The Diagnostic Role of Sputum miRNA in Non-Small Cell Lung Cancer

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Research article

Keywords: sputum miRNA, non-small cell lung cancer, diagnostic value,biomarker

Posted Date: August 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-62275/v1

Abstract

Objective: Recent studies indicated sputum miRNAs may provide a promising approach for non-small cell lung cancer (NSCLC) diagnosis. But some results were still inconsistent. So, we performed meta-analysis to evaluate the diagnostic role of sputum miRNAs for the detection of NSCLC.

Methods: Eligible studies that estimated the diagnostic accuracy of sputum miRNAs in NSCLC were searched in Pubmed, Embase and Web of Science and Chinese National Knowledge Infrastructure (CNKI). Data from the eligible studies were collected and pooled; sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratios, weighted symmetric summary ROC curve and the area under the curve (AUC) were calculated by bi-variate random effects model. The between-study heterogeneity was evaluated by Q test and I^2 statistics.

Results: 30 studies from 16 articles were included for analysis. The overall analysis yielded the sensitivity of 0.77 (95% CI: 0.73–0.81) and specificity of 0.87 (95% CI: 0.83–0.90), with an area under the SROC curve (AUC) of 0.89 (95% CI: 0.86–0.91). Subgroup analysis revealed the diagnostic accuracy in multiple miRNAs studies was higher than single miRNA (the sensitivity, specifcity and an AUC of multiple miRNAs were 0.76, 0.88 and 0.90; and for single miRNA, it was 0.74, 0.74, and 0.80). The diagnostic performance in early stage NSCLC was also very high (the sensitivity, specifcity and an AUC of stage I/II was 0.76, 0.88 and 0.91; and for stage I, it was 0.79, 0.85, and 0.87). We also found miR-210, miR-21, miR-31 and miR-126-3p might serve as potential biomarkers for lung cancer.

Conclusion: Sputum miRNAs was useful noninvasive biomarkers for NSCLC diagnosis.

Introduction

Lung cancer still remains the most common cancer and the most common cause of cancer-related death worldwide.[1] Lung cancer is the most common incident cancer and the leading cause of cancer death in China in 2015.[2] In Untied State, Lung cancer is estimated the second common cancer and the first leading causes of cancer death in 2017.[3] About 80% lung cancers were non-small cell lung cancer (NSCLC).[4] As the typically asymptomatic at early stages, most patients with NSCLC were diagnosis at locally advanced or metastatic disease. The 5-year survival rate for stage IV NSCLC is only 10%, whereas it is approximately 80% for stage IA.[5] Improve the early detection of lung cancer is very necessary and important to improve the prognosis of patients with lung cancer.

Low-dose CT (LDCT) is commonly used in lung cancer screening. Early detection of lung cancer using LDCT has demonstrated in a large randomized trial a 20% reduction in mortality in heavy smokers as compared to chest X-rays.[6] However, this strategy has several limitations including high false-positive rates, potential over-diagnosis, excessive cost and the potential harm associated with radiation exposure.[7] Existing protein biomarkers such as carcinoembryonic antigen (CEA) and CYFRA21-1 did not show sufficient sensitivity and specificity.[8] Therefore, it is important to develop early detection methods to reduce lung cancer-related deaths.

Sputum contains exfoliated airway epithelial cells, it is the most easily accessible biological fluid[9]. In recent years, sputum cytological analysis has been used for lung cancer diagnosis. Sputum cytology can identify morphologic abnormalities of bronchial epitheliums of patient with lung cancer, but poor sensitivity was exhibited. Molecular study of sputum could detect the cells containing lung tumor-associated molecular aberrations, thus providing a noninvasive approach for diagnosis of lung cancer.[10,11]

MicroRNAs (miRNAs) are short (typically 18–25 nucleotides), single-stranded and highly conserved non-coding RNAs which could negatively regulate gene expression at post-transcriptional level by binding the 3'-untranslated region of target mRNAs, resulting in either mRNA degradation or translational repression. Dysregulation of miRNAs plays crucial roles in tumorigenesis. Specific over or under expressions of miRNAs have been found to associate with particular tumor types, and thus open up a new field for molecular diagnosis of cancer.[12] Many studies suggested that miRNAs are stably present in sputum, and aberrant miRNA expression could be potentially useful for lung cancer diagnosis.[11,13] Although studies have indicated that various sets of miRNAs can be used as highly sensitive and specific markers for the detection of LC. However, conflicting results are still present due to the limited sample size and static power in individual study. Therefore, a systematic analysis of these data may be valuable to further explore the clinical applicability of miRNAs as biomarkers for the diagnosis of lung cancer.

Methods

Literature search

A comprehensive literature search was conducted in the databases of PubMed, EMBASE and Web of Science and Chinese National Knowledge Infrastructure (CNKI). The last search time was Nov 28, 2018. The following terms and combinations were used to identify studies: "microRNA", "miRNA", "lung cancer" and "lung neoplasm". Furthermore, references of retrieved articles and reviews were manually screened for additional studies.

Eligibility Criteria

The inclusion criteria were applied to identify the eligible studies: (1) articles with full texts published in English and Chinese; (2) studies investigated the diagnostic role of sputum miRNAs in NSCLC; (3) provided the diagnostic gold standard for confirming NSCLC; (4) provided sufficient data for the construction of two-by-two tables, including true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The exclusion criteria were as the follows: (1)

publications unrelated to the diagnostic values of circulating miRNAs for lung cancer; (2) studies with duplicate data reported in other studies; (3) letters, editorials, case reports or reviews.

Data Extraction And Quality Assessment

The data and information, including the name of first author, year of publication, country of study, characteristics of participants (sample size, ethnicity) were sorted. Sensitivity, specificity, TP, FP, TN and FN were extracted from included studies. The quality of each eligible study was assessed by the revised Quality Assessment of Diagnostic Accuracy Studies tool.[14]

Statistical analysis

STATA 12.0 software (StataCorp, College Station, TX, USA) is used to perform the statistical analyses. Bivariate regression model was used to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR). The DOR is an indicator of test performance. Area under the curve (AUC) was calculated to evaluate the diagnostic accuracy of miRNA in discriminating NSCLC patients from controls. The summary receiver operator characteristic (SROC) curve was plotted based on the sensitivity and specificity.[15,16] The I^2 test was conducted to analyze the heterogeneity between studies, which I^2 more than 50% indicated that there is a substantial between-study heterogeneity that existed.[17] The potential sources of heterogeneity were further identified by subgroup analyses. We assessed the publication bias in included studies by using Deek's funnel plot asymmetry test.[18]

