 1. PRISMA 2020 Workflow

Table 2. Criteria for journal grading based on indexing

Criteria	Description	Interpretation	Score
Indexed (non-ISI)	The journal is indexed in reputable databases but not ISI-indexed.	Moderate priority for inclusion	5
ISI-Indexed	The journal is indexed in the ISI (Web of Science).	High priority for inclusion	10

Table 3. Summary of journals graded

Journal Article	Indexing Status	Score
Plasma exosome-derived microRNAs profiles in patients with serofast status: A cross-sectional study	Indexed (Google Scholar)	5
Serum microRNA profiles in patients with syphilis	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Laboratory diagnostic tools for syphilis: Current status and future prospects	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Magnetic nanoparticle-assisted detection of peripheral blood miRNA biomarker characteristics in syphilis	Indexed (Google Scholar)	5
MicroRNA-101-3p downregulates TLR2 expression, leading to reduction in cytokine production by *Treponema pallidum*-stimulated macrophages	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
MicroRNA expression profiling of peripheral blood mononuclear cells associated with syphilis	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Peripheral blood mononuclear cell microRNA profiles in syphilitic patients with serofast status	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
MiR-216a-5p-containing exosomes suppress rTp17-induced inflammatory response by targeting TLR4	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
MicroRNA-101-3p, MicroRNA-195-5p, and MicroRNA-223-3p in peripheral blood mononuclear cells may serve as novel biomarkers for syphilis diagnosis	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Circulating microRNAs as potential biomarkers in the diagnosis of neurosyphilis: a case control study	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Genome-wide microRNA analysis of peripheral blood mononuclear cells reveals elevated MIR-142-3P expression as a potential biomarker for secondary syphilis	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Laboratory evaluation of a commercially available rapid syphilis test	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10

Exosomal microRNA profiles from serum and cerebrospinal fluid in neurosyphilis	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Serofast status in syphilis: Pathogenesis to therapeutics	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
MicroRNA Expression Profiling in Syphilis Patients: A Potential Diagnostic and Prognostic Biomarker Discovery Study in Manila, Philippines	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
MiR-223-3p inhibits rTp17-induced inflammasome activation and pyroptosis by targeting NLRP3	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Exosomal miR-146a-5p from Treponema pallidum-stimulated macrophages reduces endothelial cells permeability and monocyte transendothelial migration by targeting JAM-C	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10
Resurgence of syphilis: focusing on emerging clinical strategies and preclinical models	ISI-Indexed [Science Citation Index Expanded (SCIE) Database]	10

Comparatively, four journals – Plasma Exosome-Derived microRNAs Profiles in Patients with Serofast Status: A Cross-Sectional Study; MicroRNA Expression Profiling in Syphilis Patients: A Potential Diagnostic and Prognostic Biomarker Discovery Study in Manila Philippines; Serofast Status in Syphilis: Pathogenesis to Therapeutics, and Magnetic Nanoparticle-Assisted Detection of Peripheral Blood miRNA Biomarker Characteristics in Syphilis – were not indexed in the SCIE but were still indexed in other databases like PubMed Central, Google Scholar, and Embase. SCIE- indexed studies were graded higher and tagged as high priority, while a lower priority was reserved for studies not SCIE-indexed but still published in other legitimate databases.

This paper reviewed 18 journal articles, including 14 non-randomized controlled trials and 4 systematic reviews, which were screened and categorized. As summarized in Table 4, all 14 non-RCTs involved the analysis of blood samples, including whole blood, plasma and serum. Additionally, two studies incorporated human umbilical vein endothelial cells (HUVECs) alongside blood samples, while one study analyzed cerebrospinal fluid (CSF) to investigate miRNA in patients with neurosyphilis. In terms of measurement methods, seven studies utilized quantitative reverse transcription PCR (RT-qPCR), and six employed quantitative real-time PCR (qRT-PCR) to examine the regulatory mechanisms of various miRNAs across different diagnostic stages of syphilis. One study uniquely combined real-time reverse transcription PCR with both methodologies to maximize the production of sample replicates. Table 4 provides a detailed summary of miRNA regulation at different stages of syphilis, highlighting their potential as biomarkers for syphilis staging.

MicroRNAs in Syphilis Stages

Primary Syphilis

According to WHO (2024), primary syphilis is defined as the first stage of syphilis which usually presents as a hard sore around the genitals or elsewhere. It can last up to 21 days and lead to secondary syphilis if left untreated. The common testing methods available for this stage are the different serological tests that detect treponemal antibodies. Current syphilitic studies delve on the potential of miRNAs as biomarkers for the different stages of syphilis.

Based on the miRNA profiles of patients with primary syphilis in a study by Lu, et al (2017) and among the 39 differentially-expressed miRNAs and miR-6510-5p as normalization control in serum samples, specific miRNAs, namely: miR-19b-3p, miR-21-5p, miR-16-5p and miR-142- were found to be downregulated in syphilis patients. In another study by Helsey (2023), results showed that miR-1, miR-12, miR- 25 and miR-35 were upregulated and investigated to have potential in identifying true

