

## Original Article

# MicroRNAs level as an initial screening method for early-stage lung cancer: a bivariate diagnostic random-effects meta-analysis

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**Abstract:** Accumulating studies suggested that microRNAs (miRNAs) can have high diagnostic value as a non-invasive and cost-effective procedure with high sensitivity and specificity in the detection of early-stage lung cancer. However, there is inconsistency observed in the results of relevant studies. Therefore, we performed this meta-analysis to evaluate diagnostic value of miRNAs based on all related studies. A total of 38 studies from 13 included articles were used for the analysis, consisting of 510 patients and 465 healthy controls. All analyses were performed on the R 3.2.0 software. The bivariate random-effects meta-analysis model was applied to obtain the following pooled parameters: sensitivity, 0.797 (95% CI: 0.756-0.832); false positive rate, 0.296 (95% CI: 0.250-0.346); and AUC, 0.818. In addition, subgroup analyses were conducted, showing not only that a combination of multiple miRNAs as biomarkers have greater diagnostic value for early-stage lung cancer (sensitivity, false positive rate and AUC of 83%, 25.2% and 0.858, respectively) had a higher diagnostic accuracy than single miRNA (sensitivity, false positive rate and AUC of 78.3%, 31.6% and 0.799, respectively), but also that specimen from circulating system (sensitivity, false positive rate and AUC of 82.5%, 30.5% and 0.836, respectively) provide better biomarkers than specimen from non-circulating system (sensitivity, false positive rate and AUC of 73.8%, 26.5% and 0.796, respectively). In summary, the current meta-analysis suggests that miRNAs as biomarkers, particularly a combination of multiple tumor-specific miRNAs from circulating system, have moderately high clinical diagnostic value in the detection of early-stage lung cancer. However, the clinical diagnostic utilization and additional improvements of miRNAs as biomarkers for early-stage lung cancer detection still remain to be further validated by more future studies.

**Keywords:** MicroRNAs, lung cancer, early stage, diagnosis, meta-analysis

## Introduction

Lung cancer is the most leading cause of deaths in both males and females around the world, accounting for 13% (1.6 million) of the total cancer cases and 18% (1.4 million) of the death worldwide in 2008 [1]. The high mortality of lung cancer is due to the lack of effective methods for early detection [2]. The five-year survival rate of lung cancer varies largely depending on the stage of cancer [3]. So far, the lung cancer patients is often diagnosed when it develops into the advanced stages (stage III, IV), but unfortunately at that time the malignant cells have been widespread to the other organs, thus the survival rate drops to 0~14%. However, if it were diagnosed at early stages (stage I, II), the five-year survival rate would rise to 83% [4]. Thus, the early detection

is an effective method to prevent the deterioration reduces the mortality of lung cancer. Nevertheless, it's quite challenging to diagnose the cancer patients in early stage, because most of them usually appear to be asymptomatic [5, 6], making it hard to notice.

Over the past decades, scientists have made great efforts to find accurate diagnostic methods for early-stage lung cancer detection. The current screening methods mainly include histopathological biopsy, lung imaging, and biochemical tests with a number of specific biomarkers [7]. It shows a relatively good performance in cancer detection but also has its own limitations. Histopathological biopsy can be regarded as a definite diagnosis for cancer. However the invasiveness of biopsy

may cause discomfort and affect the quality of life since the patients have to subject to the biopsy multiple times a year [8]. Lung imaging, such as computerized tomography (CT), positron emission tomography (PET), X-ray, may expose a potential radiation risk to human body during the procedure [9]. Besides, the procedures mentioned above are expensive. In regard to existing biomarkers, such as carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA21-1), KRAS and TP53 genes, are closely associated with the early-stage lung cancer, which a change in their expression in vivo is found between the healthy people and cancer patients [10-12]. However, despite of their convenience and non-invasiveness, most of the discovered biomarkers fail to show a strong specificity and sensitivity for early-stage lung cancer [13]. It's necessary to find a new, effective and non-invasive method to improve the early-stage lung cancer diagnosis.