Results

Eligible studies

Initial screen identified 37 articles. After reviewing the titles and abstracts, 9 articles were excluded because they obviously did not meet our selection criteria. The remaining 28 articles were further checked by screening the full texts. In this period, 12 studies were excluded because they are reviews or meta-analyses (n=5), insufficiency information (n=3), detecting miRNA methylation (n=2) or data overlapping (n=2). As a result, 16 articles[11,13,19-32] were eligible for final meta-anlaysis. Figure 1A showed the process of studies identification. Among 16 articles, 6 articles consisted at least 2 independent studies[11,19,22,26,27,32], as a result, 30 studies from 16 articles including 2,447 subjects (1,212 patients with lung cancer and 1,235 controls) were included for analysis. These studies published from 2010 to 2018, and the sample size ranged from 30 to 315. Two studies conducted in Asian[24,27], seven studies conducted in Caucasian[13,20,25,28-31] and the remaining seven studies[11,19,21-23,26,32] conducted in mixed population. Two studies[19,26] selected healthy population as controls, 11 studies[13,20-23,25,28-32] selected cancer-free subjects as controls, and the remaining three studies[11,24,27] applied benign pulmonary disease (BPD) as controls. The detailed information of the included articles was listed in Table 1. Quality assessments results for each eligible study according to QUADAS-2 guidelines are presented in Figure 2B.

Pooled Diagnostic Accuracy of miRNA

The overall predictive accuracy of miRNAs was summarized in Table 1. The pooled sensitivity was 0.77 (95% CI: 0.73–0.81), the specificity was 0.87(95% CI: 0.83–0.90) (Figure 2), the pooled PLR was 5.9 (95% CI: 4.7–7.4), the NLR was 0.26 (95% CI: 0.22–0.30) and the DOR of 23 (95% CI: 18–29) (Table 2). The AUC value was 0.89 (95% CI: 0.86–0.91), and the corresponding SROC curve was shown in Figure 3A. The Fagan diagram was also applied to illustrate the post-test probabilities of sputum miRNAs in lung cancer diagnosis (Figure 3B).

Subgroup analyses

Various subgroup analyses according to race, miRNA profiling, source of control, histology type, sample size, publication year and stage were done. The results were summarized in Table 2

Race

For Asian population, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC were 0.81, 0.85, 5.4, 0.22, 25 and 0.90 respectively. For Caucasian, they were 0.79, 0.87, 6.0, 0.24, 25 and 0.89 respectively. For mixed population, they were 0.73, 0.89, 6.4, 0.30, 21 and 0.90 respectively (Table 2). Subgroup analysis according to race suggested the diagnostic accuracy of sputum miRNA in NSCLC was excellent in all races.

miRNA profiling

The pooled analysis suggested combined miRNA may yield higher diagnostic accuracy in distinguish NSCLC. The pooled sensitivity, specificity, PLR, NLR, DOR and AUC were 0.76 vs 0.74, 0.88 vs 0.74, 6.4 vs 2.8, 0.28 vs 0.36, 23 vs 8 and 0.90 vs 0.80 respectively for multiple miRNA vs single miRNA (Table 2 , Figure 4A and 4B, and Figure 5A and 5B).

Source of control

According the source of control, control subjects could be classified as healthy control, cancer-free control and BPD. Studies with healthy control had a pooled sensitivity of 0.73, specificity of 0.91, PLR of 8.5, NLR of 0.29, DOR of 29 and AUC of 0.77. Studies with cancer-free controls had a pooled sensitivity of 0.76, specificity of 0.86, PLR of 5.5, NLR of 0.28, DOR of 19 and AUC of 0.88. Studies with BPD controls showed a pooled sensitivity of 0.82, specificity of 0.86, PLR

of 5.7, NLR of 0.22, DOR of 26 and AUC of 0.91 (Table 2). All these subgroups showed satisfied diagnostic accuracy, suggesting sputum miRNA could not only be a tool for lung cancer screening but also be a tool for differential diagnosis.

Stage

Firstly, according to percentage of early stage disease, we divided studies as I/II≥0.6 and I/II<0.6 subgroups. Both group showed satisfied diagnostic efficacy, with pooled sensitivity of 0.76 and 0.79, specificity of 0.88 and 0.85, PLR of 6.1 and 5.3, NLR of 0.27 and 0.25, DOR of 23 and 21, AUC of 0.90 and 0.87 for I/II≥0.6 and I/II<0.6 subgroup respectively(Table 2).

We also extracted studies only focus on stage I and/or II disease for analysis. For stage I and II disease, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC were 0.76, 0.88, 6.5, 0.27, 23 and 0.90, respectively. For only stage I disease, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC were 0.75, 0.88, 6.5, 0.28, 23 and 0.90, respectively (Table 2, Figure 4C and 4D and Figure 5C and 5D). This suggested sputum miRNA could serve as an efficacy tool in early lung cancer screen.

Histology, sample size and publication year

According to the percentage of adenocarcinoma in overall NSCLC, 0.5 was selected as a cut-off to divided studies as AD≥0.5 and AD<0.5. Both studies showed satisfied diagnostic accuracy. The similar results were found in the subgroup analyses according to sample size and publication year (Table 2).

Meta-regression analyses and publication bias

Potential sources of inter-study heterogeneity were explored by meta-regression analyses. The analyses suggested race (P<0.01), miRNA profiling (P<0.01) and source of control (P<0.01) might be the potential sources of inter-study heterogeneity. The effect of samle size, publication year, age, male ratio and smoking on heterogeneity was not significant (Figure 6A). Deeks'funnel plot asymmetry test was applied to detect publication bias. No publication bias was detected in both overall (Figure 6B) and subgroup analyses (Table 2).

Potential useful sputum miRNAs identified from literature

We sorted the miRNAs reported by at least two studies that could serve as potential biomarkers. In upregulated miRNAs, 11 studies reported miR-210,[11,20-23,25-28,31,32] eight studies reported miR-21[11,13,19,22,24,25,27,32] and six studies reported miR-31.[11,21-23, 32,37] miR-205,[11,22,26] miR-182,[11,19,22] miR-200b[11,19,22] and miR-155[11,24,26] had been reported by three studies. Only miR-372[11,28] had been reported by two studies. In downregulated studies, miR-126-3p had been reported by five studies,[11,19,22,26,31] miR-486 had been reported by three studies[11,19,22] and miR-145 had been reported by two studies.[19,31] We also found some conflicting result. Two studies reported miR-375 was upregulated,[11,19] but another two studies reported it was downregulated.[22,31] miR-708 was upregulated in two studies[11,26] and downregulated in one studies.[22] Further studies should verify these conflicting results(Table 3).

