Systematic Review

The Role of miRNA as Diagnostic Biomarkers of Non-small Cell Lung Cancer through PCR-based Serum Analysis

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Abstract

Non-small cell lung cancer (NSCLC) is often diagnosed at advanced stages despite the use of conventional diagnostic biomarkers. Circulating serum microRNAs (miRNAs) offer promise as sensitive and specific tools for early detection. Following the PRISMA 2020 guidelines, a systematic review of diagnostic accuracy studies was conducted across PubMed, ScienceDirect, Cochrane, PLOS ONE, Taylor & Francis, and preprint servers, identifying seven eligible studies involving patients with NSCLC. All used PCR-based methods to detect serum miRNA expression and compared findings with traditional biomarkers, including carcinoembryonic antigen (CEA), CYFRA21-1, and squamous cell carcinoma antigen (SCCA). 11 miRNAs were evaluated individually (e.g., miR-216b, miR-762) or in panels. Panels demonstrated the highest diagnostic accuracy (up to 0.969 AUC, 89.19% sensitivity, and 98.33% specificity). This review highlights variation in diagnostic performance by miRNA source, detection platform, and clinical context. Circulating miRNAs, particularly in panels, show strong diagnostic potential. Future studies should validate these findings in multi-ethnic, treatment-naïve cohorts using standardized protocols to support clinical implementation.
Keywords: *non-small cell lung cancer, microRNA, CEA, diagnostic, PCR.*

Peran miRNA sebagai Biomarker Diagnosis Kanker Paru Sel Non-kecil melalui Analisis Serum Berbasis PCR

Abstrak

Kanker paru sel non-kecil (NSCLC) sering kali didiagnosis pada stadium lanjut meskipun telah menggunakan biomarker diagnostik konvensional. MikroRNA (miRNA) serum yang bersirkulasi menawarkan harapan sebagai alat yang sensitif dan spesifik untuk deteksi dini. Berdasarkan pedoman PRISMA 2020, tinjauan sistematis terhadap studi akurasi diagnostik dilakukan di PubMed, ScienceDirect, Cochrane, PLoS One, Taylor & Francis, dan server pracetak. Sebanyak tujuh studi diidentifikasi memenuhi syarat yang melibatkan pasien NSCLC. Seluruh studi menggunakan metode berbasis PCR untuk mendeteksi ekspresi serum miRNA dan membandingkannya dengan biomarker tradisional, termasuk carcinoembryonic antigen (CEA), CYFRA21-1, dan antigen karsinoma sel skuamosa (SCCA). Sejumlah 11 miRNA dievaluasi secara individual (misalnya, miR-216b, miR-762) atau dalam panel. Panel menunjukkan akurasi diagnostik tertinggi (AUC hingga 0,969, sensitivitas 89,19%, spesifisitas 98,33%). Ulasan ini menyoroti variasi sumber miRNA, platform deteksi, dan konteks klinis. miRNA yang bersirkulasi, terutama dalam panel, menunjukkan potensi diagnostik yang kuat. Studi di masa depan harus mevalidasi temuan ini dalam kelompok multi-etnis yang tidak memiliki pengobatan menggunakan protokol standar untuk mendukung implementasi klinis.
Kata kunci: *kanker paru bukan sel-kecil, microRNA, CEA, diagnostik, PCR.*

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with an estimated 1.8 million deaths annually and over 2.4 million new cases reported in 2022.[1] The high fatality rate is largely attributed to late-stage diagnosis, where the one-year survival rate can drop to 33–50%.[2] Non-small cell lung cancer (NSCLC), comprising around 81% of cases, includes subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.[1] While conventional diagnostic tools such as tissue biopsy and imaging modalities remain the standard, they are invasive, costly, and sometimes unsuitable for patients with comorbidities. Hence, liquid biopsy approaches—particularly serum biomarkers—are gaining traction for their non-invasiveness and diagnostic potential.[3] Among these, carcinoembryonic antigen (CEA) has shown robust diagnostic accuracy, outperforming other serum markers in various studies with AUC values consistently above 0.83.[4–6]

In recent years, microRNAs (miRNAs), short, non-coding RNAs that regulate gene expression, have emerged as promising biomarkers for NSCLC. Their expression patterns can reflect tumor behavior, including cell proliferation, angiogenesis, and drug resistance.[7] miRNAs are stable in serum and detectable in early-stage disease, enabling potential improvements in early diagnosis and treatment monitoring.[8] They may also play a role in predicting resistance to agents such as cisplatin.[9] Quantitative reverse-transcriptase PCR (qRT-PCR) remains the gold standard for detecting circulating miRNAs, offering high sensitivity and specificity from minimal serum volume.[10–12] This platform enables precise quantification of miRNA signatures, distinguishing malignant from non-malignant conditions.

Despite encouraging results, miRNA performance variability across studies requires a comprehensive evaluation. Therefore, this systematic review aims to assess the diagnostic accuracy of serum-derived miRNAs measured via PCR in NSCLC and to compare their performance against traditional markers, specifically CEA. While CEA is widely used in clinical settings, its limited sensitivity and specificity, especially in early-stage NSCLC, highlight the urgent need for more reliable diagnostic alternatives such as circulating miRNAs.

Methods

Study Eligibility

In this systematic review, two reviewers (AS, KM) conducted a comprehensive search for relevant studies using the Chrome browser on a Windows operating system. The initial search was performed on October 23, 2024, across several reputable databases, namely PubMed, ScienceDirect, and Cochrane. A second search was conducted on June 4, 2025, targeting additional sources, including PLOS One, Sage Journals, and Taylor & Francis Online. A third search was performed on June 8, 2025, to capture relevant grey literature, including records from ClinicalTrials.gov, Europe PMC (preprints), medRxiv, and bioRxiv. Both searches adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 (PRISMA 2020) guidelines to identify all clinical studies evaluating serum-based PCR detection of miRNA as a diagnostic tool for NSCLC.[13] Details of the search strategies are presented in Table 1. The review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD42024609038) and has been published.

Inclusion Criteria

Studies were included based on a structured PICO framework. The population of interest comprised patients diagnosed with NSCLC. The intervention involved detecting serum-based miRNA expression using PCR methods. The primary comparison was made against using CEA as a diagnostic biomarker. The primary outcomes assessed included diagnostic performance measures such as sensitivity, specificity, and area under the curve (AUC).