Table 4. Summary of findings

Author/s, (Year)	MicroRNA	Specimen	Method of Measurement	Syphilis Stage/ Condition	Regulation	Purpose
Liu, J., Zhang, R., Lian, T., *et al,* 2023	miR-197-3p, miR-4485-5p, miR-1273 g-3p	Plasma	Quantitative Reverse Transcription PCR	SF	Upregulated	Diagnostic Stage Markers (for SF vs. SC)
	miR-1908-3p			SF	Upregulated	Diagnostic Stage Marker (for SF vs. HC)
				HC	Downregulated	
	miR-4419b			SS & SC	Downregulated	Diagnostic Stage Marker (for SS & SC vs. SF)
Lu, P., Fang, C., Cheng, Q., *et al,* 2017	miR-19b-3p, miR-21-5p, miR-16-5p, miR-142-3p	Serum	Quantitative Reverse Transcription PCR	PS, SS, & Latent Syphilis	Downregulated	Infection Biomarkers (for Syphilitic Patients vs. HC)
Zhang,Z., Hu, W., Yu, J., *et al,* 2022	miR-125a-3p	Serum	Quantitative Reverse Transcription PCR	Early Latent Syphilis & SS	Downregulated	Diagnostic Stage Marker (for HC vs. ELS and SS)
	miR-185-3p			Early Latent Syphilis & SS	Upregulated	Diagnostic Stage Marker (for HC vs. ELS; ELS vs. SS)
	miR-660-3p			Early Latent Syphilis	Upregulated	Diagnostic Stage Marker (for HC vs. SS; ELS vs. SS)
				SS	Downregulated	
Huang, T., Yang, J., Zhang, J., *et al,* 2020a	miR-101-3p	Peripheral Blood	Quantitative Real-Time PCR	PS & SF	Upregulated	Diagnostic Stage Marker (for PS and SF vs. HC)
				HC	Downregulated	
	miR-21-5p, miR-19b-3p, miR-16-5p, miR-142-3p	Plasma		SS	Downregulated	Diagnostic Stage Markers
Huang, T., Zhang, J., Ke, W., *et al,* 2020b	miR-195-5p	Peripheral Blood	Quantitative Reverse Transcription PCR	PS & SF	Upregulated	Diagnostic Stage Marker (for SF vs. SC and HC; PS vs. HC)
						Immune Suppression Marker in SF patients
	miR-223-3p, miR-589-3p			SF	Upregulated	Diagnostic Stage Markers (for SF vs. SC and HC)

	miR-338-5p			SF	Upregulated	Diagnostic Stage Marker
Jia, X., Liu, X., Wang, Z., *et al,* 2020	np-miR-128, np-miR-244	Peripheral Blood	Quantitative Real-Time PCR	SS & SF	Downregulated	Diagnostic Stage Markers
	np-miR-163			SS	Downregulated	Diagnostic Stage Marker
	np-miR-5			SS	Upregulated	Diagnostic Stage Marker
Peng, R., Shang, S., Zhao, L., *et al,* 2019	miR-216a-5p	Plasma HUVECs	Quantitative Reverse Transcription PCR	PS, SS, & Latent Syphilis	Downregulated	Infection Biomarkers (for Syphilitic Patients vs. HC)
Yang, J., Huang, T., Zhao, P., *et al,* 2021	miR-195-5p	Peripheral Blood	Quantitative Reverse Transcription PCR	PS, SF, SC, & Latent Syphilis	Upregulated	Diagnostic Stage Marker
	miR-223-3p			PS, SS, & Latent Syphilis	Upregulated	Diagnostic Stage Marker
	miR-101-3p			SF, SC, & Latent Syphilis	Upregulated	Diagnostic Stage Marker
				SS	Downregulated	
Wu, K., Zhang, S., Bao, C., *et al,* 2021	hsa-miR-21-5p, hsa-miR-21-3p, hsa-miR-503-5p, hsa-miR-148a-3p, hsa-miR-664b-5p, hsa-miR-1288-3p, hsa-miR-6131, hsa-miR-6752-3p, hsa-miR6512-5p	Peripheral Blood	Quantitative Real-Time PCR	SS & NS (higher in SS)	Upregulated	Diagnostic Stage Markers (SS & NS vs. HC)
	hsa-miR-6848-3p, hsa-miR-4664-3p, hsa-miR-1237-3p, hsa-miR-1539			SS & NS (higher in HS)	Upregulated	Diagnostic Stage Markers (SS & NS vs. HC)
Yang, Z., Yang, X., Gan, Z., *et al,* 2021	miR-142-3p (most significant) miR-3180, miR-675-5p, miR-98-5p, miR-3195, miR-6132, miR-4498, miR-5100, miR-6860, miR-1229-5p	Peripheral Blood	Quantitative Real-Time PCR	SS	Upregulated	Diagnostic Stage Markers (SS vs. HC)

Chen, H., Zhou, Y., Wang, Z., *et al,* 2019	miR-210-5p	Serum CSF	Quantitative Real-Time PCR	NS	Upregulated (Internal Ref.)	Diagnostic Stage Marker (NS vs. Non-NS)
	miR-590-5p, miR-570-3p, miR-570-5p				Upregulated (Serum & CSF)	Diagnostic Stage Markers (NS vs. Non-NS)
	miR-21-5p				Upregulated (CSF)	Diagnostic Stage Marker (NS vs. Non-NS)
	miR-93-3p				Downregulated (Serum & CSF)	Diagnostic Stage Marker (NS vs. Non-NS)
	miR-7–5p, miR-1307–5p, miR-203a-3p				No Significant Difference in Serum or CSF	No Difference Between CSF-Negative and CSF-Positive Samples (not affected by Neurosyphilis)
	miR-16, miR-23b-3p				Altered (Serum > CSF)	
	miR-27b-5p				Altered (Serum < CSF)	
Helsey, B., n.d	miR-1, miR-2, miR-12, miR-15, miR-21, miR-25, miR-32	Peripheral Blood	Quantitative Reverse Transcription PCR	N/A	Upregulated	Diagnostic Marker for Syphilis
	miR-50, miR-60				Downregulated	
Long, F., Kou, C., Li, K, *et al,* 2020	miR-223-3p	Serum HUVECs	Real-Time Reverse Transcription PCR	N/A	Downregulated	Diagnostic Marker for Syphilis Protective Against *T. pallidum* Infection
Hu, W., Xu, B., Zhang, J., *et al,* 2020	miR-146a-5p	Plasma	Quantitative Real-Time PCR	PS	Upregulated	Infection Biomarker by Decreasing Monocyte Transendothelial Migration