MiRNAs, a family of small non-coding RNAs with about 22 nucleotides [14], act as a post-transcriptional regulator in the gene expression, and are implicated in a wide range of physiological processes related to tumorigenesis, such as cell proliferation, differentiation and apoptosis [15]. It's effortless to extract the miRNAs from human body without any invasive procedure since they are found in tissues and body fluids, such as serum, urine, plasma, sputum etc. In addition, miRNAs show strong stability and resistance to RNase digestion, boiling, extended storage, extremes of PH, and multiple freeze-thaw cycles [16]. Until now, over 1,400 miRNAs have been reported [17] and most of them are proven to have a great diagnostic value for lung cancer detection, even at early stage [18-20]. Furthermore, accumulating evidences have demonstrated that miRNAs as biomarkers have relatively high specificity and sensitivity for the early-stage lung cancer detection [21-23]. MiRNAs have the advantages of high accuracy, convenience and non-invasiveness over the conventional diagnostic methods, which have a promising perspective as effective biomarkers in lung cancer diagnosis.

A number of studies have suggested the potential association between the abnormal expression of miRNAs and lung cancer [24-26], but the specificity and sensitivity of miRNAs as biomarkers are under debate. There are inconsis-

tent results shown in the relevant studies, which make it necessary to assess the diagnostic value of miRNA comprehensively. Therefore, we conducted a meta-analysis of all relevant articles to evaluate the performance of miRNA in early-stage lung cancer detection. To the best of our knowledge, this is the first meta-analysis to fully appraise the diagnostic value of miRNAs in early-stage lung cancer.

### Materials and methods

#### *Literature search*

This analysis was conducted according to the guidelines for diagnostic meta-analysis [27]. Cochrane library, Chinese National Knowledge Infrastructure (CNKI), Chinese Biomedical Literature Database (CBM), Web of science, Embase, PubMed and Google scholar were searched up to April 5, 2015 without language restrictions, using the following search terms: "at the early stages" or "of early stage"; "lung tumor" or "lung cancer"; "microRNAs" or "miRNAs"; "diagnosis" or "diagnosis value"; "sensitivity" or "specificity" or "ROC curve". Besides, the reference lists of relevant review were manually browsed in order to retrieve the additional articles related to the study.

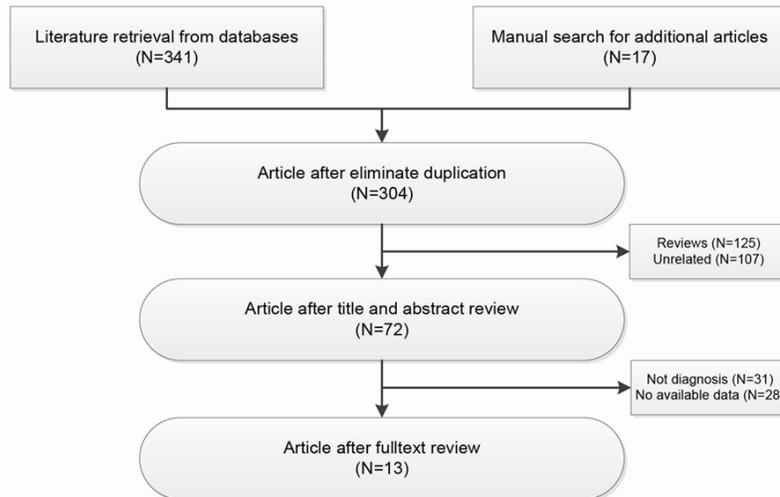
#### *Inclusion and exclusion criteria*

Studies qualified to be included in our meta-analysis have to fulfill the following criteria: (1) studies concerning the diagnostic value of miRNAs for early-stage lung cancer; (2) studies using the gold standard to confirm the diagnosis of the early-stage lung cancer patients; (3) studies providing adequate data to create two-by-two tables, including true positive, false positive, true negative and false negative. Exclusion criteria were as follows: (1) studies irrelevant to the diagnostic value of miRNAs for early-stage lung cancer; (2) studies citing duplicate data from other studies; (3) studies in the form of letters, editorials, reviews or case reports.