To be eligible for inclusion in this systematic review, studies had to meet the following criteria: 1) involve a study population consisting of patients diagnosed with NSCLC; 2) assess serum-based

miRNA expression as a biomarker using PCR methods; 3) include a comparison with CEA as a diagnostic marker; 4) report primary outcomes evaluating the diagnostic performance of miRNA and its comparison in detecting NSCLC, including sensitivity, specificity, and AUC; and 5) published in the English language.

Exclusion Criteria

The exclusion criteria for studies in this systematic review were as follows: 1) use of non-human subjects (e.g., animal models or in vitro experiments without human serum); 2) focus on diseases other than NSCLC; 3) use of analytical methods other than PCR-based techniques for miRNA detection; 4) absence of serum specimens; 5) absence of a comparison with CEA; 6) duplicate publications; and 7) incomplete reporting or insufficient data for extraction and quality assessment.

Study Selection

Following the study search, the first step involved screening the titles and abstracts of all identified records using Rayyan, a web-based tool for systematic review screening.[14] Studies that did not meet the eligibility criteria were excluded at this stage. The remaining studies underwent full-text review to assess their compliance with predefined inclusion and exclusion criteria. This phase thoroughly evaluated each study's methodology, population, intervention, outcomes, and clinical relevance. When essential data was missing or unclear, reviewers emailed corresponding authors to request the information. Studies with no response were excluded. A subset of studies was selected for inclusion in the qualitative synthesis.

Data Extraction

After study selection, the eligible data will be extracted and compiled into a synthesis table. The extracted data will include: 1) the first author's name and year of publication; 2) the region where the study was conducted; 3) study design; 4) population; 5) age characteristics of the population; 6) sample characteristics and size; 7) type of PCR method used; 8) types of miRNA evaluated; 9) miRNA-related outcomes; and 10) CEA-related outcomes.

The outcomes will encompass validation data, including primary outcomes (sensitivity, specificity, AUC, 95% confidence interval (CI), and p-value). Data extraction will be performed independently by two reviewers (AS, KM) and subsequently validated by three additional reviewers (KL, AR, AA). The finalized data will be presented in tabular format.

Critical Review Method

Five reviewers (AS, KM, KL, AR, AA) assessed the selected studies' quality and risk of bias using the Quality Assessment of Diagnostic Accuracy Studies 2 tool. Each study was evaluated across four key domains: patient selection, index test, reference standard, and flow and timing. For each domain, the risk of bias and concerns regarding applicability were judged as "Low," "High," or "Unclear".[15] The results of this evaluation will be summarized in a table to provide an overview of the methodological quality of the included studies.

Results

Eligible Studies

After searching for studies across various databases (bioRxiv, ClinicalTrials.gov, Cochrane Library, Europe PMC [preprint], medRxiv, PLOS One, PubMed, Sage Journals, ScienceDirect, and Taylor & Francis Online) using specific keywords, a total of 267 studies were identified. Studies were screened based on the eligibility criteria outlined in the methods section. Six duplicates were found and removed. At least five reviewers (AS, KM, KL, AR, AA) independently selected the remaining 261 studies based on titles and abstracts alone. A total of 249 studies were excluded for not fulfilling the previously established inclusion criteria. Finally, 12 studies were further screened through full-text review. Two studies were found to be inaccessible, and three had incomplete data, leaving seven studies that met all the criteria. A complete overview of the screening results is presented in Figure 1.