PS, Primary Syphilis; SS, Secondary Syphilis; NS, Neurosyphilis; SF, Serofast; SC, Serologically Cured; HC, Healthy Control; ELS, Early Latent Syphilis; miR, MicroRNA; np, Nucleus Pulposus; CSF, Cerebrospinal Fluid; HUVECs, Human Umbilical Vein Endothelial Cells; PCR, Polymerase Chain Reaction

positives for syphilis. Higher diagnostic accuracy was calculated using the panel of miR-1, miR-12, and miR-25, in comparison to using the miRNAs by themselves. Although both studies included primary syphilitic patients as part of the study population, the reported data were inadequate to conclude that those distinct miRNAs were specific to primary syphilis.

According to Yang, et al (2021), miR- 195-5p and miR-223-3p were significantly upregulated in patients suffering from primary and secondary syphilis. Together, they show a potential in distinguishing patients of primary syphilis from healthy controls with high sensitivity (100.0%) and specificity (94.4%).

In peripheral blood mononuclear cells (PBMCs) samples, high levels of miR-101-3p were identified in samples of patients with primary syphilis resulting in serofast states (Huang, et al, 2020). Peng, et al (2019) found miR-216a-5p, contained in exosomes, and described it to be significantly correlated to disease progression and decreased in syphilis patients more than in healthy controls. And similar to miR-216a-5p, miR-146a-5p was studied in *T. pallidum* - stimulated macrophages derived from HUVECs, and high levels were associated with syphilis patients (Hu, et al, 2020). In the studies of Peng, et al (2019) and Hu, et al (2019), there was no conclusion about the miRs mentioned being specific solely to primary syphilis.

Table 5. Relevant differentially expressed miRs in primary syphilis

Author/s, (Year)	microRNA	Specimen	Method of Measurement	Regulation
Lu, P., Fang, C., Cheng, Q., et al, 2017	miR-19b-3p miR-21-5p miR-16-5p miR-142-3p	Serum	Quantitative Reverse Transcription PCR	Downregulated
Helsey, B., n.d.	miR-1 miR-12 miR-25 miR-35	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated
Huang, T., Yang, J., Zhang, J., et al, 2020	miR-101-3p	Peripheral Blood and Plasma	Quantitative Real-time PCR	Upregulated
Huang, T., Zhang, J., Ke, W., et. al, 2020b	miR-195-5p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated
Peng, R., Shang, S., Zhao, L., et al, 2019	miR-216a-5p	Plasma and HUVECs	Quantitative Reverse Transcription PCR	Downregulated
Yang. J., Huang T., Zhao, P., et al, 2021	miR-195-5p miR-223-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated
Hu, W., Xu, B., Zhang, J., et al, 2020	miR-146a-5p	Plasma	Quantitative Real-time PCR	Upregulated

Secondary Syphilis

WHO (2024) states that secondary syphilis symptoms include a non-itchy rash found on the palms and soles of the feet. A gray or white lesion may also appear in areas that are warm and moist at the sight of the chancre, especially in the labia and anus. In comparison to primary syphilis, there is a wider dissemination and bacteremia of *T. pallidum*.

Latent Syphilis

Latent syphilis is the stage of syphilis wherein there are no obvious manifestations of symptoms unlike primary, secondary, and tertiary stages, but is described to be seroreactive (CDC, n.d.). It also remains undetected and untreated unless primary and secondary syphilis were initially detected. The most common methodology in detecting latent syphilis is serology. Nontreponemal tests are said to be reliable in detecting early latent syphilis, however, sensitivity decreases over time (Luo, et al, 2021).

Based on the study of Zhang, et al (2022), miR-185-3p and miR-660-3p expressions discerned distinction between early latent syphilis and secondary syphilis. MiR-125a-3p, in combination with miR-660-3p was able to discriminate early latent syphilis, secondary syphilis, and the normal population from one another.

