Clin. Lab. 2017;63:XXX-XXX

ORIGINAL ARTICLE

Long Non-Coding RNA Expression Signature Hallmarks Promising Efficacy in Identification of Human Non-Small Cell Lung Cancer: a Meta-Analysis Study

Hongyun Yang [*], Yanyan Han [*], Lele Wu, Chaojun Wu

[*] *Equal contributors*
Department of Clinical Laboratory, Affiliated Hospital of Taishan Medical University, Tai'An 271000, China

SUMMARY

Background: **The long non-coding RNAs (lncRNAs) are significantly altered in an expanding list of malignant neoplasms, suggesting that they might be popularized as potential biomarkers for cancer detection. This study sought to validate the diagnostic efficacy of lncRNA expression signature(s) as potential biomarker(s) for non-small cell lung cancer (NSCLC) diagnosis.**
Methods: **We conducted the online databases search for all eligible studies. A quantitative meta-analysis was performed using Stata 12.0 and Meta-Disc 1.4 statistical programs. Sensitivity analysis and a meta-regression test were applied to deeply trace the underlying heterogeneity sources.**
Results: **Eight cohorts comprised 775 NSCLC patients and 630 matched controls were included. Our data manifested that lncRNA expression profiling harbored a pooled sensitivity of 0.77 (95% CI: 0.71 - 0.82) and specificity of 0.86 (95% CI: 0.80 - 0.90) in discriminating NSCLC cases from cancer-free individuals, along with an AUC (area under the curve) value of 0.88. Further subgroup analysis revealed that paralleled testing of lncRNAs (sensitivity, specificity, and AUC of 0.90, 0.80 and 0.96, respectively) substantially strengthened the diagnostic efficacy as compared with the single testing pattern (sensitivity, specificity, and AUC of 0.71, 0.77 and 0.82, respectively). Other stratified analysis of ethnicity, histology type, and test matrix also presented robust results.**
Conclusions: **Altogether, our results indicate that lncRNA expression signature(s) might be applicable as complementary biomarker(s) for the identification of NSCLC.**
(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170325)

Correspondence:
Dr. Chaojun Wu
Department of Clinical Laboratory
Affiliated Hospital of
Taishan Medical University
No. 706, Taishan Street
Tai'An City, 271000, Shandong Province
China
Phone: +86 0538-6236505
Fax: +86 0538-6236505
Email: chaojunwu126@126.com

Manuscript accepted March 29, 2017

KEY WORDS

long non-coding RNA, non-small cell lung cancer, diagnosis, meta-analysis

INTRODUCTION

Lung cancer has a poor prognosis with a frustrating 5-year overall survival rate less than 18% [1]. According to the newly issued cancer statistical data, lung cancer continues to be the leading cause of cancer-related deaths in China [2]. Non-small cell lung cancer (NSCLC) accounts for the majority of all lung cancer types [1,3], but most of the cases are often diagnosed in late stages, resulting in unfavorable clinical outcomes. Early diagnosis and treatment remains the main ap-

proach in achieving satisfactory prognosis among the NSCLC patients. In consequence, it is urgently needed to identify and develop potential biomarkers with greater diagnostic values for NSCLC.

The discovery of long non-coding RNAs (lncRNAs) has broadened the horizons in cancer investigation. LncRNA is classified as one kind of endogenous RNAs possessing a structural sequence of more than 200 nucleotides, but has no protein-coding frame(s) [4]. Since their discoveries, lncRNAs are found to be involved in diverse biological processes, such as gene expression, cell growth, apoptosis, migration, and so forth [5-7]. Importantly, an increasing number of lncRNAs have been shown to participate in the occurrence and development of multiple cancers, especially in NSCLC [8-17]. Of note, the markedly altered expression status of lncRNAs in NSCLC suggested that they might be popularized as potential biomarkers or indicators for cancer identification [9-16]. Notwithstanding, it seems that different lncRNA expression patterns yielded different accuracy in conforming NSCLC. For instance, it is reported that single testing of lncRNA-MALAT1 confers a relatively low sensitivity of 56%, but a high specificity of 96% in discriminating NSCLC patients from cancer-free individuals [15]. Nevertheless, a subset of five-lncRNA panels hallmarked an estimated sensitivity of 96.8% and specificity of 92.1%, revealing a high efficacy of lncRNAs for NSCLC [14]. Thus, it can be seen that individual studies with small sample sizes often lead to inconsistent results and compromise the final study accuracy. Herein, we established standardized inclusion and exclusion criteria, and conducted this meta-analysis to derive a more precise estimation of the diagnostic accuracy of lncRNA expression signature for NSCLC.