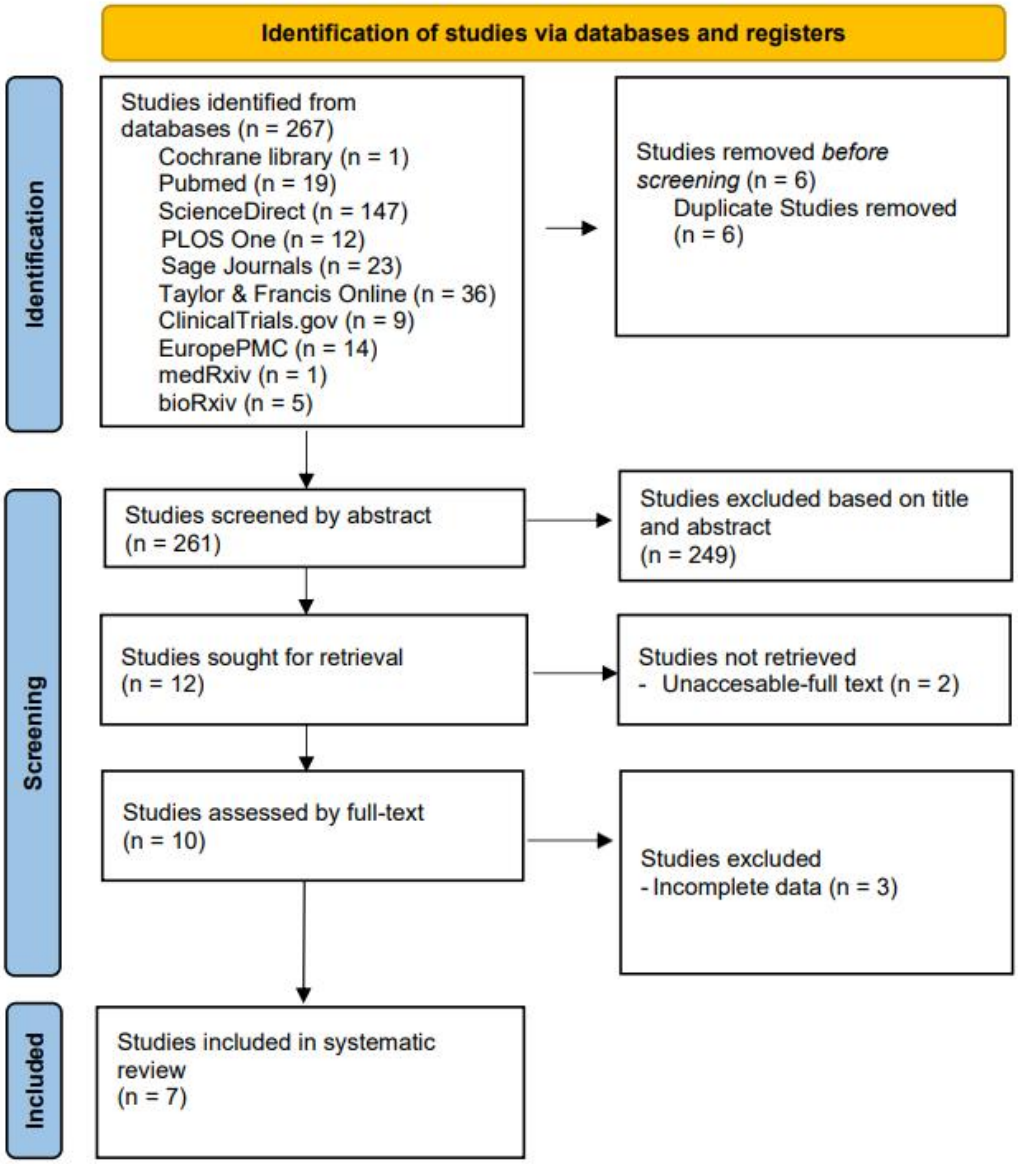

Figure 1. The Results of The Study Selection

Study Characteristics

Seven observational studies from China were included, with sample sizes ranging from 108 to 309 participants. Age categorizations varied by study: most divided samples into groups younger or older than 60 years, while Zhang et al[16] used cutoff points at 53 and 61 years. Control and NSCLC patient groups were the primary samples analyzed. Four studies classified lung cancer subtypes into adenocarcinoma (AD), squamous cell carcinoma (SCC), and large cell carcinoma (LCC).[17–20] Almost all studies employed the qRT-PCR method for miRNA analysis, except one that used Taqman-based qRT-PCR. The miRNAs investigated included miR-10a-5p, miR-196a-5p, miR-762, miR-185, miR-216b, miR-9-5p, miR-21-5p, miR-223-3p, miR-17-5p, miR-29c, and miR-429.[16–22]

Traditional biomarkers such as CEA, CYFRA21-1, and squamous cell carcinoma antigen (SCCA) were also assessed alongside miRNAs. Several studies evaluated combined biomarker panels integrating miRNAs and conventional markers. A comprehensive summary of the study characteristics and biomarker performances is provided in Tables 2 and 3.

Critically Reviewed Studies

The quality assessment of included studies was performed using the QUADAS-2 tool.[15] Overall, the studies showed low risk of bias across most domains, including index test, reference standard, flow, and timing, as well as low concerns regarding applicability in patient selection, index test, and reference standard domains. However, all studies demonstrated an unclear risk of bias in the patient selection domain. Notably, Zhang et al[16] were rated with a high risk of bias in the reference standard and flow and timing domains. Detailed results of the quality assessment and risk of bias are summarized in Table 4.

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Table 1. The Search Strategy

Database	Search Type	Search Terms/Keywords	Search Filters/ Limits	Date of Search	Number of Records Retrieved
Cochrane Library	Free-text + boolean	nsclc AND microrna AND cea AND diagnostic AND serum	Clinical Trials	October 23, 2024	1
PubMed	Free-text + boolean	non-small-cell-lung cancer AND microRNA AND cea AND diagnostic AND PCR	-	October 23, 2024	19
ScienceDirect	Free-text + boolean	“(Non-Small-Cell-Lung Cancer)” AND “(microRNA)” AND “(CEA)” AND “(Diagnostic)” AND “(PCR)”	Research Article	October 23, 2024	147
PLOS One	Free-text + boolean	nsclc AND microrna AND cea AND diagnostic AND serum	Research Article	June 4, 2025	12
Sage Journals	Free-text + boolean	nsclc AND microrna AND cea AND diagnostic AND serum	Research Article	June 4, 2025	23
Taylor & Francis Online	Field-specific boolean	[All: nsclc] AND [All: microrna] AND [All: cea] AND [All: diagnostic] AND [All: serum] AND [Article Type: Article]	Article	June 4, 2025	36
ClinicalTrials.gov	Filter search	Lung Cancer | Other terms: miRNA | Completed studies | Observational studies	Clinical Trials	June 8, 2025	9
Europe PMC (preprint)	Boolean + Filter search	mir AND nsclc AND serum AND (SRC: PPR)	Preprint	June 8, 2025	14
medRxiv	Free-text	mirna cea nsclc	Preprint	June 8, 2025	1
bioRxiv	Free-text	mirna cea nsclc	Preprint	June 8, 2025	5
TOTAL					267

Table 2. Characteristics of Included Studies

Study (Author, Year)	Country	Population (n)	Study Design	Age (n)	Sample [Validation Set] (n)				PCR Method
					Control	NSCLC			
						AD	SCC	LCC	
Bao et al (2018)[17]	China	155	Observational	≤60 (85) >60 (70)	75	50	23	7	qRT-PCR
Chen et al (2020)[18]	China	148	Observational	<60 (62) ≥60 (86)	60	84	64	0	qRT-PCR
Liu et al (2020a)[19]	China	226	Observational	<60 (85) ≥60 (61)	80	88	58	0	qRT-PCR
Liu et al (2020b)[20]	China	165	Observational	<60 (49) ≥60 (56)	60	45	60	0	qRT-PCR
Yang et al (2018)[21]	China	154	Observational	57.03±9.52 (104) 55.05±8.47 (50)	50		104		qRT-PCR
Zhang et al (2019)[16]	China	309	Observational	≤53 (26) >53 (21) ≤61 (33) >61 (39)	47		72		qRT-PCR
Zhu et al (2014)[22]	China	108	Observational	<60 (34) ≥60 (36)	48		70		Taqman-based qRT-PCR

AD (adenocarcinoma); LCC (large cell carcinoma); LC (lung cancer); NSCLC (non-small cell lung cancer); PCR (polymerase chain reaction); qPCR (quantitative polymerase chain reaction); qRT-PCR (quantitative reverse transcriptase polymerase chain reaction); SCC (squamous cell carcinoma); SCLC (small-cell lung cancer)