#### *Data extraction*

Two investigators independently read the full text of all included studies carefully and then extracted the following information to this meta-analysis: (1) basic characteristics of studies, including the first author's name, publication

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**Figure 1.** Detailed flow diagram indicating the filtering process of included studies.

year, original country; (2) description of study population, including ethnicity, sample size, mean age, patient spectrum, source of control, stage of early-stage lung cancer; (3) relevant information of biomarkers, including miRNAs profiled, specimen test method; and (4) diagnostic results, including sensitivity, specificity, true positive, false positive, true negative, and false negative.

## Statistical analysis

All analyses were performed on the R 3.2.0 software. The bivariate random-effects meta-analysis model was applied to obtain the following pooled parameters with corresponding 95% confidence intervals (CIs): sensitivity and false positive rate [28, 29]. We then extracted the sensitivity and specificity of individual studies to generate the summary receiver operator characteristic (SROC) curve and to calculate the area under the SROC curve (AUC), which is a comprehensive evaluation for the accuracy of diagnosis [30]. The value of AUC can also be a parameter to assess the discrimination, which ranges from 0.5 (random discrimination) to 1.0 (ideal discrimination) [31]. Heterogeneity of all studies was measured by the X-squared statistics. A *P* value of X-squared less than 0.05 indicated significant between-study heterogeneity, a random-effect model was used. To further find out the possible sources of heterogeneity, subgroup analysis was carried out based on relevant information of our included studies.

## Results

### Literature search

The results of the literature search are listed in **Figure 1**. We obtained a total of 358 articles in the initial search, among which 54 duplicate studies were excluded. 304 studies are left for further examination. After reviewing titles and abstracts, 125 letters, reviews and meta-analysis as well as 107 irrelevant studies were removed. Therefore, only 72 articles are qualified for full-text review. After careful reading,

59 were excluded: 31 are not correlated with to the diagnosis of early-stage lung cancer and 28 don't provide adequate data. Finally, 13 studies were included in our meta-analysis [2, 3, 20-26, 32-35].

### Baseline characteristics of included studies

The main characteristics of the 13 articles are summarized in **Table 1**. In this meta-analysis, 38 studies from 13 articles were used to evaluate the diagnostic value of miRNAs for the detection of early-stage (stage I, II) lung cancer, with a total of 510 patients and 465 healthy controls included. All the included articles were published during 2010 to 2014. The ethnicities of population include Asian, Caucasian and African. 25 studies investigated the diagnostic value of single particular miRNA while the other 13 studies focused on the combination of multiple miRNAs in early-stage cancer detection. The specimens of 24 studies were taken from circulating system, while the other 14 studies were belonged to non-circulating system.

### Diagnostic accuracy of miRNAs for early-stage lung cancer

We constructed a forest plots for the overall studies, the X-squared for sensitivity and specificity are 168.6473 ( $P < 0.05$ ) and 153.6486 ( $P < 0.05$ ) respectively, thus conducting a random-effect model. Moreover, the evaluated results are as follows: sensitivity, 0.797 (95% CI: 0.756-0.832); false positive rate, 0.296

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**Table 1.** Summary of articles assessing the diagnostic value of miRNAs for the early stage lung cancer