Discussion

The current studies comprehensively investigated the diagnostic accuracy of sputum miRNAs in NSCLC by sorting current evidence. We found sputum miRNA showed satisfied diagnostic accuracy with a pooled sensitivity of 0.77 and specificity of 0.87, with corresponding PLR 5.9 and NLR 0.26. The pooled DOR was 23 and the AUC was 0.89. Even in various subgroup analyses, the diagnostic accuracy was still very high, suggesting sputum miRNAs might be potential biomarker to distinguish lung cancers from controls.

Previous studies investigated the diagnostic role of circulating miRNAs on lung cancer by meta-analysis[33–36]. According to their report, the pooled sensitivity ranged from 0.76 to 0.83, specificity ranged from 0.77 to 0.84, AUC ranged from 0.83 to 0.90. Our study showed sputum miRNAs have an equivalent diagnostic accuracy when compared with circulating miRNAs. However, sputum was more noninvasive than blood. As a result, sputum miRNAs might serve as more noninvasive and efficacy biomarkers for lung cancer diagnosis.

Previous meta-analysis by Liao et al. published in 2014 had investigated the diagnostic role of sputum miRNAs in lung cancer.[37] Only 8 studies with 514 lung cancer patients and 491 controls were included in their analyses. They found the pooled sensitivity, specificity, PLR, NLR, DOR and AUC of sputum miRNAs were 0.70, 0.89, 5.6, 0.35, 17.5 and 0.83, respectively. Our analyses yielded higher diagnostic accuracy when compared with the previous study. This may because our analysis included more studies and more samples than previous work. After 2014, 8 studies were published. In our work, we included 16 articles consisted of 1,212 patients with lung cancer and 1,235 controls. Our sample size was extremely larger than previous study; as a result, our result was more robust and reliable. Also, our analysis was more comprehensively and presented more detailed and useful information. We conducted subgroup analyses by race, miRNA profiling, the source of control, histology type, sample size, publication year and stage.

In subgroup analysis by the source of control, we also demonstrated sputum miRNA could not only screen lung cancer from healthy population, but also distinguish lung cancer from cancer-free/BPD patients. Lung cancer screen in early stage was very important because it could significantly improve the prognosis of this lethal disease. As a result, we conducted subgroup analysis by stage. We found both I/II ≥ 0.6 (0.90) and I/II<0.6 (0.87) subgroup showed good diagnostic performance. We also extract studies only investigated stage I/II and stage I disease. We found sputum miRNA could distinguished stage I/II and stage I NSCLC from controls correctly. This suggested sputum miRNAs could serve as useful biomarkers for NSCLC screen. Other subgroups such as histology type, sample size and publication year also showed satisfied diagnostic accuracy, suggesting sputum miRNA was efficacy and reliable in lung cancer diagnosis.

Although the diagnostic performance of sputum miRNA was excellent, but the certain miRNAs applied in individual studies were not uniform. These may limited the application of sputum miRNAs. We sorted certain miRNAs used in each study, and we found miR-210, miR-21, miR-31 and miR-126-3p was most frequently applied miRNAs. Our studies supplied some evidence that these miRNA might serve as useful potential sputum biomarkers. Future studies should further verify these miRNAs and applied as a high efficacy panel to improve the diagnostic accuracy. Another problem was the cutoff value was various among each studies. Established standard reference for these useful sputum miRNAs was necessary for their further utility. Also, combining sputum miRNAs with CT screening, sputum cytology and serum tumor protein markers, such as cytokeratin 19 fragment (CYFRA21-1), squamous cell carcinoma antigen (SCC) and carcinoembryonic antigen (CEA) could yield more satisfied diagnostic performance.

High heterogeneity was existed in our analysis. Meta-regression analysis was done to explore the potential sources of heterogeneity. We found race, miRNA profling and the source of control might be the major cause of heterogeneity. Also, lacking uniform cutoff values and certain miRNAs in each study was the one of the major source of heterogeneity. This should be acknowledged and interpreted with caution.

Conclusion

The present studies supported sputum miRNAs was a useful noninvasive biomarkers for NSCLC diagnosis. Combinations of miRNAs may yield satisfied diagnostic accuracy. miR-210, miR-21, miR-31 and miR-126-3p was most frequently applied miRNAs in current evidence and might serve as useful sputum miRNAs in the diagnosis of NSCLC. However, the clinical application of miRNA profiling for NSCLC detection still needs further validation by future studies.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Zaoxiu Hu put forward the idea and designed the research; All authors contributed to literature searching, data extraction and analysis; Zaoxiu Hu wrote the manuscript; Yunchao Huang and Yonghe Zhao revised the manuscript; The authors approved the final version of the manuscript.

Funding

This research is supported by Scientific research fund project of Yunnan Provincial Department of Education, China (grant number 2019J1282), and National Key Research and Development Program of China (grant number 2017YFC0907902).

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Characteristics of included studies.