Table 3. The Comparison of Diagnostic Accuracy

Study (Author, Year)	MicroRNA Biomarker [Validation Set]							Traditional Biomarker [Validation Set]						Panel Biomarker [Validation Set]					
	Type	Regulation	Sensitivity (%)	Specifity (%)	95% IC	AUC	p-value	Type	Sensitivity (%)	Specifity (%)	95% IC	AUC	p-value	Type	Sensitivity (%)	Specifity (%)	95% IC	AUC	p-value
Bao et al. (2018)[17]	miR-10a-5p miR-196a-5p	Up Up	65.98 67.86	72.71 77.57	0.627–0.791 0.711–0.860	0.709 0.785	<0.0001 0.0018	CEA	62.75	74.16	0.685–0.835	0.76	0.0029	Panel 1	76.34	79.26	0.733–0.869	0.801	<0.0001
Chen et al. (2020)[18]	miR-762	Up	72.97	93.33	1.216–4.794	0.874	<0.001	CEA CYFRA21-1	75.00 74.32	91.67 91.80	NR NR	0.826 0.841	NR NR	Panel 2	89.19	98.33	NR	0.969	NR
Liu et al. (2020a)[19]	miR-185	Down	68	78.7	0.709–0.856	0.79	<0.05	CEA	82	53.7	0.600–0.766	0.687	NR	Panel 3	86	70	0.730–0.872	0.808	NR
Liu et al. (2020b)[20]	miR-216b	Down	86.7	75	NR	0.84	NR	CEA CYFRA21-1 SCCA	83.8 81.0 71.4	74.0 73.3 68.3	NR NR NR	0.808 0.813 0.698	NR NR NR	Panel 4	89.5	88.3	NR	0.925	NR
Yang et al. (2018)[21]	miR-9-5p miR-21-5p miR-223-3p	Up Up Up	78.8 68.3 76.9	58.0 78.0 80.0	0.627–0..776 0.69–0.83 0.668–0.811	0.706 0.765 0.744	<0.001 <0.001 <0.001	CEA CYFRA21-1 SCCA	54.8 64.4 35.6	96.0 70.0 92.0	0.673–0.816 0.658–0.803 0.534–0.693	0.749 0.735 0.616	<0.001 <0.001 0.0109	Panel 5a Panel 5b Panel 5c	76.9 55.8 82.7	84.0 92.0 88.0	0.754–0.88 0.736–0.866 0.852–0.932	0.824 0.807 0.886	<0.001 <0.001 <0.001
Zhang et al. (2019)[16]	miR-17-5p	Up	66.7	76.6	0.649–0.814	0.738	<0.001	CEA CYFRA21-1 SCCA	26.4 59.7 26.4	93.6 78.7 95.7	0.523–0.704 0.593–0.776 0.541–0.721	0.617 0.684 0.634	<0.05 <0.05 <0.05	Panel 6	76.4	76.6	0.766–0.904	0.844	<0.05
Zhu et al. (2014)[22]	miR-29c miR-429	Up Down	65.7 54.3	74.1 81.2	0.584–0.759 0.623–0.793	0.676 0.713	0.0004 <0.0001	CEA	22.9	96.8	0.482–0.666	0.576	0.1493	Panel 7a Panel 7b Panel 7c	NR NR NR	NR NR NR	0.622–0.792 0.616–0.787 0.713–0.865	0.712 0.707 0.797	<0.0001 0.0007 <0.0001

AUC (area under curve); CEA (carcinoembryonic antigen); CYFRA21-1 (cytokeratin 19 fragment); IC (interval confidence); NR (not reported); P-value (probability value); qPCR (quantitative polymerase chain reaction); SCCA (squamous cell carcinoma antigen);

Panel 1 = "miR-10a-5p + miR-196a-5p + CEA"; Panel 2 = "miR-762 + CEA + CYFRA21-1"; Panel 3 = "miR-185 + CEA"; Panel 4 = "miR-216b + CEA + CYFRA21-1 + SCCA"; Panel 5a = "miR-9-5p + miR-21-5p + miR-223-3p"; Panel 5b = "CEA + CYFRA21-1 + SCCA"; Panel 5c = "miR-9-5p + miR-21-5p + miR-223-3p + CEA + CYFRA21-1 + SCCA"; Panel 6 = "miR-17-5p + CEA + CYFRA21-1 + SCCA"; Panel 7a = "miR-29C + CEA"; Panel 7b = "miR-429 + CEA"; Panel 7c = "miR-29C + miR-429 + CEA"

Table 4. The Result of Critical Review of Eligible Studies Based on the "QUADAS-2"[15]

Study	Risk of Bias				Applicability Concern		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Bao et al[17]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;
Chen et al[18]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;
Liu et al[19]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;
Liu et al[20]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;
Yang et al[21]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;
Zhang et al[16]	unclear risk	low risk;	low risk;	high risk	low risk;	low risk;	low risk;
Zhu et al[22]	unclear risk	low risk;	low risk;	low risk;	low risk;	low risk;	low risk;

MicroRNA Expressions (Single Biomarkers)

Seven eligible studies discussed different types of miRNA, with 11 types of miRNA.[16–22] In each study, serum miRNA was used as a potential biomarker for NSCLC. Among the 11 types of miRNA, there was a variation among sensitivity (54.3%–86.7%), specificity (58.0%–93.3%), and AUC (0.676–0.874). The lowest sensitivity came from miR-429 (54.3%), the lowest specificity came from miR-9-5p (58.0%), and the lowest AUC came from miR-29c (0.676).[21,22] Conversely, the highest sensitivity came from miR-216b (86.7%), the highest specificity came from miR-762 (93.3%), and the highest AUC came from miR-762 (0.874).[18,20] Three studies reported the downregulation of specific miRNAs (miR-185, miR-216b, and miR-429) in NSCLC patients, indicating a significant decrease in their expression compared to control groups.[19,20,22] In contrast, eight studies identified the upregulation of several miRNAs (miR-10a-5p, miR-196a-5p, miR-762, miR-9-5p, miR-21-5p, miR-223-3p, miR-17-5p, and miR-29c, showing significantly higher expression levels in NSCLC patients relative to healthy controls or adjacent normal tissues.[16–18,21,22] Complete details of miRNA expression can be found in Table 3.

Seven studies have evaluated CEA serum levels as potential biomarkers for NSCLC.[16–22] The reported sensitivity ranged from 22.9% to 83.8%, specificity from 53.7% to 96.8%, with AUC values spanning 0.576 to 0.826. The p-values were significant in three studies (0.0029, <0.001, <0.05), while one study reported non-significant results (0.1493), and the remaining three studies did not report p-values.[16–22] The highest diagnostic accuracy was observed in Chen et al,[18] where CEA demonstrated a sensitivity of 72.97%, specificity of 93.33%, and an AUC of 0.874, suggesting excellent discriminative ability for NSCLC. Conversely, the lowest performance was noted in Zhu et al,[22] with a sensitivity of 22.9%, specificity of 96.8%, and an AUC of 0.576, indicating moderate utility as a standalone biomarker. Complete diagnostic values for CEA are summarized in Table 3.