The integration of miR-101-3p, miR-195- 5p and miR-223-3p were found to be upregulated and were considered markers for latent syphilis. Although

Table 6. Relevant differentially expressed miRs in secondary syphilis

Author/s, (Year)	microRNA	Specimen	Method of Measurement	Regulation
Zhang,Z., Hu, W., Yu, J., et al, 2022	miR-125a-3p miR-660-3p	Serum	Quantitative Reverse Transcription PCR	Downregulated
	miR-185-3p	Serum	Quantitative Reverse Transcription PCR	Upregulated
Jia, X., Liu, X., Wang, Z., et al, 2020	np-miR-5	Peripheral Blood and Plasma	Quantitative Real-time PCR	Upregulated
	np-miR-128 np-miR-244 np-miR-163	Peripheral Blood and Plasma	Quantitative Real-time PCR	Downregulated
Yang. J., Huang T., Zhao, P., et al, 2021	miR-223-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated
	miR-101-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Downregulated
Yang, Z., Yang, X., Gan, Z., et al, 2021	miR-142-3p (most significant) miR-3180 miR-675-5p miR-98-5p miR-3195 miR-6132 miR-4498 miR-5100 miR-6860 miR-1229-5p	Peripheral Blood	Quantitative Real-time PCR	Upregulated

these three microRNAs enhanced the reliability of diagnosing latent syphilis in comparison to healthy individuals, their diagnostic limitations include being unable to distinguish latent syphilis from the serofast stage. They were nonspecific as they were also upregulated in primary and secondary syphilis (Yang, et al, 2021). But Huang, et al (2020) states that miR-101-3p was defined to have a higher expression in serofast than latent syphilis.

Neurosyphilis

According to NIH (2024), neurosyphilis is an infection that can emerge in people with syphilis, especially if left untreated. This infection targets the CNS, particularly the brain, spinal cord, and brain coverings.

MiR-590-5p, miR-570-3p, and miR-570- 5p were markedly elevated in both serum and CSF, while miR-

21-5p showed upregulation in CSF only which suggests CNS-related specificity. On the contrary, miR-93-3p showed downregulation in both serum and CSF (Chen, et al, 2019). In a study of Wu, et al (2020) where peripheral blood mononuclear cells (PBMCs) were used as the specimen, miR- 6512-5p and miR-1288-3p expression were also similarly downregulated.

Furthermore, miR-210-5p has shown potential to be used as an internal control in future studies due to its consistent expression. Thus, miR-210-5p can ensure that any observed changes in an experiment can be regulated and checked (Chen, et al, 2019). Wu, et al (2020) also demonstrated that the combined analysis of nine miRNAs – hsa-miR-21-5p, hsa-miR-21-3p, hsa-miR-503-5p, hsa-miR-148a-3p, hsa-miR-664b-5p, hsa-miR-1288-3p, hsa-miR-6131, hsa- miR-6752-3p, and hsa-miR6512-5p – that are elevated more in secondary syphilis than in neurosyphilis and four miRNAs – hsa-miR-6848- 3p, hsa-miR-4664-3p, hsa-miR-1237-3p, hsa- miR-1539 – that are elevated more in neurosyphilis than in secondary syphilis improved diagnostic accuracy.

Table 7. Relevant differentially expressed miRs in latent syphilis

Author/s, (Year)	microRNA	Specimen	Method of Measurement	Regulation
Zhang,Z., Hu, W., Yu, J., et al, 2022	miR-125a-3p	Serum	Quantitative Reverse Transcription PCR	Downregulated
	miR-185-3p miR-660-3p	Serum	Quantitative Reverse Transcription PCR	Upregulated
Yang. J., Huang T., Zhao, P., et al, 2021	miR-195-5p, miR-223-3p miR-101-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated

Table 8. Relevant differentially expressed miRs in neurosyphilis

Author/s, (Year)	microRNA	Specimen	Method of Measurement	Regulation
Wu, K., Zhang, S., Bao, C., et al, 2021	hsa-miR-21-5p hsa-miR-21-3p hsa-miR-503-5p hsa-miR-148a-3p hsa-miR-664b-5p hsa-miR-1288-3p hsa-miR-6131 hsa-miR-6752-3p hsa-miR6512-5p hsa-miR-6848-3p hsa-miR-4664-3p hsa-miR-1237-3p hsa-miR-1539	Peripheral Blood	Quantitative Real-time PCR	Upregulated
Chen, H., Zhou, Y., Wang, Z., et al, 2019	miR-210-5p miR-590-5p miR-570-3p miR-570-5p miR-21-5p	Serum and CSF	Quantitative Real-Time PCR	Upregulated
	miR-93-3p	Serum and CSF	Quantitative Real-Time PCR	Downregulated

Serofast Syphilis

Recent studies have shown the clinical importance of differentiating patients with serofast status from patients with latent syphilis. Currently, there is no consistent definition of serofast status in syphilis. However, clinicians generally characterize serofast as having a low- titer (less than 4-fold decrease) after months of treatment (Liu, et al 2023). As such, Huang, et al (2020) agrees that the difficulty of distinguishing serofast and latent stages of syphilis is due to their similar serological manifestations and the absence of clinical symptoms.

A study by Liu, et al (2023) identified several potential exosome-derived miRNAs, including miR-197-3p, miR-4485-5p, miR- 1273g-3p, and miR-1908-3p as potential diagnostic markers, with miR-1908-3p showing the highest diagnostic accuracy in distinguishing serofast patients from serologically cured patients or healthy controls. A familiar miRNA, miR-101-3p, was documented to be upregulated in serofast compared to secondary and untreated syphilis. MiR-195-5p, miR-223-3p and miR-589-3p showed significant upregulation in serofast patients as well. MiR-195-5p, in particular, was higher in serofast patients compared to secondary patients and healthy controls. While miR-223-3p and miR-589-3p exhibited higher expression levels in serofast patients compared to secondary syphilis patients only (Huang, et al 2020).