Study,year	Country (ethnicity)	No. of cases/control	Mean age (case/control)	Male ratio (case/control)	Smoking status (pack-years) (case/control)	AD ratio	Stage(I/II ratio)	Source of controls	miRNA
Xie Y,2010[13]	USA(Caucasian)	23/17	68.1/45.5	0.78/0.71	40.5 /16.2	0.56	I-IV(0.35)	CF	miR-21
Yu L,2010[19], stage I cohort	USA(mixed)	36/36	68.2/66.7	0.56/0.56	28.6/28.8	1.00	I(1.00)	HC	miR-48 and 375
Independent cohort	USA(mixed)	64/58	67/65	0.58/0.54	30.9/27.7	0.52	I-IV(0.48)	HC	miR-48
Roa,2012[20]	Canada(Caucasian)	24/6	69/54	0.67/0.80	92.0/40.0	0.71	I-II(1.00)	CF	miR-21,
Anjuman,2013[21]	USA(mixed)	39/42	65.6/62.3	0.59/0.60	48.5/46.7	0.54	I(1.00)	CF	miR-48
Shen J,2014[22], training Set	USA(mixed)	66/68	64/67	0.56/0.62	53.0/49.0	0.41	I-IV(0.66)	CF	miR-21,126, 5p,708,
Testing Set	USA(mixed)	64/73	66/64	0.64/0.66	55/50	0.47	I-IV(0.66)	CF	miR-21
Li N, 2013[23]	USA(mixed)	35/40	68.9/65.7	0.63/0.65	48.4/20.8	0.54	I(1.00)	CF	miR-21
Yang X,2013[24]	China(Asian)	24/24	60.5/57.8	0.67/0.71	40.0/15.0	0.33	I-IV(0.38)	BPD	miR-21,
Kim,2015[25]	Canada(Caucasian)	21/10	70/59	0.81/0.80	44.0/40.0	0.62	I-II(1.00)	CF/HC	miR-21,
Xing L,2010[26], case-control cohort	USA(mixed)	48/48	67.5/65.9	0.58/0.58	30.6/29.3	0	I(1.00)	HC	miR-20 429
Independent cohort	USA(mixed)	67/55	68/65	0.69/0.64	31.1/26.8	0	I-IV(0.45)	HC	miR-20 429
Xing L,2015[11],Training set	USA(mixed)	60/62	67.3/66.4	0.63/0.63	37.3/32.6	0.45	I-II(1.00)	Beingin SPN	miR-21,
Internal testing set	USA(mixed)	67/69	66.4/64.9	0.64/0.67	36.9/34.6	0.45	I-II(1.00)	Beingin SPN	miR-21,
External testing set	USA(mixed)	76/79	68.7/67.9	0.64/0.67	39.3/32.7	0.45	I-II(1.00)	Beingin SPN	miR-21,
Su Y,2016[27], training set	China(Asian)	117/174	66.5/65.4	0.80/0.79	45.8/44.7	0.54	I(1.00)	Beingin SPN	miR-21,
Testing set	China(Asian)	144/171	66.3/65.2	0.79/0.79	44.4/43.3	0.54	I(1.00)	Bingin SPN	miR-21,
Razzak,2016[28]	Canada(Caucasian)	43/10	70.0/58.0	0.62/0.80	40.1/41.7	0.54	I-IV(0.49)	CF	miR-21,
Sheervalilou,2017[29]	Iran(Caucasian)	30/30	NR	0.60/0.50	50.0/50.0	0.43	I-III(0.73)	CF	miR-10
Bagheri,2017[30]	Iran(Caucasian)	17/17	NR	0.88/0.88	NR	0.65	I-IV(0.29)	CF	miR-22
Bagheri,2018[31]	Iran(Caucasian)	30/30	62.9/60.5	0.87/0.87	26.7/13.3	0.80	I-IV(0.50)	CF	miR-145,126 and 375
Su J,2018[32], discovery cohort	USA(mixed)	68/66	64.5/64.7	0.59/0.58	34.6/33.3	0.54	I-IV(0.62)	CF	miR-31,
Validation cohort	USA(mixed)	49/50	65.2/64.7	0.61/0.60	35.5/35.2	0.53	I-IV(0.61)	CF	miR-31,

Note: AD, adenocarcinoma; NR, not report; CF, cancer-free; HC, healthy control; BPD, benign pulmonary disease; SPN, solitary pulmonary nodule

Table 2. the main results of meta-analysis.

Analysis	No. of studies	SEN (95%CI)	SPE (95%CI)	PLR (95%CI)	NLR (95%CI)	DOR (95%CI)	AUC (95
Overall	30	0.77(0.73,0.81),I^2=54.0%,p<0.001	0.87(0.83,0.90),I^2=70.2%,p<0.001	5.9(4.7,7.4)	0.26(0.22,0.30)	23(18,29)	0.89(0.8
Subgroup 1: race							
Asian	5	0.81(0.77,0.85),I^2=0.0%,p=0.86	0.85(0.81,0.88),I^2=0.0%,p=0.71	5.4(4.3,6.8)	0.22(0.17,0.28)	25(17,36)	0.90(0.8
Caucasian	14	0.79(0.70,0.86),I^2=62.8%,p<0.001	0.87(0.76,0.93),I^2=80.3%,p<0.001	6.0(3.3,10.7)	0.24(0.17,0.33)	25(13,46)	0.89(0.8
Mixed	11	0.73(0.68,0.77),I^2=43.3%,p=0.06	0.89(0.86,0.91),I^2=10.1%,p=0.35	6.4(5.1,8)	0.30(0.26,0.36)	21(15,29)	0.90(0.8
Subgroup2: miRNA profiling							
Single miRNA	38	0.74(0.67,0.79),I^2=72.2%,p<0.001	0.74(0.68,0.78),I^2=77.8%,p<0.001	2.8(2.4,3.3)	0.36(0.30,0.43)	8(6,10)	0.80(0.7
Multiple miRNA	11	0.76(0.72,0.79),I^2=47.6%,p=0.01	0.88(0.86,0.90),I^2=0.0%,p=0.74	6.4(5.4,7.5)	0.28(0.24,0.32)	23(18,29)	0.90(0.8
Subgroup3: source of control							
HC	4	0.73(0.67,0.79),I^2=0.0%,p=0.71	0.91(0.83,0.96),I^2=71.6%,p=0.01	8.5(4.0,17.9)	0.29(0.23,0.38)	29(12,70)	0.77(0.7
Cancer-free control	18	0.76(0.68,0.82),I^2=61.0%,p<0.001	0.86(0.79,0.91),I^2=78.8%,p<0.001	5.5(3.8,8.0)	0.28(0.22,0.36)	19(13,29)	0.88(0.8
BPD	8	0.82(0.78,0.85),I^2=0.0%,p=0.98	0.86(0.83,0.88),I^2=0.0%,p=0.89	5.7(4.7,6.9)	0.22(0.18,0.26)	26(19,36)	0.91(0.8
Subgroup4: histology type							
AD>50%	16	0.79(0.72,0.84),I^2=60.2%,p<0.001	0.86(0.78,0.91),I^2=79.57%,p<0.001	5.5(3.7,8.2)	0.25(0.20,0.32)	22(15,33)	0.88(0.8
AD<50%	14	0.76(0.72,0.80),I^2=23.1%,p=0.19	0.88(0.85,0.90),I^2=0.0%,p=0.46	6.5(5.3,8.0)	0.27(0.23,0.31)	24(18,32)	0.91(0.8
Subgroup5: sample size							
>100	10	0.77(0.72,0.81),I^2=56.2%,p=0.01	0.87(0.85,0.89),I^2=0.0%,p=0.62	6.0(5.0,7.2)	0.27(0.22,0.32)	22(17,30)	0.90(0.8
<100	20	0.77(0.71,0.83),I^2=54.5%,p<0.001	0.87(0.79,0.91),I^2=76.8%,p<0.001	5.8(3.8,8.6)	0.26(0.21,0.33)	22(14,34)	0.88(0.8
Subgroup6:publication year							
>2015	17	0.81(0.75,0.86),I^2=57.7%,p<0.001	0.86(0.79,0.90),I^2=76.7%,p<0.001	5.6(4.0,8.0)	0.22(0.17,0.28)	25(19,34)	0.90(0.8
<2015	13	0.71(0.67,0.75),I^2=10.75%,p=0.34	0.89(0.85,0.92),I^2=37.0%,p=0.09	6.3(4.7,8.7)	0.32(0.28,0.37)	20(13,29)	0.77(0.7
Subgroup7: stage							
I/II>0.6	18	0.76(0.72,0.80),I^2=52.2%,p=0.01	0.88(0.85,0.90),I^2=0.0%,p=0.91	6.1(5.2,7.2)	0.27(0.23,0.32)	23(17,29)	0.90(0.8
I/II<0.6	12	0.79(0.69,0.86),I^2=60.0%,p<0.001	0.85(0.72,0.93),I^2=83.5%,p<0.001	5.3(2.8,9.8)	0.25(0.18,0.35)	21(11,40)	0.87(0.8
Subgroup8: stage I/II							
Stage I/II patients	16	0.76(0.72,0.80),I^2=23.1%,p=0.19	0.88(0.85,0.90),I^2=0.0%,p=0.46	6.5(5.3,8.0)	0.27(0.23,0.31)	24(18,32)	0.91(0.8
Stage I patients	8	0.75(0.69,0.80),I^2=40.7%,p=0.11	0.88(0.85,0.91),I^2=28.8%,p=0.20	6.5(4.9,8.6)	0.28(0.22,0.35)	23(16,34)	0.90(0.8