CYFRA-21-1 Expression (Single Biomarker)

Four investigations have explored serum levels of cytokeratin 19 fragment (CYFRA-21-1) for diagnosing NSCLC.[16,18,20,21] These studies report sensitivity values ranging from 59.7% to 81.0%, with specificity between 70.0% and 91.8%. The AUC estimates span from 0.684 to 0.841. All findings indicate statistically significant differences ($p<0.05$), except for Chen et al[18] and Liu et al[20] who didn't report. Chen et al18 reported the most robust performance, who found a sensitivity of 74.32%, specificity of 91.80%, and an AUC of 0.841, demonstrating strong discriminative capacity for NSCLC detection. Conversely, Zhang et al[16] observed a sensitivity of 59.7%, specificity of 78.7%, and an AUC of 0.684, suggesting moderate diagnostic effectiveness. Detailed

diagnostic metrics for CYFRA-21-1 are provided in Table 3.

SCCA Expression (Single Biomarker)

Three studies have examined serum levels of SCCA to aid in diagnosing NSCLC.[16,20,21] These studies report sensitivity rates ranging from 26.4% to 71.4%, with specificity values between 68.3% and 95.7%. The reported AUC falls within the range of 0.616 to 0.698, indicating a moderate to high ability to distinguish between diseased and healthy individuals. Two findings show statistically significant differences ($p < 0.05$), except for Liu et al[20] who didn't report. The most notable performance was documented by Liu et al,[20] who achieved a sensitivity of 71.4%, specificity of 68.3%, and an AUC of 0.698. On the other hand, Yang et al[21] reported a sensitivity of 35.6%, specificity of 92.0%, and an AUC of 0.616, indicating a moderate level of accuracy. Complete diagnostic statistics are provided in Table 3.

Panel Biomaker

Seven studies have assessed the combined use of multiple biomarkers as a panel to improve diagnostic accuracy in NSCLC.[16–22] Eleven panels typically include a combination of serum proteins, genetic markers, or other molecular indicators. Reported sensitivities for these panels range from 55.8% to 89.50%, specificities vary from 70.0% to 98.33%, while Zhu et al[22] did not report both.[9] The AUC values in these studies generally fall between 0.707 and 0.969, indicating high discriminative power. Most studies demonstrated statistically significant differences ($p < 0.05$), with three not reporting their p-values.[18–20] The best AUC value of 0.969 was achieved by Chen et al,[18] who combined the biomarkers miR-762, CEA, and CYFRA21-1. This panel demonstrated excellent diagnostic performance, with a sensitivity of 89.19% and specificity of 98.33%. On the other hand, the lowest AUC value of 0.707 was reported by Zhu et al[22], who used a combination of miR-429 and CEA biomarkers as part of Panel 7b, with no reported sensitivity and specificity. A complete summary of diagnostic statistics is provided in Table 3.

Discussion

MicroRNA

miRNA is an RNA molecule that does not code for proteins, usually spanning 21 to 25 nucleotides, and regulates gene expression after transcription. This molecule binds to specific messenger RNA (mRNA), inhibiting translation and gene suppression. MiRNAs are extensively dispersed in eukaryotic cells, taking part in various biological activities, including cell growth, development, proliferation, and apoptosis. The abnormal regulation of miRNA expression is associated with various diseases, particularly cancer, where changes in miRNA levels can serve as potential biomarkers for diagnostic and prognostic purposes.[7,18]

In the context of NSCLC, miRNAs are increasingly recognized for their dual roles as oncogenes or tumor suppressors, depending on the genes they regulate.[23] This review reported several miRNAs across studies as differentially expressed in NSCLC. Among those upregulated were miR-10a-5p, miR-196a-5p, miR-762, miR-9-5p, miR-21-5p, miR-223-3p, miR-17-5p, and miR-29c.[16–18,21,22] These miRNAs are frequently associated with tumor-promoting effects, such as enhancing proliferation, angiogenesis, metastasis, and resistance to therapy.[24] Conversely, downregulated miRNAs, including miR-185, miR-216b, and miR-429, often act as tumor suppressors.[19,20,22] Their reduced expression can result in the loss of cell cycle control and increased oncogenic signaling.[24] While this review focuses on a select group of miRNAs identified in included studies, many others are known to be involved in NSCLC, and their clinical application remains an area of active investigation.

MicroRNA in Serum as New NSCLC Diagnostic Biomarkers

Based on Table 3, 11 types of miRNAs are significantly expressed in patients with NSCLC, one of which is miR-762, reported by Chen et al[18] with an AUC of 0.874. Other miRNA types also have the potential to serve as an NSCLC diagnostic biomarker. Their detection capabilities

may be further enhanced by combining them simultaneously.

A total of three studies reported significant downregulation of miR-185, miR-216b, and miR-429 in NSCLC tissues compared to adjacent non-tumor tissues.[19,22,25] MiR-185 has been shown to function as a tumor suppressor by directly targeting AKT1, thereby inhibiting cell proliferation, migration, and invasion in NSCLC cells. Additionally, miR-185-5p has been implicated in regulating the MALAT1/MDM4 axis, further supporting its role in tumor suppression.[26]

MiR-216b is significantly downregulated in the serum exosomes of NSCLC patients, and its low expression correlates with advanced TNM stage, lymph node metastasis, poor tumor differentiation, and reduced overall and disease-free survival.[20] Functionally, miR-216b acts as a tumor suppressor, targeting key oncogenes such as FOXM1, SOX9, and c-Jun, inhibiting cell proliferation, migration, invasion, and promoting apoptosis. Its levels were found to increase after surgical resection, and persistently low levels were associated with a higher likelihood of postoperative metastasis. Multivariate analysis confirmed serum exosomal miR-216b as an independent prognostic factor for NSCLC.[20]

Similarly, miR-429 is also downregulated in NSCLC serum and associated with advanced TNM stage and lymph node metastasis, suggesting a role in tumor progression.[22] MiR-429 regulates epithelial-mesenchymal transition (EMT) by targeting transcription factors like ZEB1 and ZEB2, thereby influencing cell invasion and migration.[26] Kaplan–Meier survival analysis further reveals that patients with low miR-429 expression have significantly shorter overall survival, underscoring its potential as a prognostic biomarker.[27]

MiR-10a-5p is upregulated in NSCLC tissues and serum, as demonstrated by Bao et al.[17] Its higher expression significantly correlates with advanced TNM stage and lymph node metastasis. Functionally, miR-10a-5p acts as an oncogenic miRNA by targeting the tumor suppressor PTEN, activating the AKT/ERK signaling pathway, which promotes tumor cell proliferation and metastasis. This mechanistic role aligns with findings by Yu et al,[28] who reported that miR-10a-5p enhances NSCLC progression via PTEN suppression. Overexpression of miR-10a-5p has also been observed in other cervical, thyroid, and AML cancers, supporting its broader oncogenic role. Despite its oncogenic behavior, the exact regulatory networks involving miR-10a-5p remain incompletely understood and warrant further functional validation in NSCLC.[17]

MiR-196a-5p is also significantly overexpressed in NSCLC tissue and serum, with elevated levels associated with later tumor stages and lymph node metastasis, as Bao et al[17] reported. It promotes tumorigenesis by targeting HOXA5, a gene involved in epithelial differentiation and tumor suppression, thereby facilitating increased cell proliferation and invasion. Liu et al[29] confirmed this mechanism in NSCLC cells, and other studies have reported similar oncogenic roles of miR-196a-5p in gastric and colorectal cancer. Additionally, miR-196a-5p has been linked to chemoresistance, with lower expression enhancing cisplatin sensitivity in NSCLC cells.[30] These findings support its potential as a prognostic marker and therapeutic target in NSCLC management.