Yang, et al (2021) confirmed that miR- 195-5p and miR-101-3p were upregulated across various stages of syphilis. MiR-101-3p was upregulated in patients with serofast status (2.68-fold higher) and latent syphilis (4.76-fold higher), and patients who were serologically cured (2.38-fold higher). Similarly, miR-195-5p and miR-223-3p were both upregulated (4.92- and 6.32- fold, respectively) in patients with primary syphilis, latent syphilis (3.61- and 6.28-fold, respectively), serofast status (3.94- and 3.39-fold, respectively), and patients who were serologically cured (3.20- and 3.86-fold, respectively). Combined, miR-223-3p and miR- 195-5p exhibited biomarker potential for differentiating patients with serofast status from healthy controls, with 93.9% sensitivity and 83.3% specificity. However, the ROC curve analysis showed that neither miR-195-5p, miR- 223-3p, nor miR-101-3p, could differentiate patients with latent syphilis from the serofast status.

Additionally, a separate study of Jia, et al (2020) that investigated miRNA expression in PBMCs, miR-338-5p, np-miR-128, and np-miR-244 were differentially expressed in serofast patients. MiR-338-5p was upregulated in serofast patients, while np-miR-128 and np-miR- 24 were downregulated.

Table 9. Relevant differentially expressed miRs in serofast patients

Author/s, (Year)	microRNA	Specimen	Method of Measurement	Regulation
Liu, J., Lian, t. et al, 2023	miR-197-3p miR-4485-5p miR-1273 g-3p miR-1908-3p	Plasma	Quantitative Reverse Transcription PCR	Upregulated
Huang, T., Yang, J., Zhang, J., et al, 2020	miR-101-3p	Peripheral Blood and Plasma	Quantitative Real-time PCR	Upregulated
Huang, T., Zhang, J., Ke, W., et al, 2020b	miR-195-5p miR-223-3p miR-589-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated
Jia, X., Liu, X., Wang, Z., et al, 2020	miR-338-5p	Peripheral Blood	Quantitative Real-time PCR	Upregulated
	np-miR-128 np-miR-244	Peripheral Blood	Quantitative Real-time PCR	Downregulated
Yang. J., Huang T., Zhao, P., et al, 2021	miR-195-5p miR-101-3p	Peripheral Blood	Quantitative Reverse Transcription PCR	Upregulated

Serological Tests and MicroRNA in Diagnosing Syphilis

Serological tests, including non- treponemal assays (RPR and VDRL) and treponemal assays (TPPA, FTA-ABS, etc.), represent the current standard for syphilis diagnosis. These methods are widely used due to their high sensitivity and specificity, particularly during the secondary and latent stages of the disease (Luo, et al, 2021). However, based on the study of Wu, et al (2020) and Cao, et al (2024), their utility is limited in certain scenarios, such as distinguishing between active and treated infections in serofast status or detecting neurosyphilis in asymptomatic patients. Rapid serologic tests have been developed like the Syphilis Health Check (SHC) and offer convenience, but have varying reliability across demographic groups (Pereira, et al, 2018). So far, it has been established that while tests can remain the current standard, progress can be made to better the identification of syphilis, especially neurosyphilis.

Recent advances in molecular diagnostics for syphilis can represent this paradigm. MicroRNAs (miRNAs) control gene expression by binding to complementary sequences within the 3' untranslated region (3' UTR) of target messenger RNAs (mRNAs), causing translational repression or mRNA degradation (Cao, et al, 2024). Though miRNAs are mostly recognized to silence genes, they are capable of promoting gene upregulation by preventing target mRNAs from being degraded by inhibiting negative regulators or by promoting translation efficiency under certain cellular conditions (Xiong, et al, 2023). With regard to downregulation, miRNAs cause gene silencing by two primary mechanisms: mRNA degradation in the case of near-perfect complementarity, and translational inhibition in the case of partial complementarity that inhibits protein synthesis without causing degradation of the mRNA (Cao, et al, 2024).

Altered miRNA profiles in exosomal and peripheral blood samples from serofast patients have been proposed as biomarkers for differentiating these cases from active infections, addressing a major limitation of serological methods, as demonstrated in the findings of Liu, et al (2023) and Jia, et al (2020). While traditional approaches often require invasive CSF analysis in neurosyphilis identification, specific miRNAs like miR-590-5p and miR-570- 3p have been identified as potential non-invasive biomarkers in serum and/or CSF samples (Chen, et al, 2019). Functional studies also demonstrate that miR-101-3p and miR-216a-5p regulate immune responses by modulating Toll-like receptor (TLR) pathways. These pathways, particularly TLR2 and TLR4, are critical in syphilis pathogenesis and immune evasion by *T. pallidum*.

The distinct expression profiles offer an opportunity to improve diagnostic accuracy, particularly in challenging cases where serological tests may yield inconclusive results. Magnetic nanoparticle-assisted methods have only further enhanced the sensitivity and specificity of miRNA (Zhang, et al, 2022). While serological tests remain indispensable for their accessibility and established use, integrating miRNA profiling into clinical practice could significantly enhance syphilis diagnostics. By combining traditional methods with miRNA- based approaches, it is possible to improve detection accuracy, enable stage-specific diagnosis, and tailor treatment strategies, optimizing patient outcomes and addressing limitations in current diagnostic tools.

Reporting Bias Assessment

The systematic review used ROBIS (Risk of Bias in Systematic Reviews) as the primary tool to assess the risk of bias. The overall risk of bias is low, though minor concerns regarding date and language restrictions were noted. The systematic review process is methodologically sound, with clear pre-specified eligibility criteria and rigorous data collection and synthesis methods. Lastly, the conclusions drawn in the review are likely well-supported by the evidence, and efforts to minimize bias were clearly implemented.