Note: CF, cancer-free; HC, healthy control; BPD, benign pulmonary disease; AD, adenocarcinoma; SEN, sensitivity; SPE, specificity; PLR, positive likelihood ratio (PLR); NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the curve.

Table 3.The differentially expressed miRNAs with a consistent direction reported in at least two studies

miRNAs	Accession	Mature sequence	Source	Direction of expression	No of studies reported	Reference
Upregulated						
hsa-miR-210-5p	MIMAT0026475	AGCCCCUGCCCACCGCACACUG	literature	↑	11	11,20-23,25-28,31,32
hsa-miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	literature	↑	8	11,13,19,22,24,25,27,32
hsa-miR-31-5p	MIMAT0000089	AGGCAAGAUGCUGGCAUAGCU	microarray	↑	6	11,21-23, 32,37
hsa-miR-205-5p	MIMAT0000266	UCCUUCAUUCCACCGGAGUCUG	microarray,literature	↑	3	11,22,26
hsa-miR-182-5p	MIMAT0000259	UUUGGCAAUGGUAGAACUCACACU	literature	↑	3	11,19,22
hsa-miR-200b-3p	MIMAT0000318	UAAUACUGCCUGGUAAUGAUGA	microarray	↑	3	11,19,22
hsa-miR-155-5p	MIMAT0000646	UUAAUGCUAAUCGUGAUAGGGGUU	literature	↑	3	11,24,26
hsa-miR-372-5p	MIMAT0026484	CCUCAAAUGUGGAGCACUAUUCU	microarray	↑	2	11,28
Downregulated						
hsa-miR-126-3p	MIMAT0000445	UCGUACCGUGAGUAAUAAUGCG	microarray,literature	↓	5	11,19,22,26,31
hsa-miR-486-5p	MIMAT0002177	UCCUGUACUGAGCUGCCCCGAG	microarray	↓	3	11,19,22
hsa-miR-145-5p	MIMAT0000437	GUCCAGUUUUCCCAGGAAUCCCU	microarray,literature	↓	2	19,31
Conflicting						
hsa-miR-375-5p	MIMAT0037313	GCGACGAGCCCCUCGCACAAACC	microarray	↑	2	11,19
				↓	2	22,31
hsa-miR-708-5p	MIMAT0004926	AAGGAGCUUACAAUCUAGCUGGG	microarray	↓	2	11,26
				↑	1	22

Figures

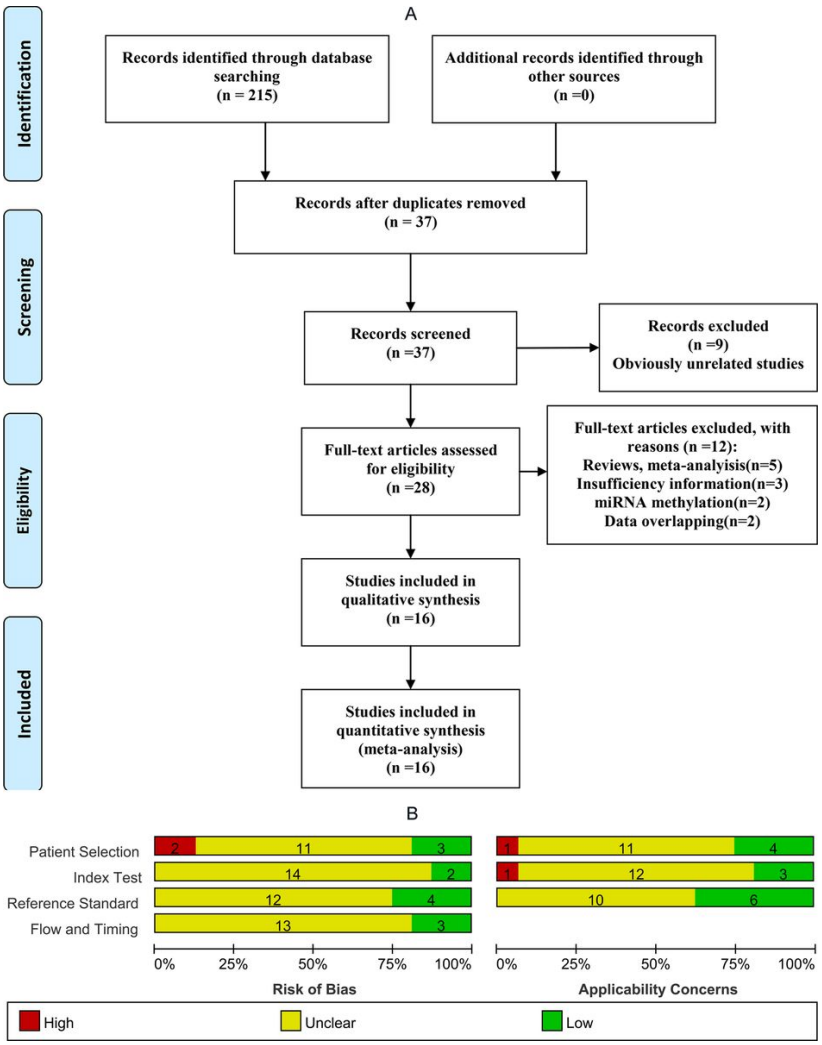

Figure 1

Flow chart of the study selection process (A) and quality of included studies according to QUADAS-2 guidelines (B).