MiR-762 is upregulated in NSCLC serum and significantly correlates with advanced stage, lymph node metastasis, poor differentiation, gefitinib resistance, and reduced overall and relapse-free survival. Functionally, it acts as an oncomiR by promoting cell proliferation and S-phase progression, while its knockdown inhibits tumor growth in vitro. Bioinformatic analysis links miR-762 to cancer-related pathways such as Ras and TNF signaling. These findings are consistent with studies in other cancers, including breast and head and neck, where miR-762 targets genes like IRF7, PHLPP2, and FOXO4, reinforcing its oncogenic role.[18]

MiR-9-5p is significantly upregulated in the serum of NSCLC patients and shows association with lymphatic and distant metastasis, suggesting its potential role in tumor dissemination.[21] Functionally, miR-9-5p acts as an oncomiR by promoting cancer progression by targeting tumor suppressors such as SOX7, which contributes to enhanced invasion and adhesion of lung cancer

cells.[31] Although results in other cancers are mixed, its overexpression in NSCLC aligns with findings by Xu et al,[32] who linked elevated miR-9 levels to poor prognosis.

MiR-21-5p, one of the most widely studied oncomiRs, is also significantly upregulated in NSCLC serum and correlates with advanced TNM stage and T factor, particularly in squamous cell carcinoma subtypes.[21] It promotes tumor progression by targeting PTEN, PDCD4, and other tumor suppressors, enhancing proliferation, survival, and metastasis.[33] Its diagnostic performance was moderate (AUC = 0.765), and its expression patterns are consistent with other studies highlighting miR-21's utility as a diagnostic and prognostic biomarker in lung cancer.

MiR-223-3p is upregulated in NSCLC serum and lung cancer cell lines (e.g., A549), and enhances tumor progression via activation of the NF-κB signaling pathway.[21,34] Although its expression did not correlate with specific clinicopathological features in this study, its diagnostic performance (AUC = 0.744) was notable, especially in the Xuanwei population (AUC = 0.752). Prior studies by Zhang et al[31] and Geng et al[35] have reported even higher diagnostic accuracy, reinforcing its role as a potential biomarker in lung cancer detection.

MiR-29c is significantly upregulated in the serum of NSCLC patients and shows strong diagnostic potential with an AUC of 0.887, particularly effective in detecting early-stage disease and small tumors (<3 cm).[22] Biologically, miR-29c has dual roles depending on cancer type; while it functions as a tumor suppressor in some contexts by targeting genes like DNMT3A and MCL1, in NSCLC, its upregulation may reflect a compensatory or cancer-type–specific regulatory response.[22]

Most included studies employed qRT-PCR to quantify serum or exosomal miRNA expression in NSCLC patients, typically using SYBR-based detection and the $2^{-\Delta\Delta Ct}$ method. While this provides a reliable baseline, several methodological differences may influence the results' sensitivity, reproducibility, and comparability. For example, Liu et al[20] used the mirVana kit and performed triplicate testing with NanoDrop-based quality control, which improves analytical accuracy and minimizes random error. Meanwhile, Liu et al[20] and Zhang et al[16] focused on exosomal miRNAs, requiring an additional exosome isolation step (ExoQuick), which may enhance specificity by capturing vesicle-enclosed miRNAs protected from RNase degradation. Furthermore, Zhang et al[16] used endogenous miR-16-5p for normalization instead of synthetic spike-ins; this approach reflects physiological conditions more accurately and avoids variability due to synthetic control recovery. Zhang's use of GeneCopoeia's all-in-one PCR kits also streamlines the process but may differ in amplification efficiency compared to commonly used SYBR systems.[16] While methodologically valid, these variations underscore the need for standardized miRNA assessment protocols to ensure consistency and comparability in future diagnostic research.

Variation Gap in Diagnostic Accuracy Across Studies

The variations in sensitivity and specificity between studies may be due to a combination of biological, technical, and population factors. For example, the sensitivity difference between miR-429 (54.3%), and miR-216b (86.7%) is possibly influenced by the type of miRNA studied, specimen source, and study approach. MiR-429 is extracted from total serum and shows more prognostic potential, while miR-216b, derived from serum exosomes, is more stable and specific to tumor cells, with a larger sample size and more even stage distribution, which improves diagnostic accuracy.[20,22] On the other hand, the difference in specificity between miR-9-5p (58.0%) and miR-762 (93.33%) may also be explained by variations in population and methodological approaches. Yang's study involved the local population of Xuanwei with unique environmental factors, a smaller sample size, and comparators limited to cancer-free individuals without benign lung disease control, which may decrease specificity.[21] Meanwhile, Chen et al[18] used standardized RNA isolation and normalization methods, and more representative healthy controls. In addition, miR-9-5p expression is known to vary across cancer

types, which may affect the consistency of its detection.[21] Thus, this variation gap reflects the importance of biomarker selection, sample source, study population, and laboratory methods in assessing the diagnostic value of miRNAs for NSCLC.