First author	Year	Country	Ethnicity	Case		Control		Stage	miRNAs profiled	Specimen
				No.	Age	No.	Age			
Yu L [21]	2010	USA	Caucasian/African	36	68.2	36	66.7	Stage I	miR-486, -126, -145, -21, -182, -200b, -375	Sputum
Bianchi F [41]	2011	Italy	Caucasian	22	n.a.	30	n.a.	Stage I	a set of 34 miRNAs	Serum
Foss KM [23]	2011	USA	Caucasian	22	65.0	31	64.0	Stage I	miR-1254 and -574-5p	Serum
				11	62.0	11	62.0	Stage I	miR-1254 and -574-5p	Serum
Shen J [42]	2011	USA	Caucasian/African	15	n.a.	29	n.a.	Stage I	miR-21, -126, -210, -486-5p	Plasma
Ma Y [44]	2012	USA	Caucasian	76	n.a.	110	n.a.	Stage I-II	miR-125b	Serum
Roa WH [22]	2012	Canada	Caucasian	24	68.8	6	44.6	Stage I-II	miR-21, -143, -155, -210, -372	Sputum
Li GJ [43]	2012	China	Asian	18	n.a.	14	n.a.	Stage I	miR-494, -22, -200b	Serum
Abd-El-Fattah AA [23]	2013	Egypt	African	65	54.1	37	50.1	Stage I-II	miR-21, -155, -182, -197,	Serum
Cazzoli R [26]	2013	Italy	Caucasian	10	66.1	10	65.6	Stage I	miR-378a, -379, -139-5p, -200-5p	Plasma
				40	70.5	15	66.7	Stage I	miR-151ap, -30a, -200b-5p, -629, -100, -154-3p	Plasma
Ma J [25]	2013	USA	Caucasian/African	36	66.7	38	64.6	Stage I	miR-21-5p, and -335-3p	Plasma
Ulivi P [2]	2013	Italy	Caucasian	86	68.1	24	65.0	Stage I-II	miR-328 and -361	Blood
				86	68.1	24	65.0	Stage I-II	miR-339 and -140	Blood
Li N [45]	2014	USA	Caucasian	35	40.0	68.9	65.7	Stage I	miR-31 and -210	Sputum
Zhu W [27]	2014	China	Asian	36	n.a.	36	n.a.	Stage I	miR-29c and -429	Tissue

n.a. not availables.

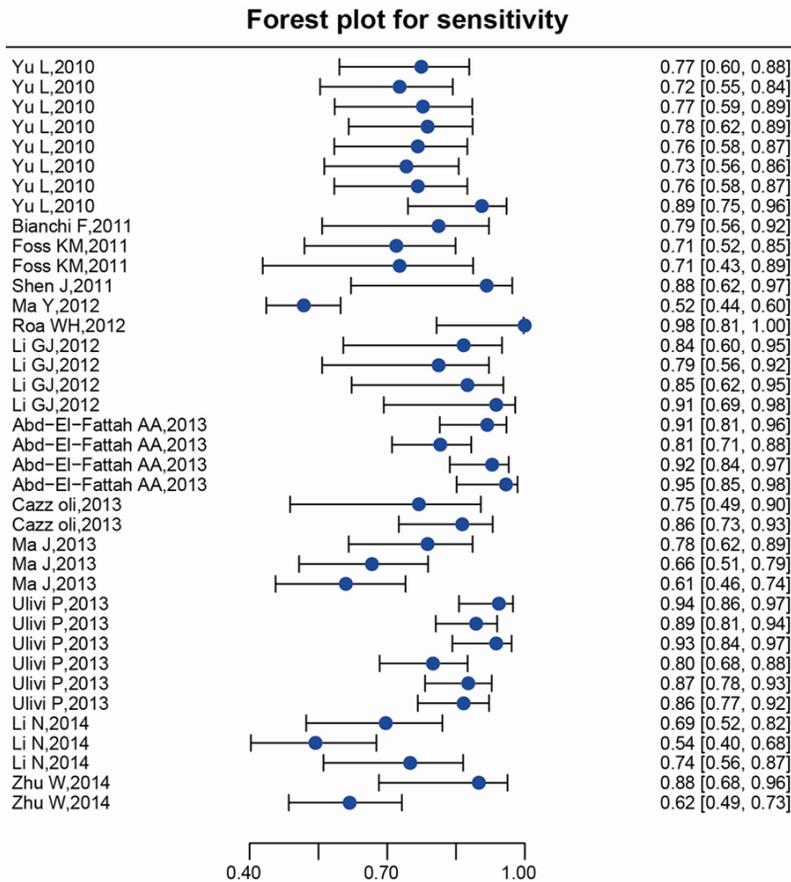


Figure 2. Forest plots of estimates of sensitivity.