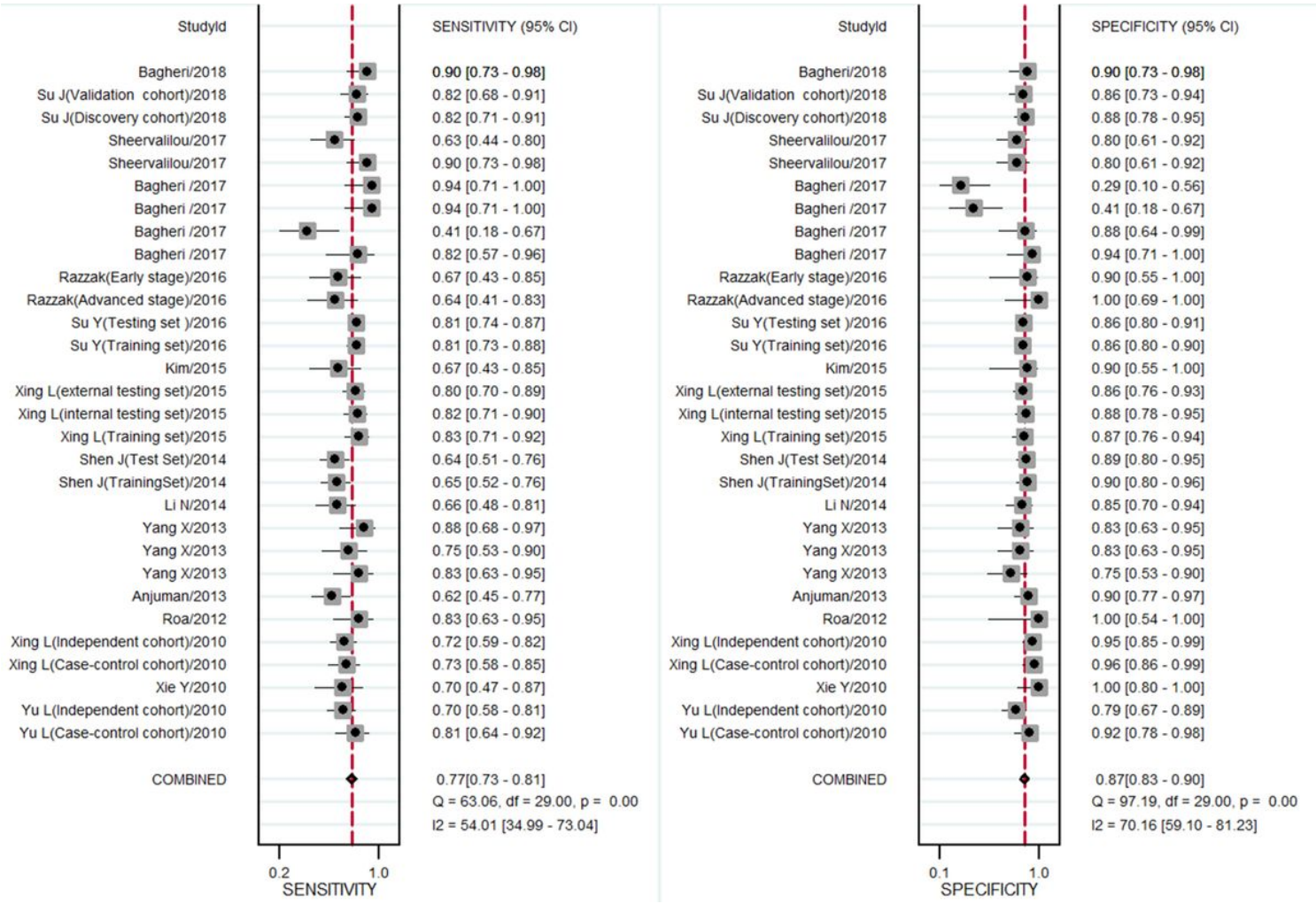

Figure 2

Forest plots of sensitivity and specificity for sputum miRNAs in the diagnosis of NSCLC patients in overall population.