MATERIALS AND METHODS

Study search

To obtain eligible studies, we systematically searched the online PubMed, EMBASE, and BioMed Central databases from inception to March 1st, 2017. The search items included "long non coding RNA OR lncRNA", "lung cancer OR lung tumor OR lung carcinoma OR pulmonary tumor", "non-small-cell lung cancer OR non-small-cell lung carcinoma OR NSCLC", and "sensitivity OR specificity OR ROC OR AUC OR area under the curve OR diagnosis". Article references were also manually searched for other relevant publications.

Inclusion and exclusion criteria

The inclusion criteria were predefined as: (1) studies assessing the diagnostic value of lncRNA(s) in confirming NSCLC; (2) studies clearly addressing the patient and control sizes; and (3) diagnostic parameters included true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN) were available or could be calculated indirectly. Publications were excluded according to the following criteria: (1) studies unrelated to the diagnostic utility of lncRNA(s) for NSCLC; (2) data insufficient to establish the 2 x 2 table; (3) studies with a sample number less than 20; (4) studies failing to state the control types; and (5) non-English articles, reviews, basic research, letters and comments, meta-analyses, etc.

Data extraction and article quality evaluation

Data were collected by two reviewers (Hongyun Yang and Yanyan Han) and the information included: (1) name of the first author and publication year, (2) country and ethnicity, (3) sample sizes and control sources, and (4) test method, reference gene, sensitivity, and specificity, etc. Any disagreement from the data extractors were finally resolved by group discussion.

Article quality of all enrolled studies was judged in terms of the newly issued Quality Assessment for Studies of Diagnostic Accuracy (QUADAS) II checklist [18], in which the concerns for risk of bias and applicability were defined as "low", "high" or "unclear", corresponding to an evaluation score of "1", "0" and "0", respectively.

Statistical analysis

We reported this meta-analysis following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [19]. All statistical analyses were undertaken based on Stata 12.0 (Stata Corporation, College Station, TX, USA) and Meta-disc 1.4 (XI Cochrane Colloquium, Barcelona, Spain) programs. Study heterogeneity was assessed by Cochran's-Q and I^2 tests as well as L'Abbe and Galbraith plots, and $p < 0.01$ or $I^2 > 50\%$ were deemed as statistically significant. The parameters as sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and AUC with corresponding 95% CIs were combined using a random-effects model when significant heterogeneity was detected among studies; otherwise, a fixed-effects model will be chosen [20]. Bias from article publication was checked via Deek's funnel plot asymmetry test, and $p < 0.05$ was treated as the significant level.

RESULTS

Article enrollment and study characteristics

As graphed in Figure 1, a total of 562 relevant records were obtained from the online databases after an elimination of the duplicated articles. All of the studies received a detailed review of article abstracts, and 256 records were accordingly excluded because the contents were unrelated to our topic. The remaining 306 publications received further evaluations and 298 of them were eliminated due to the status of reviews (n = 24), basic studies (n = 167), prognosis related studies (n = 93), and meta-analysis (n = 14). Finally, only 8 cohorts [9-16], comprising 34 individual studies seemed to meet the

Table 1. Main features of the included studies.

First author	Year	Country	Sample size		Individual studies	Sample version	Control sources	LncRNA signature	Test method	Reference gene	QUADAS score
			NSCLC	Controls							
Hu et al. [9]	2016	China	120	120	2	Plasma	Cancer-free	SPRY4-IT1, ANRIL, NEAT1	RT-qPCR	Unclear	6
Pan et al. [10]	2015	China	125	125	1	Tissue	Para-carcinoma	NEAT1	RT-qPCR	GAPDH	5
Tantai et al. [12]	2015	China	32	30	3	Tissue	Normal tissue	XIST, HIF1A-AS1	RT-qPCR	GAPDH	5
Tang et al. [11]	2015	China	252	155	2	Plasma	Cancer-free	RP11-397D12.4, AC007403.1, ERICH1-AS1	RT-qPCR	U6	6
Wang et al. [13]	2015	China	60	60	2	Tissue	Para-carcinoma	UCA1	RT-qPCR	GAPDH	5
Weber et al. [15]	2015	Germany	45	25	12	Plasma	Cancer-free	MALAT1	RT-qPCR	GAPDH, HPRT1	6
Wang et al. [14]	2015	China	50	50	10	Tissue	Cancer-free	A panel of 10 lncRNAs	RT-qPCR	GAPDH	6
Yu et al. [16]	2015	China	91	65	2	Tissue	Cancer-free	A panel of 64 lncRNAs	RT-qPCR	β-actin	5

NSCLC - non-small cell lung cancer, RT-qPCR - reverse transcription quantitative real-time polymerase chain reaction, QUADAS - quality assessment for studies of diagnostic accuracy.

aim of this study and were enrolled for the meta-analysis.