(95% CI: 0.250-0.346). The corresponding overall SROC curve is presented in Figure 4, with an AUC of 0.818, demonstrating that miRNAs level could be used as an initial screening method in discriminating patients with early-stage lung cancer from controls.

*Subgroup analysis*

In this meta-analysis, subgroup analysis based on miRNAs profiled was performed to identify the potential sources of heterogeneity.

The analysis of 25 studies based on single miRNA in early-stage lung cancer diagnosis presented a pooled sensitivity of 0.783 (95% CI: 0.730-0.827), false positive rate of 0.316 (95% CI: 0.256-0.381), while the rest of studies focusing on the diagnostic value of a combination of multiple miRNAs showed us results with higher accuracy than the former, the results are listed as follows: sensitivity, 0.830 (95% CI: 0.767-0.878), false positive rate, 0.252 (95% CI: 0.191-0.324). According to Figure 5, we

found that the AUC of these two subgroups are 0.799 and 0.830, respectively, indicating that multiple miRNAs could be better biomarkers than single miRNA in lung cancer diagnosis.

The analysis of 24 studies whose specimen come from circulating system showed a pooled sensitivity of 0.825 (95% CI: 0.774-0.866), false positive rate of 0.305 (95% CI: 0.235-0.386), while the other studies which specimen belonged to non-circulating system manifested a pooled sensitivity of 0.738 (95% CI: 0.680-0.789), false positive rate, 0.265 (95% CI: 0.225-0.311). According to Figure 6, we found that the AUC of these two subgroups are 0.836 and 0.796, respectively, pointing out that specimen coming from circulating system could provide a

more sensitive biomarkers than specimen from non-circulating system.

**Discussion**

Lung cancer causes high mortality in the world, largely due to the late diagnosis [34]. As we know, the five-year survival rate of early-stage lung cancer is far higher than advanced-stage lung cancer [4]. Therefore, accurate diagnosis at the early stages of cancer plays a very important role in reducing the high mortality, scientists have devoted to finding diagnostic tools and biomarkers for the past decades. However, the current diagnostic methods are unsatisfactory for early-stage cancer diagnosis, which includes histopathological biopsy, lung imaging, and biochemical tests with a number of biomarkers [7]. Their disadvantages can be summarized as follows: (1) histopathological biopsy: high-cost, high rate of false positives, invasiveness; (2) lung imaging: potential hazards from radiation, expensive; (3) biochemical tests: low of reproducibility [36, 37]. The tech-