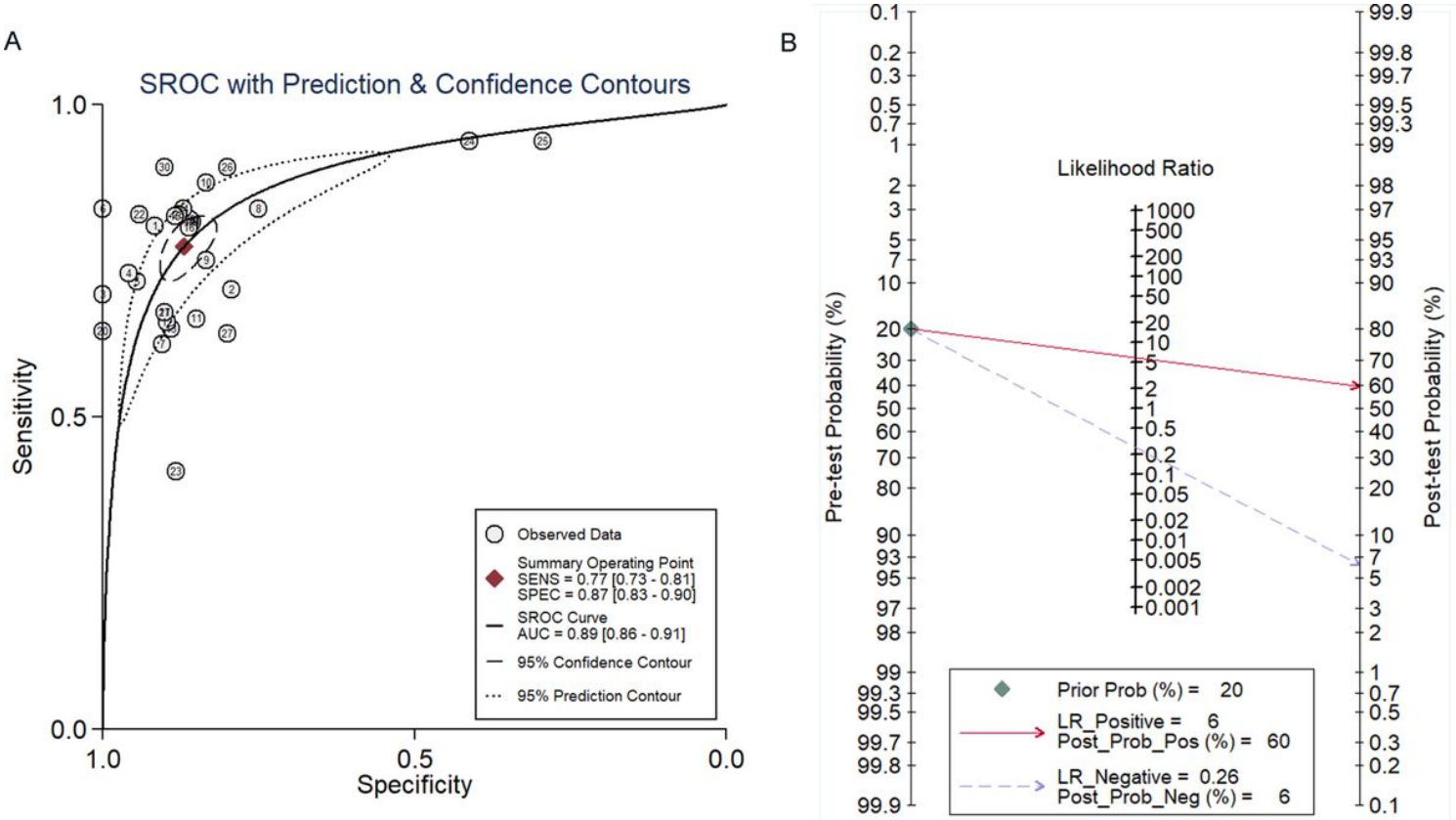

Figure 3

The summary receiver operator characteristic (SROC) curves of sputum miRNA for the diagnosis of NSCLC patients in overall population(A) and Fagan diagram evaluating the positive likelihood ratio (PLR) and negative likelihood ratio (NLR)(B).

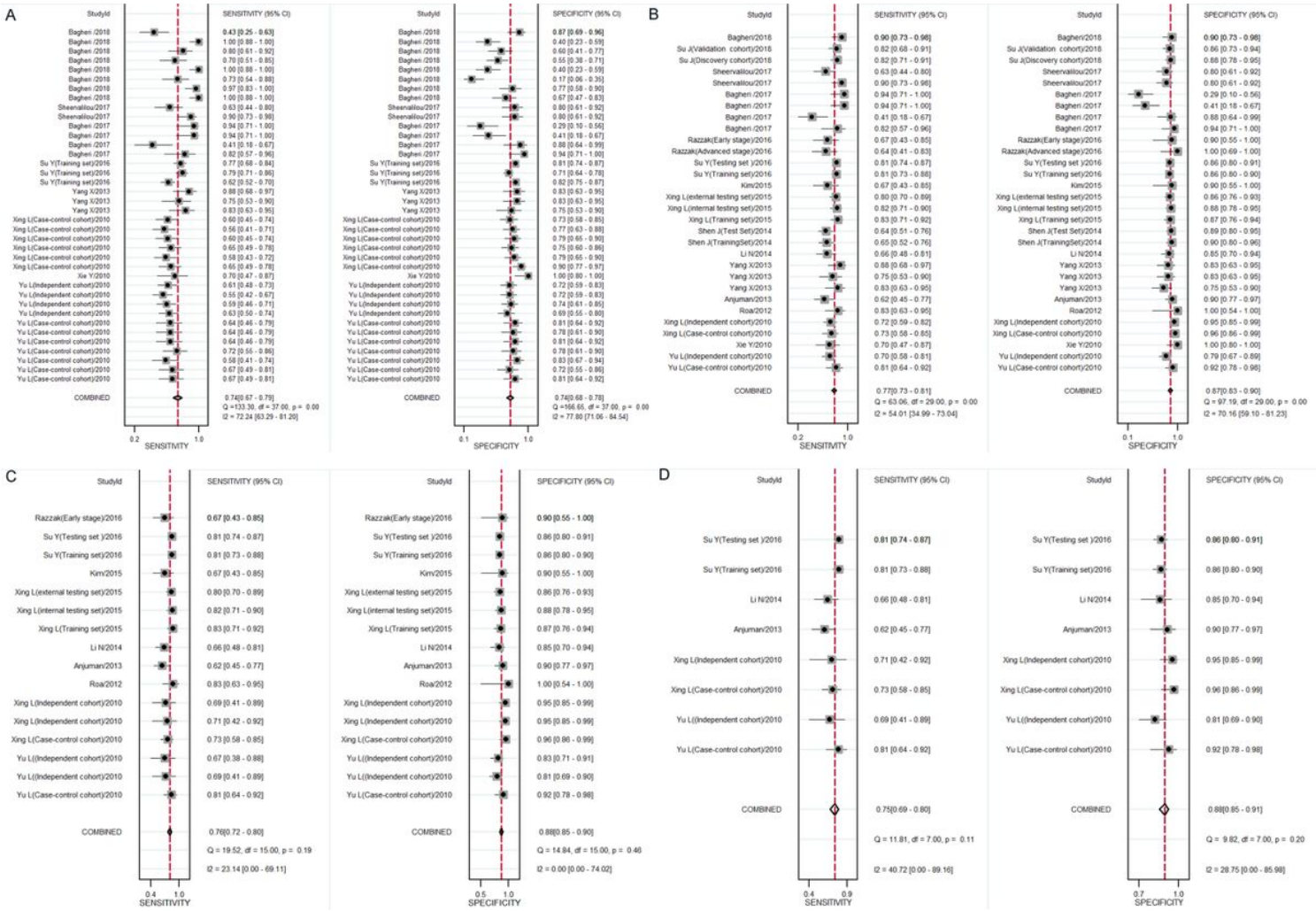

Figure 4

Forest plots of sensitivity and specificity for sputum miRNAs in the diagnosis of NSCLC by single miRNA (A), multiple miRNAs (B) and in stage I/II (C) and stage I disease (D).