DISCUSSION

Key Findings

A detailed synthesis of findings revealed consistent patterns in miRNA expression associated with syphilis progression. In primary syphilis, miR-19b-3p, miR-21-5p, and miR-16-5p are consistently downregulated, with Lu, et al (2017) reporting significantly lower serum levels in syphilis patients compared to healthy controls. Conversely, miR-195-5p and miR-223-3p exhibit upregulation in both primary and secondary syphilis. Yang, et al (2021) identified miR-223-3p as a highly sensitive biomarker

distinguishing active from serofast states, reinforcing its diagnostic relevance.

Several studies highlight the potential of miRNAs in improving syphilis diagnostics. Huang, et al (2020) and Zhang, et al (2022) suggest that combining miR-195-5p and miR-223-3p enhances diagnostic accuracy, supporting a multi-miRNA approach over single biomarker assessments.

Standardization remains a key challenge in miRNA research. Variability in diagnostic criteria, sample collection timing, and treatment history contributes to inconsistencies across studies. For instance, defining serofast status, a condition where nontreponemal antibody levels decrease but remain reactive post-treatment, varies across literature, affecting miRNA interpretation. A standardized approach to defining syphilis stages and post-treatment statuses is crucial for improving the reliability of miRNA-based diagnostics.

In addition to their value in diagnostics, miRNAs hold promise as prognostic biomarkers and therapeutic targets in syphilis. Hu, et al (2020) identified miR-2, miR-15, and miR-21 as positively correlated with syphilis progression, while miR-50 and miR-60 exhibited inverse associations, highlighting a complex regulatory network. Notably, miR-101-3p, which is significantly upregulated in serofast patients, downregulates Toll-like receptor 2 (TLR2). This suggests that miRNA-targeted therapies could modulate immune responses, opening new avenues for syphilis treatment.

The collective evidence reinforces the diagnostic and prognostic potential of miRNAs in syphilis. While some inconsistencies exist in expression profiles, multiple studies support their role in distinguishing disease stages and predicting progression. Future research should prioritize longitudinal studies to elucidate miRNA dynamics throughout infection and treatment. Integrating miRNA profiling with traditional serological tests could significantly enhance diagnostic accuracy, enabling stage-specific detection and personalized therapeutic strategies. Ultimately, advancing miRNA-based diagnostics could transform syphilis management by overcoming the limitations of existing diagnostic tools.

MicroRNA Patterns Across Syphilis Stages

Primary syphilis is characterized by localized infection, typically presenting as a painless chancre. During this stage, several microRNAs have been identified as potential biomarkers, offering insights into host-pathogen interactions and immune responses. Studies indicate that miR-19b-3p, miR-21-5p, and miR- 16-5p are significantly downregulated in patients with primary syphilis compared to healthy controls. These miRNAs are associated with immune response regulation and inflammation suppression, suggesting their reduced expression may contribute to an impaired host defense mechanism (Lu, et al, 2017).

MiR-1, miR-12 and miR-25 are upregulated in primary syphilis, indicating their potential involvement in pathogen recognition and host immune defense mechanisms. In a recent study by Helsey (2023), combining these three upregulated miRNAs exhibited superior diagnostic accuracy compared to individual miRNAs, achieving an area under the curve (AUC) of 0.95, indicating their potential use in early syphilis detection.

MiR-223-3p expression has shown inconsistent findings across different studies. While Huang, et al (2020) and Yang, et al (2021) reported an upregulation of miR-223-3p in syphilis patients, another study by Long, et al (2020) observed a downregulation of the same miRNA. These discrepancies may arise due to variations in study populations, including sex, ethnicity and underlying health conditions, which could influence miRNA expression. Furthermore, differences in methodologies, miRNA profiling techniques and statistical thresholds could contribute to variability between studies (Helsey, 2023).

Notably, additional research has indicated that miR-142-3p is significantly elevated in patients with secondary syphilis and plays a role in immune suppression. While its involvement in primary syphilis remains unclear, it highlights the potential for miRNAs to act as stage-specific biomarkers. In the study of Yang, et al (2021), a number of microRNAs were identified as specific biomarkers for secondary syphilis. The most significant of these was miR- 142-3p, followed by miR-3180, miR-675-5p, miR-98-5p, miR-3195, miR-6132, miR-4498, miR-5100, miR-6860, and miR-1229-5p. These microRNAs were found to be upregulated in secondary syphilis, providing a contrast to healthy controls and indicating their potential as diagnostic markers for this stage of infection. MiR-142-3p showed the most significant fold change, suggesting its strong association with the host immune response to *T. pallidum*. Patients with erythema-positive lesions had higher miR-142-3p levels than those without skin lesions, suggesting a

possible role in inflammatory responses. Regarding clinical correlation and population differences, miR-142- 3p levels were higher in males than females. Also, the elderly group showed higher miR-142-3p expression compared to younger individuals. Although the difference between the results was not statistically significant, it shows that variations in study populations could influence miRNA expression.

MiR-195-5p has also been identified as a key biomarker in syphilis, particularly in immune suppression and disease progression. In the same study, it was upregulated in secondary syphilis and serofast states, making it useful for distinguishing these conditions from primary syphilis. Individually, both miR-223-3p and miR- 195-5p have high specificity for syphilis stages, but their combined analysis significantly improves diagnostic precision. Using both of them together enables better differentiation between primary and secondary syphilis (Yang, 2021).