The significant differences in sensitivity and specificity between several NSCLC biomarker panels, such as between the CEA + CYFRA21-1 + SCCA panel (55.8%) and the miR-216b + CEA + CYFRA21-1 + SCCA panel (89.5%), as well as between the miR-185 + CEA panel (70.0%) and the miR-762 + CEA + CYFRA21-1 panel (98.33%) highlight the impact of biomarker composition, sampling methods, timing, and study population characteristics, reflecting the influence of biomarker composition, sampling method, time of collection, and study population characteristics.[18–21] Liu et al[20] study incorporating the exosomal miRNA miR-216b was shown to significantly improve sensitivity over the classic panel without miRNAs, whereas Chen et al[18] demonstrated improved specificity through the combination of miR-762 with two classic markers. In addition, other factors contributing to these variations include post-therapy sampling and exosomal serum isolation in Liu et al[20] study, compared to total blood specimens and environmentally exposed populations in Yang et al[21]. The choice of miRNA type was also important, with miR-762 showing a strong correlation with NSCLC clinical parameters, whereas miR-185 did not provide such strong clinical value, thus explaining the variation gap between panels.[19]

Measurement Methods for Circulating miRNAs in NSCLC Studies

Accurate quantification of circulating miRNAs is essential in evaluating their diagnostic utility for NSCLC. Most of the included studies employed qRT-PCR to measure serum or exosomal miRNA levels, but with methodological variations that could influence diagnostic performance. Two main qRT-PCR detection chemistries were used: SYBR Green and TaqMan. SYBR Green is a cost-effective, fluorescence-based intercalating dye that binds to all double-stranded DNA, including non-specific products like primer-dimers.[36] Its simplicity and affordability make it a popular choice, as exemplified by Bao et al,[17] who used SYBR-based qRT-PCR for measuring miR-10a-5p and miR-196a-5p in serum samples. However, the lack of specificity requires careful primer design and melt curve validation. In contrast, TaqMan probes, used in studies like Zhu et al[22] and Liu et al[20], offer higher specificity through sequence-specific fluorescent probes, reducing false positives. Recent studies continue to evaluate both methods; for instance, Poel et al,[37] Marzi et al,[38] and Want et al[39] have validated SYBR-based qRT-PCR as analytically efficient for circulating miRNA profiling, though standardization remains challenging. These methodological differences highlight the need for harmonized protocols to ensure cross-study comparability in miRNA biomarker research.

Traditional Biomarkers in NSCLC

Traditional biomarkers such as CEA, CYFRA21-1, and SCCA become detectable in the blood due to molecular changes occurring in lung cancer cells.[4] These changes include abnormal activation of signaling pathways, cytoskeletal damage, and cellular stress responses that disrupt normal barriers and allow intracellular proteins to leak or be actively secreted into the bloodstream. Their presence in serum reflects ongoing cancer biology and helps identify tumor type and progression.[40–42]

CEA is a glycoprotein involved in cell adhesion and is normally repressed after fetal development.[43] Although first identified and extensively studied in colorectal cancer, the mechanisms regulating CEA expression and release are shared across various epithelial malignancies, including NSCLC. In NSCLC, especially adenocarcinoma, CEA becomes re-expressed through oncogenic signaling pathways such as the RAS/MAPK and PI3K/AKT cascades, which activate transcription factors like c-Myc and STAT3, leading to increased CEACAM5 gene expression.[40] Additionally, loss of cell polarity, a hallmark of epithelial cancers, causes CEA to mislocalize from the apical membrane toward the basolateral surface. This occurs due to disruption of tight junctions and polarity-regulating proteins such as PAR3 and aPKC, facilitating CEA

cleavage by phospholipases and matrix metalloproteinases (MMPs) and its subsequent release into the circulation.[44–46] Therefore, its serum elevation reflects increased production and altered cellular architecture within the tumor.

CYFRA21-1 is a soluble fragment of cytokeratin 19 (KRT19), a cytoskeletal protein essential for structural stability in epithelial cells.[47] In NSCLC, especially squamous subtypes, tumor cells experience high turnover and undergo frequent apoptosis and necrosis, which activates intracellular proteases.[41] Enzymes such as caspase-3 (during apoptosis), calpain (in calcium-mediated stress), and cathepsins (released from lysosomes) cleave full-length KRT19 into smaller, soluble fragments like CYFRA21-1. These fragments are then released from damaged cells into the extracellular space and enter the bloodstream due to disrupted membrane integrity and leaky tumor vasculature. Thus, serum CYFRA21-1 levels increase in response to active tumor cell death and cytoskeletal breakdown, especially in squamous tumors where KRT19 is highly expressed.[48–50]

SCCA belongs to the serpin family of protease inhibitors, specifically the isoforms SERPINB3 (SCCA1) and SERPINB4 (SCCA2). These proteins inhibit cysteine and serine proteases, such as cathepsins and chymotrypsin, to protect squamous epithelial cells from protease-mediated damage.[51] In squamous NSCLC, SERPINB3/B4 expression is upregulated as part of the squamous differentiation program, which is regulated by transcription factors like ΔNp63, SOX2, and NOTCH1. These serpins support tumor survival by blocking apoptosis, stabilizing lysosomes, and reducing cellular stress. When tumor cells experience oxidative stress or damage, SCCA can be passively leaked or secreted in exosomes, entering the bloodstream.[42,52,53] Because SERPINB3/B4 are tightly linked to the identity of squamous epithelial cells, their overexpression and release into serum indicate squamous lineage commitment, tumor aggressiveness, and treatment resistance.

Evaluating Single miRNAs Against Traditional NSCLC Biomarkers

Although traditional serum biomarkers such as CEA, CYFRA21-1, and SCCA remain widely used in NSCLC diagnostics, their diagnostic accuracy is variable and often limited, as measured by the AUC. For instance, CEA achieved a relatively high AUC of 0.76 in Bao et al[17] and 0.808 in Liu et al[20], demonstrating moderate discriminative ability. In some cases, this performance surpassed individual miRNAs; miR-185, for example, Liu et al[19] reached an AUC of 0.79, only slightly higher than CEA, while miR-29c in Zhu et al[22] had a lower AUC of 0.676 compared to CEA's 0.76 in Bao et al.[17]

Despite these exceptions, single miRNAs generally demonstrated higher or comparable AUC values in multiple independent studies. MiR-762 reached an AUC of 0.874, significantly exceeding the combined AUCs of CEA (0.826) and CYFRA21-1 (0.841) in the same dataset.[18] Similarly, miR-196a-5p showed an AUC of 0.785 in Bao et al,[17] outperforming CEA (0.76). Furthermore, miR-429 yielded an AUC of 0.713 in Zhu et al,[22] notably superior to CEA's AUC of 0.576 within the same study. miR-17-5p, evaluated in Zhang et al,[16] achieved an AUC of 0.738, compared to CEA's 0.617 in the same study, further emphasizing the higher diagnostic accuracy of certain miRNAs.

In summary, despite occasional exceptions, single miRNAs often show superior or comparable diagnostic accuracy to traditional biomarkers. Their biological relevance and ability to detect disease early on enhance their clinical value. These findings support the growing view that circulating miRNAs offer a more precise and informative biomarker class for NSCLC diagnosis.