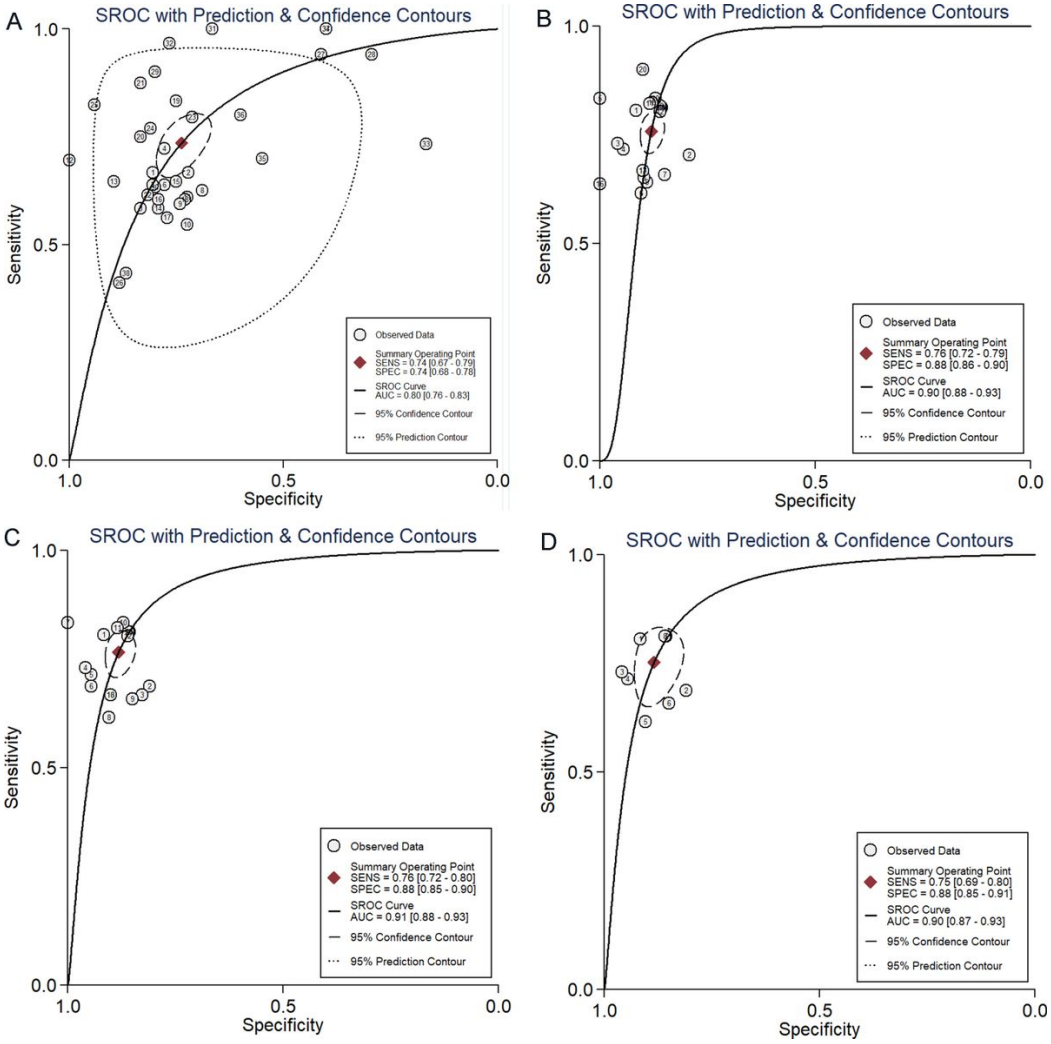

Figure 5

The summary receiver operator characteristic (SROC) curves of sputum miRNA for the diagnosis of NSCLC by single miRNA (A), multiple miRNAs (B) and in stage I/II (C) and stage I disease (D).

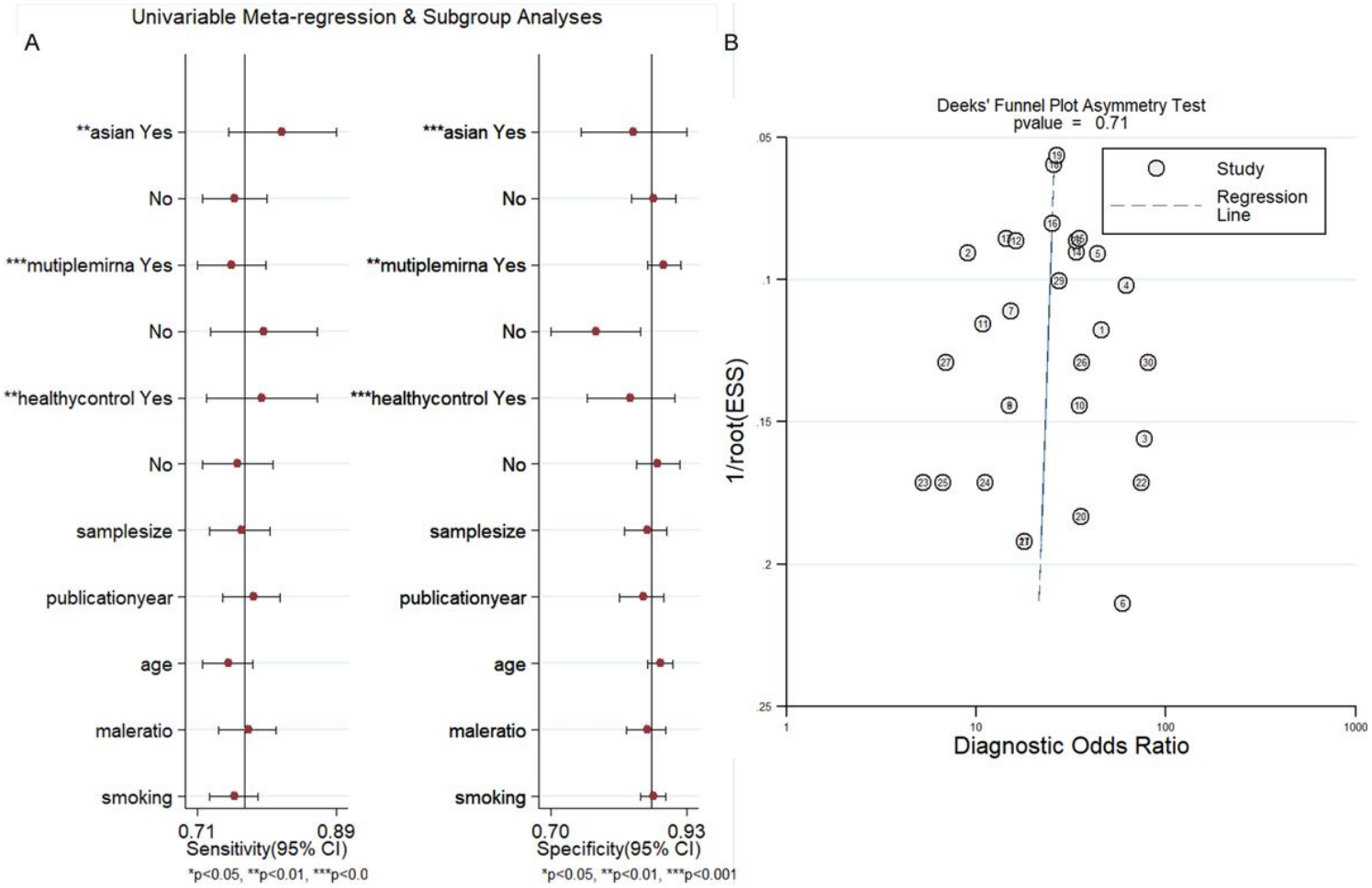

Figure 6

Forest plots of multivariable meta-regression analyses for sensitivity and specificity (A) and Deeks' linear regression test of funnel plot asymmetry (B) in overall population.