Neurosyphilis is a severe complication of syphilis that occurs when *T. pallidum* invades the central nervous system (CNS). It can develop at any stage of syphilis but is most commonly seen in untreated or inadequately treated cases. Diagnosing neurosyphilis remains challenging due to its reliance on cerebrospinal fluid (CSF) analysis. MicroRNAs have emerged as potential non-invasive biomarkers for neurosyphilis detection. As discovered by Chen, et al, (2019), miR-590-5p, miR-570-3p, and miR-570-5p were found to be significantly elevated in both serum and CSF samples of neurosyphilis patients, suggesting their potential as CNS-specific biomarkers. On top of that, miR- 21-5p was specifically upregulated in CSF samples, indicating its strong association with CNS involvement in neurosyphilis. In a similar study of neurosyphilis by Wu, et al, (2021), miR- 503-5p and miR-664b-5p were upregulated in PBMCs, suggesting a systemic immune response to neurosyphilis.

Wu, et al (2021) also identified several microRNAs as potential biomarkers for differentiating secondary syphilis and neurosyphilis from healthy controls. Specifically, they found that hsa-miR-21-5p, hsa-miR-21-3p, hsa-miR-503-5p, hsa-miR-148a-3p, hsa-miR- 664b-5p, hsa-miR-1288-3p, hsa-miR-6131, hsa-miR-6752-3p, and hsa-miR-6512-5p were upregulated in both secondary syphilis and neurosyphilis, with higher expression levels in secondary syphilis, distinguishing it from healthy individuals.

Conversely, miR-93-3p exhibited decreased expression in both serum and CSF, with a more pronounced reduction in serum. This downregulation may indicate its role in immune suppression and potential involvement in blood- brain barrier disruption (Chen, et al, 2019). Moreover, miR-6512-5p and miR-1288-3p were found to be significantly downregulated in PBMCs, suggesting a consistent association with progressive neurosyphilis pathology.

Both studies performed receiver operating characteristic (ROC) analysis to evaluate the diagnostic accuracy of miRNA- based neurosyphilis detection. Wu, et al (2021) found that a 13-miRNA panel achieved 100% accuracy in distinguishing neurosyphilis from healthy controls, while a subset of miRNAs, including miR-503-5p and miR-148-3p, distinguished neurosyphilis from secondary syphilis with 96.8% accuracy. Chen, et al (2019) demonstrated that exosomal microRNA panels, particularly miR-590-5p and miR-570-3p, significantly improved neurosyphilis detection accuracy. They are considered strong candidates for CNS-specific markers due to their consistent elevation in neurosyphilis patients.

A combination of PBMC-derived and exosomal microRNAs can improve early neurosyphilis detection. The use of miR-570-3p and miR-590-5p as CNS-specific markers may allow for blood-based diagnostics, reducing the reliability of lumbar punctures. However, validation in larger cohorts is necessary as both studies used relatively small sample sizes.

Longitudinal studies are needed to track miRNA changes over time and validate their role in neurosyphilis progression. Furthermore, these advancements emphasize the potential use of microRNAs as non-invasive instruments in the diagnosis and management of neurosyphilis, creating much more accessible and precise diagnostic tools.

Studies utilizing ROC curve analysis have demonstrated that specific miRNA expressions, such as miR-185-3p and miR-660- 3p were highly accurate for distinguishing patients with early latent syphilis from those with secondary syphilis, thereby improving stage- specific diagnostics. While miR-125a-3p alone was highly accurate in distinguishing the normal population from syphilis patients, when combined with miR-660-3p, the accuracy and specificity in distinguishing the normal population from patients with ELS, and ELS from those with SS increased significantly (Zhang, et al, 2022).

Serofast syphilis, in contrast to latent syphilis, remains a poorly defined clinical state with no universally accepted criteria. Reports indicate that

15% to 41% of treated syphilis patients develop SF, though these rates vary based on the stage of infection, pretreatment antibody levels, and the timing of post-treatment assessments. While serofast does not indicate an active infection or high infectivity, it remains diagnostically challenging due to their persistent serological reactivity, making it difficult to differentiate it from latent syphilis or residual immune responses to *Treponema pallidum*.

Recent studies have identified several miRNAs associated with serofast syphilis, providing new insights into its pathogenesis and potential biomarkers for its detection. Among these, miR1908-3p has emerged as the most promising diagnostic marker, demonstrating high diagnostic accuracy, sensitivity, and specificity with an AUC value of 0.901, 92.3% sensitivity, and 91.7% specificity, in differentiating serofast patients from serologically cured individuals and healthy controls. When combined, all four miRNAs – miR-197- 3p, miR-1908-3p, miR- 1273g-3p, and miR-4485-5p, showed a strong capacity to differentiate SF from SC or HC, with an AUC value of 0.904–0.949, 92.3 – 100% sensitivity, and 83.3–91.7% specificity (Liu, et al, 2023).

Other miRNAs such as miR-195-5p, miR- 223-3p, and miR-589-3p, have also been identified as key regulators of the serofast state (Huang, et al, 2020). A different study confirmed the role of miR-195-5p, miR-223-3p, and miR-101-3 p showing that these were consistently upregulated across various syphilis stages, including primary, latent, serofast, and serologically cured states. While the combination of miR-223-3p and miR-195-5p exhibited strong biomarker potential, showing 93.9% sensitivity and 83.3% specificity in distinguishing serofast patients from healthy individuals (Yang, et al, 2021).