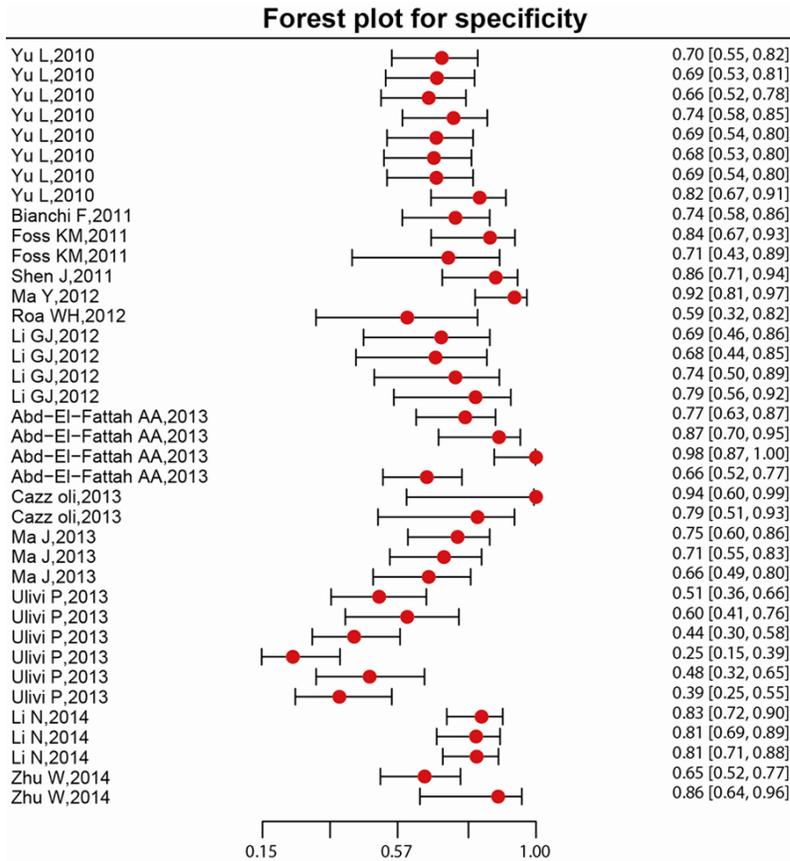


Figure 3. Forest plots of estimates of specificity.