Study characteristics are summarized in Table 1. The 8 eligible studies contained a study population of 775 NSCLC patients and 630 matched controls. All NSCLC cases had a clear diagnosis by pathological examination, and the controls included healthy [12], cancer-free [9, 11,14-16], and para-carcinoma samples [10,13]. All tissue or blood samples were obtained from the patients before undergoing either therapy. The ethnic population involves Europeans and Asians. Test of all lncRNAs were enabled by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), and the reference genes included GAPDH [10,12-15], β-actin [16], HPRT1 [15], and U6 [11].

Study quality and heterogeneity

All included studies received a comprehensive evaluation of article quality according to the 7-item QUADAS II checklist [18]. As shown in Figure 2 and Table 1, all studies retained a judge score higher than 5, suggesting a relatively high quality of the enrolled studies. For the heterogeneity analysis, the Cochran's-Q test presented an estimated p-value of 0.000 for the overall pooled study, accompanied by $I^2 = 98.73\%$, displaying significant heterogeneity among studies. On the other hand, heterogeneity evaluated by L'Abbe and Galbraith plots also showed obvious heterogeneity distribution among studies (Supplement Figure 1). As a result, a random-effects model was employed for the finally meta-analysis.

Diagnostic performance

The pooled AUC of lncRNA expression profiling in identification of NSCLC were estimated to be 0.88, where the combined sensitivity and specificity were 0.77 (95% CI: 0.71 - 0.82) and 0.86 (95% CI: 0.80 - 0.90), respectively (Figure 3). Additionally, lncRNA signature retained a PLR of 5.52 (95% CI: 3.91 - 7.81), NLR of 0.27 (95% CI: 0.22 - 0.34), and DOR of 20.43 (95% CI: 13.28 - 31.21), displaying an overall high efficacy in confirming NSCLC.

Subgroup analysis

Subgroup analyses were carried out according to lncRNA testing pattern (single or in parallel), ethnicity, tu-

Table 2. Subgroup analysis of the lncRNA testing efficacy for NSCLC based on testing pattern, ethnicity, histology type and test matrix.

Subgroups	AUC	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	Publication bias p-value
Ethnicity							
Asian	0.87	0.82 (0.80 - 0.84)	0.74 (0.72 - 0.77)	3.32 (2.67 - 4.12)	0.23 (0.18 - 0.31)	15.69 (10.09 - 24.38)	0.505
European	0.82	0.55 (0.50 - 0.60)	0.93 (0.90 - 0.96)	9.00 (4.45 - 18.19)	0.49 (0.44 - 0.55)	19.23 (10.92 - 33.88)	0.371
Testing pattern							
Single	0.82	0.71 (0.68 - 0.73)	0.77 (0.75 - 0.80)	3.34 (2.69 - 4.16)	0.37 (0.32 - 0.44)	12.17 (8.83 - 16.78)	0.000
Parallel	0.96	0.90 (0.87 - 0.93)	0.80 (0.76 - 0.83)	6.59 (3.20 - 15.10)	0.13 (0.07 - 0.23)	61.26 (20.31 - 184.78)	0.413
Histology types							
Squamous carcinoma	0.66	0.63 (0.50 - 0.74)	0.95 (0.87 - 0.99)	11.02 (4.18 - 29.04)	0.40 (0.29 - 0.54)	28.56 (9.26 - 88.10)	/
Adeno-carcinoma	0.85	0.77 (0.74 - 0.81)	0.75 (0.71 - 0.78)	2.94 (2.37 - 3.64)	0.32 (0.25 - 0.41)	11.54 (8.77 - 15.19)	0.767
Sample type							
Plasma	0.90	0.87 (0.83 - 0.90)	0.74 (0.69 - 0.78)	3.85 (2.40 - 6.19)	0.19 (0.14 - 0.25)	20.51 (11.49 - 36.59)	0.426
Tissue	0.85	0.78 (0.75 - 0.80)	0.74 (0.71 - 0.76)	3.09 (2.39 - 3.99)	0.27 (0.20 - 0.37)	12.63 (7.37 - 21.66)	0.372

PLR - positive likelihood ratio, NLR - negative likelihood ratio, DOR - diagnostic odds ratio, AUC - area under the curve.

mor histology type, and test matrix. The results are exemplified in Table 2. The data displayed that paralleled testing of lncRNAs substantially increased the diagnostic efficacy versus single lncRNA analysis (AUC: 0.96 versus 0.82; sensitivity: 0.90 versus 0.71; specificity: 0.80 versus 0.77; PLR: 6.59 versus 3.34; NLR: 0.13 versus 0.37; DOR: 61.26 versus 12.17). When stratified by ethnicity, it revealed that the Asian-based lncRNA signature achieved better diagnostic efficacy than the European-based analysis (AUC: 0.87 versus 0.82). Furthermore, a comparison of lncRNA expression patterns according to histology type manifested that testing of lncRNAs in adenocarcinoma hallmarked a superior efficacy than that in squamous cell carcinoma (AUC: 0.85 versus 0.66). We finally stratified the study by test matrix. The pooled AUC values in plasma- and tissue-based tests were 0.90 and 0.85, respectively (Table 2).