Among the identified miRNAs, miR-101- 3p stands out for its potential to differentiate serofast syphilis from latent syphilis. MiR-101-3p has been significantly upregulated in SF patients compared to secondary and untreated syphilis cases. This miRNA has been shown to downregulate toll-like receptor 2 (TLR2) expression, leading to reduced cytokine production that may impair bacterial clearance and contribute to the persistence of antibody titers in serofast patients. This mechanism suggests that miR-101-3p levels may influence the progression of syphilis into different subtypes, particularly in determining whether a patient develops a serofast state or transitions into latent syphilis. One of the most notable findings from Huang, et al (2020), is that miR- 101-3p is upregulated in serofast patients compared to those with latent syphilis.

Current serological tests are unable to differentiate between these two conditions, leading to diagnostic challenges. However, measuring miR-101-3p levels offers a potential molecular approach to making this distinction, as its expression is significantly higher in serofast patients than in those with latent syphilis. Despite this promising finding, a 2021 ROC study curve analysis by Yang, et al (2021), found that neither miR-195-5p, miR-223-3p, nor miR-101-3p could reliably differentiate latent syphilis from serofast status. This indicates that while these miRNAs show strong diagnostic potential, additional studies are needed to validate miRNAs potential for differentiating between latent syphilis and serofast state.

Diagnostic Accuracy of MicroRNA-based Assays and Conventional Diagnostic Methods

The diagnosis of syphilis relies primarily on serological tests, which include non- treponemal tests (NTTs) like the Rapid Plasma Reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) test, as well as treponemal tests (TTs) such as the *Treponema pallidum* particle agglutination assay (TPPA) and enzyme immunoassays (EIAs). However, these tests have limitations in sensitivity and specificity, particularly in cases of early or latent syphilis, serofast status, and reinfections (Luo, et al, 2021).

On the subject of the diagnostic performance of MicroRNA-based assays, detecting syphilis at various stages has shown high specificity and high sensitivity. This result is of great significance since its performance surpasses the traditional serological test assay in potentially distinguishing the active form of syphilis from cured syphilis. The miRNA panels enhance diagnostic precision by minimizing false positives and false negatives observed in serological testing.

In spite of its promising findings, several challenges must be addressed before integrating miRNA-based assays in clinical practice. Addressing these challenges could make the advances more reliable and efficient. The challenges to be addressed include the need for standardization since there is apparent variability in miRNA expression across different studies which could help improve and establish reliable diagnostic thresholds. Moreover, there are

Table 10. Sensitivity and specificity of syphilis diagnostic tests and microRNA biomarkers

Test Type	Sensitivity	Specificity	Limitations
Non-Treponemal Tests (RPR, VDRL)	High in secondary syphilis (97-100%) but lower in primary syphilis (62-78%)	Moderate	Prone to biological false positives and serofast status
Treponemal Tests (TPPA, FTA-ABS, EIA, CIA)	100% in secondary syphilis, lower in latent syphilis (86-98%)	High (99%)	Cannot differentiate between active and past infections
MicroRNA-based Assays (miRNA qPCR, RNA-seq)	High (miR-101-3p, miR-195-5p, and miR-223-3p can distinguish syphilis patients from non-syphilis patients)	High (specific miRNA profiles can differentiate serofast from cured states)	Requires further validation but offers a non-invasive biomarker approach

still diagnostic limitations for the miRNAs so the selection must be thorough to ensure high sensitivity, high specificity and a good quality diagnostic tool. Given that serological tests maintain their status as the standard due to their accessibility, cost-effectiveness and broad applicability to the disease, incorporating miRNA profiling holds a promising potential to enhance early disease detection, staging and treatment accuracy, and treatment monitoring. This is also a good alternative especially for neurosyphilis detection as it uses an invasive diagnostic procedure. In addition, future research should further validate the capabilities of these biomarkers in larger cohorts and focus on exploring point-of-care applications to improve syphilis diagnostics.

CONCLUSION

The findings of this systematic review highlight the potential of microRNAs as promising biomarkers for syphilis detection, offering a novel approach to improving diagnostic accuracy. Given the diagnostic challenges posed by *Treponema pallidum*, particularly its ability to remain latent and mimic other diseases, current diagnostic methods often lack sensitivity and specificity. The analysis of existing literature suggests that distinct microRNA expression profiles may serve as reliable indicators of syphilis infection, allowing earlier detection and better disease monitoring. However, discrepancies exist in the reported expression patterns of certain microRNAs, with some studies presenting conflicting findings regarding their correlation with disease progression. These inconsistencies may arise due to methodological variations, differences in patient populations, or the timing of sample collection. Furthermore, microRNA expression may fluctuate at different disease stages or following treatment, complicating result interpretation. Addressing these inconsistencies requires standardized study protocols to ensure uniformity in microRNA profiling. While preliminary studies demonstrate encouraging results, further large-scale and well-designed research is necessary to validate the clinical utility of microRNA-based assays. By synthesizing current evidence, this review underscores the need for continued research, which could ultimately lead to more sensitive, rapid and non-invasive diagnostic tools for syphilis detection.

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FOOTNOTES

Supplementary Reports

The authors have completed the Data Extraction Forms and Screening Eligibility Forms, PRISMA 2020 checklist, and ROBIS tool and its guidance documents. Available to view at https://rb.gy/6ov956

Conflicting Interest

The authors have no conflicts of interest to declare.

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