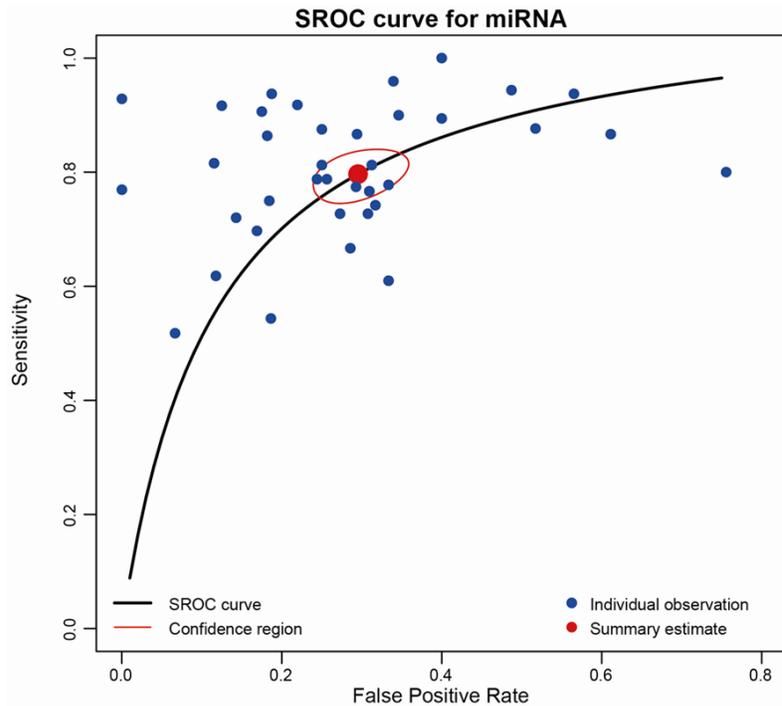


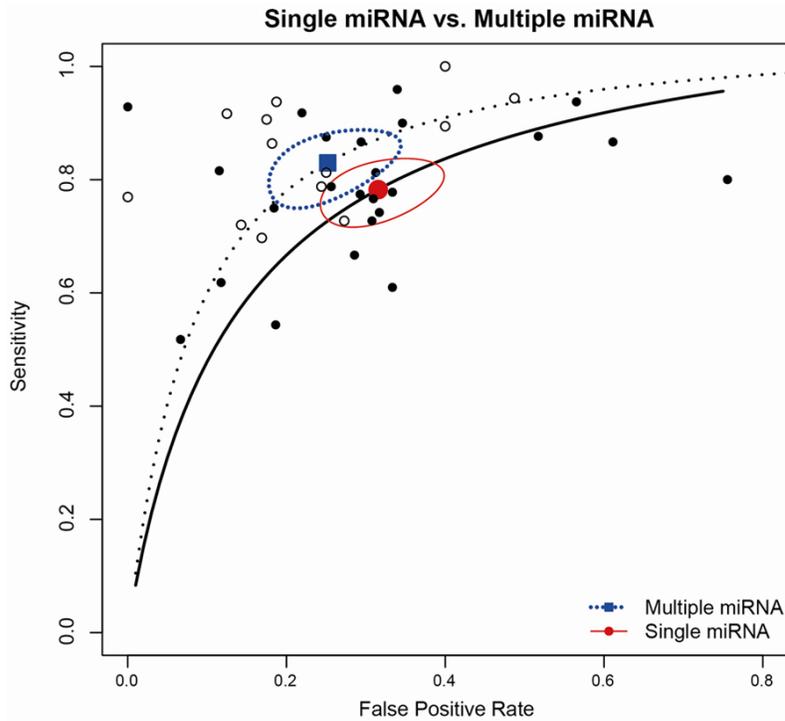
Figure 4. Summary receiver operator characteristic (SROC) curve appraising the accuracy of miRNAs as biomarkers for the diagnosis of early-stage lung cancer.

niques mentioned above present a low sensitivity and specificity. Thus, it is urgent and crucial to find minimally invasive or non-invasive biomarkers with high sensitivity, specificity and reproducibility in the detection of early-stage lung cancer.

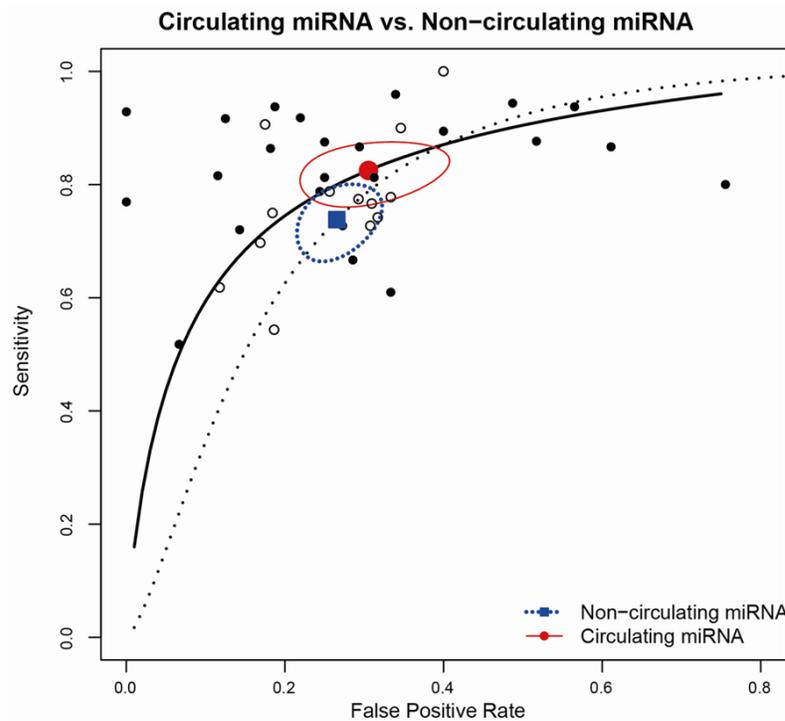
A variety of researches reported the great potential of miRNA as powerful biomarkers in lung cancer diagnosis since the abnormal expression of miRNA is discovered in lung cancer patients. Meanwhile, the miRNAs play important role in the regulation of gene expression, which involve a number of biological processes [15, 38]. Furthermore, miRNAs exist in body fluids, tissues or excrement, which can be easily extracted to examine and facilitate the diagnostic tests.