Diagnostic Advantage of Panel Biomarkers in NSCLC Serum

Whether miRNA or traditional protein-based, single biomarkers often demonstrate moderate diagnostic utility in NSCLC. For instance, in a study by Bao et al,[17] miR-196a-5p yielded an AUC of 0.785, marginally outperforming CEA, which reached an AUC of 0.76. However, when miR-10a-5p, miR-196a-5p, and CEA were combined into a panel (Panel 1), the diagnostic performance increased, with an AUC of 0.801. Similarly, Chen et al[18] reported that miR-762 alone achieved an AUC of 0.874, surpassing traditional markers CEA (0.826) and CYFRA21-1 (0.841). When combined as Panel 2 (miR-762 + CEA + CYFRA21-1), the AUC improved significantly to 0.969, indicating excellent diagnostic accuracy.

Liu et al[19] demonstrated that miR-185, a downregulated tumor suppressor miRNA, had an AUC of 0.79, higher than CEA alone (AUC 0.687). Integrating miR-185 and CEA (Panel 3) further increased diagnostic accuracy (AUC 0.808). In study by Yang et al,[21] a miRNA-only panel comprising miR-9-5p, miR-21-5p, and miR-223-3p (Panel 5a) yielded an AUC of 0.824, superior to the individual AUCs of any single miRNA in the panel. Adding CEA, CYFRA21-1, and SCCA to this panel (Panel 5c) enhanced the AUC to 0.886, further supporting the combinatorial advantage.

These data collectively indicate that while select miRNAs may outperform traditional biomarkers individually, integrating multiple biomarkers, particularly across mechanistic classes, results in consistently higher diagnostic performance. The superior accuracy of biomarker panels likely arises from their ability to capture a broader spectrum of tumor biology.[23] miRNAs reflect upstream gene regulatory alterations, while traditional protein markers indicate downstream effects such as cytoskeletal degradation, altered differentiation, and apoptosis. This multimodal strategy reduces diagnostic gaps caused by tumor heterogeneity and enhances robustness across histological subtypes, supporting the clinical value of panel-based biomarker approaches in NSCLC.[54]

Standardization, Validation, and Clinical Applicability of miRNA Panels in NSCLC

Although miRNA panels show strong diagnostic potential, clinical implementation is hindered by the lack of standardized protocols and comprehensive validation. Technical inconsistencies, ranging from blood collection and RNA extraction to normalization strategies, complicate reproducibility across studies. Harmonizing pre-analytical and analytical steps, aligned with MIQE or equivalent frameworks, is essential for reliable quantification.[5]

Large, multicenter cohorts are needed for clinical validation to evaluate diagnostic accuracy across NSCLC subtypes and stages. Metrics such as AUC, sensitivity, and specificity should be benchmarked against traditional biomarkers like CEA, CYFRA21-1, and SCCA. Moreover, subgroup analyses by age, histology, and smoking status are crucial for generalizability. These formats can be modeled in the Hu et al[55] study.

Beyond diagnosis, miRNA panels may hold value as prognostic tools. Certain miRNAs correlate with tumor stage, metastasis, or treatment resistance, suggesting a role in outcome prediction and therapeutic monitoring. However, their clinical utility must be confirmed through prospective trials, which show that they improve decision-making and patient outcomes.[56] Until these requirements are met, miRNA panels remain promising but investigational tools in NSCLC care.

Strengths and Novelty of The Study

This systematic review presents several strengths and novel contributions. First, it is among the few reviews focusing specifically on PCR-based circulating miRNA biomarkers for NSCLC diagnosis. It offers a comprehensive comparative evaluation of individual and panel miRNAs versus traditional markers such as CEA. Unlike prior reviews, this study emphasizes diagnostic metrics (sensitivity, specificity, AUC) and explores variation gaps between studies concerning miRNA type, source, platform, and clinical context.[57] The inclusion of both individual and composite miRNA biomarkers, along with contextual interpretation of biological and methodological heterogeneity, provides nuanced insights for translational applications. Furthermore, the review incorporates recent studies published within the last five years, enhancing its relevance to current clinical research. While meta-analysis was not feasible, the qualitative synthesis highlights gaps in current literature and proposes future directions for methodological standardization and cross-population validation, which are critical steps toward clinical implementation of miRNA-based diagnostics.

Study Limitations and Future Directions

This review has several limitations. Meta-analysis was not optimal due to substantial heterogeneity in miRNA targets, control

definitions, study designs, and diagnostic outcomes. Incomplete reporting, such as missing confidence intervals and unclear p-values, further limited data synthesis and cross-study comparability.

All included studies were conducted in China, raising concerns of geographical and ethnic bias. miRNA expression is known to vary by genetic background and environmental exposure, thus limiting the generalizability of the findings. Validation in multi-ethnic and international cohorts is crucial for broader clinical relevance.

Quality assessment revealed that most studies showed low risk of bias, though Zhang et al[16] had concerns about the flow and timing. Inconsistent reference standards complicate comparison, especially when using and combining traditional biomarkers. The absence of standardized thresholds for both miRNAs and comparator markers may have introduced variability in diagnostic accuracy.

Future research should adopt standardized protocols, include multi-center cohorts, and validate panels in diverse populations. Incorporating longitudinal designs and outcomes such as progression, recurrence, and survival could support clinical utility. Advances in computational modeling may also refine miRNA panel selection and support individualized diagnostic strategies.

Conclusion

The function of miRNA as a diagnostic biomarker for NSCLC based on PCR is superior to conventional biomarkers. Despite the variability observed, researchers still regard miRNA as a possible biomarker in diagnosing NSCLC because several miRNAs exhibit high sensitivity and specificity. Combining multiple miRNAs in biomarker panels is often employed to enhance diagnostic accuracy, addressing the limitations of individual miRNA sensitivity and specificity. It positions miRNA as an effective biomarker, particularly when combined, although further standardization and additional research are necessary to ensure its accuracy in clinical settings.

In conclusion, while miRNA demonstrates significant potential as a diagnostic tool for NSCLC, conducting comprehensive studies that include diverse patient populations and standardized methodologies is essential. This will help to validate the findings and facilitate the implementation of miRNA-based diagnostics in clinical practice. Furthermore, understanding the biological roles of specific miRNAs in cancer progression could lead to more targeted and effective treatment strategies, enhancing patient outcomes in NSCLC. As research continues to evolve, miRNA may play an increasingly vital role in early discovery and management of lung cancer.

Conflict of Interest

There are no conflicts of interest.

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