The diagnostic value of miRNAs for early-stage lung cancer diagnosis has been widely studied for several years. Roa et al. showed that a combination of 5 miRNAs had a high sensitivity (83.3%) and specificity (100%) in the detection of early-stage lung cancer [21]. However, Ulivi et al. found that using miRNAs as biomarkers for the early stage diagnosis only have a low accuracy, with a sensitivity of 60.0% and a specificity of 44.0%, which suggested that miRNAs may not be qualified to be specific biomarkers. Individual studies have reached discrepant results [25, 32, 35], thus a meta-analysis are urgently needed to evaluate the diagnostic value of

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**Figure 5.** Summary receiver operator characteristic (SROC) curves of subgroup analysis, showing the difference of diagnostic accuracy between single miRNA and multiple miRNAs as biomarkers.



**Figure 6.** Summary receiver operator characteristic (SROC) curves of subgroup analysis, showing the difference of diagnostic accuracy between circulating miRNAs and non-circulating miRNAs as biomarkers.

miRNAs for early-stage lung cancer.

To the best of our knowledge, this is the first meta-analysis focusing on the diagnostic proficiency of miRNAs assays for early-stage lung cancer, which was based on 38 studies from 13 articles. We conducted a bivariate random-effects meta-analysis, the outcomes indicate an AUC of 0.818 with a moderately high sensitivity of 0.797 (95% CI: 0.756-0.832) and false positive rate of 0.296 (95% CI: 0.250-0.346). Thus, miRNAs may be the potential diagnostic tool for early-stage cancer, but more tests are required for further examination.

In this meta-analysis, subgroup analysis was conducted to explore the potential sources of heterogeneity. The results revealed that a combination of multiple miRNAs as biomarkers have better diagnostic performance for early-stage lung cancer than single miRNA. The pooled sensitivity for multiple miRNAs and single miRNA are 0.830 (95% CI: 0.767-0.878) and 0.783 (95% CI: 0.730-0.827), respectively, and meanwhile the pooled false positive rate are 0.252 (95% CI: 0.191-0.324) and 0.316 (95% CI: 0.256-0.381), respectively. **Figure 4** showed us the AUC of these two subgroups are 0.858 and 0.799, respectively. Based on the subgroup results, we then made further tests to assess the diagnostic value of a combination of multi-

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ple miRNAs for early-stage lung cancer. Forest plots of sensitivity and specificity were conducted and shown in **Figures 2** and **3**. Further investigation and research must be carried out to illuminate the mechanisms.

Our meta-analysis have higher accuracy and credibility than each individual studies on the research of miRNAs as biomarkers in early-stage lung cancer diagnosis. Firstly, we searched in several databases and carefully retrieve those articles that meet the inclusion criteria to guarantee the high quality of included studies. Secondly, we conducted the subgroup analyses to find the underlying sources of heterogeneity. However, certain limitation in our research must be taken into account, which can be summarized as follows: (1) some of the related studies may be missed during the literature search even if we have already performed a comprehensive search; (2) most of the selected studies focuses on stage I or stage I/II lung cancer patients, these are no studies concentrating on stage II, which make it difficult to find out the diagnostic performance of miRNAs in stage II (3) sample size is relatively small (4) 10 patients and 465 healthy controls, so further research based on a larger sample should be carried out to validate the results. Despite the disadvantages mentioned above, our meta-analysis is the first meta-analysis to fully evaluate the diagnostic proficiency of miRNAs in early-stage lung cancer.

In summary, the current meta-analysis suggests that miRNAs as biomarkers, particularly a combination of multiple tumor-specific miRNAs and coming from circulating system, have moderately high clinical diagnostic value in the detection of early-stage lung cancer. As shown in the subgroup analysis, multiple miRNAs are more sensitive and specific indicators than single miRNA as screening tools for early-stage lung cancer diagnosis. However, there is a lot further examination required to be made to confirm the diagnostic accuracy of miRNA before it can be widely used as biomarkers in the clinical diagnosis.

### Disclosure of conflict of interest

None.

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