Influence assay and meta-regression

Our influence analysis identified no single studies as deviations, suggesting a homogeneous distribution of the enrolled studies (Figure 4). A meta-regression test was employed to deeply trace the underlying causes of study heterogeneity with 7 pre-specified covariates: test matrix, histology types, ethnicity, patient and control numbers, reference gene, and QUADAS score [21]. Our analysis revealed that the histology type ($p = 0.01$) and control size ($p = 0.01$) were likely to be the underlying factors in giving rise to study heterogeneity (Supplement Table 1).

Publication bias

Publication bias among studies assessed by Deek's funnel plot asymmetry test showed a p-value of 0.424 for the overall combined study, hinting that no obvious bias was generated from different publications (Figure 5). The p-values of publication bias in subgroup studies are summarized in Table 2.

DISCUSSION

Non-small cell lung cancer (NSCLC) represents approximately 80% of all carcinomas of the lungs [2]. The 5-year survival rate of NSCLC remains unsatisfactory due to the fact that most cases are diagnosed in advanced or late stages following diagnosis [3]. In principal, tumor biomarkers should be feasible for detecting cancer in early stages. However, the conventional blood tumor markers, such as carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and cytokeratin 19 fragment 21-1 (CYFRA 21-1), etc., are all incapable of discerning lung cancer in early stages, but the levels of which are always influenced by some non-specific fac-

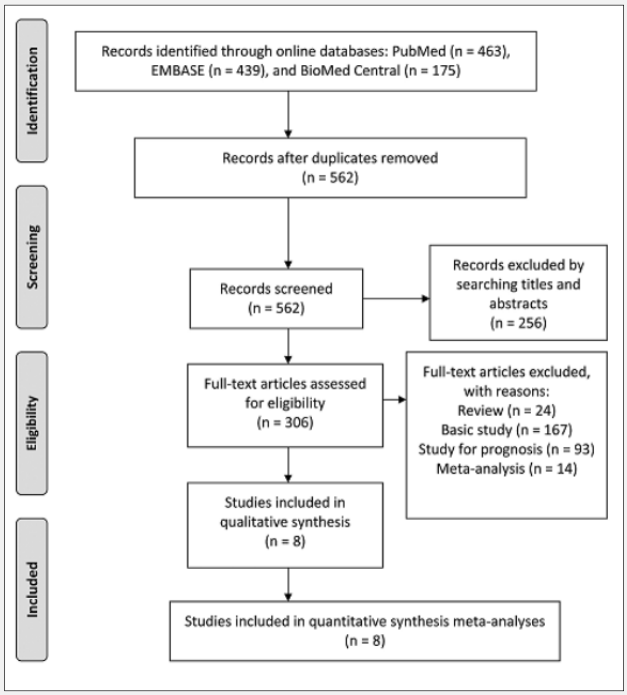


Figure 1. Flow diagram of study enrollment procedure.

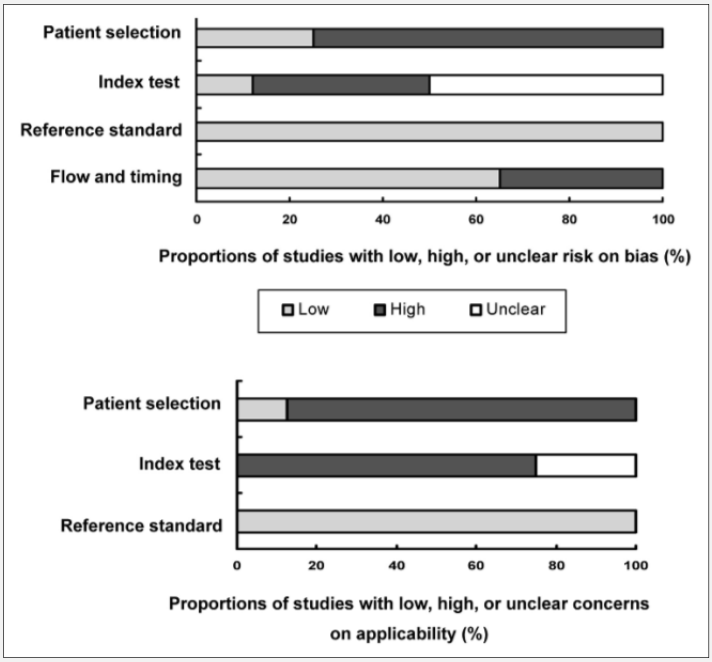
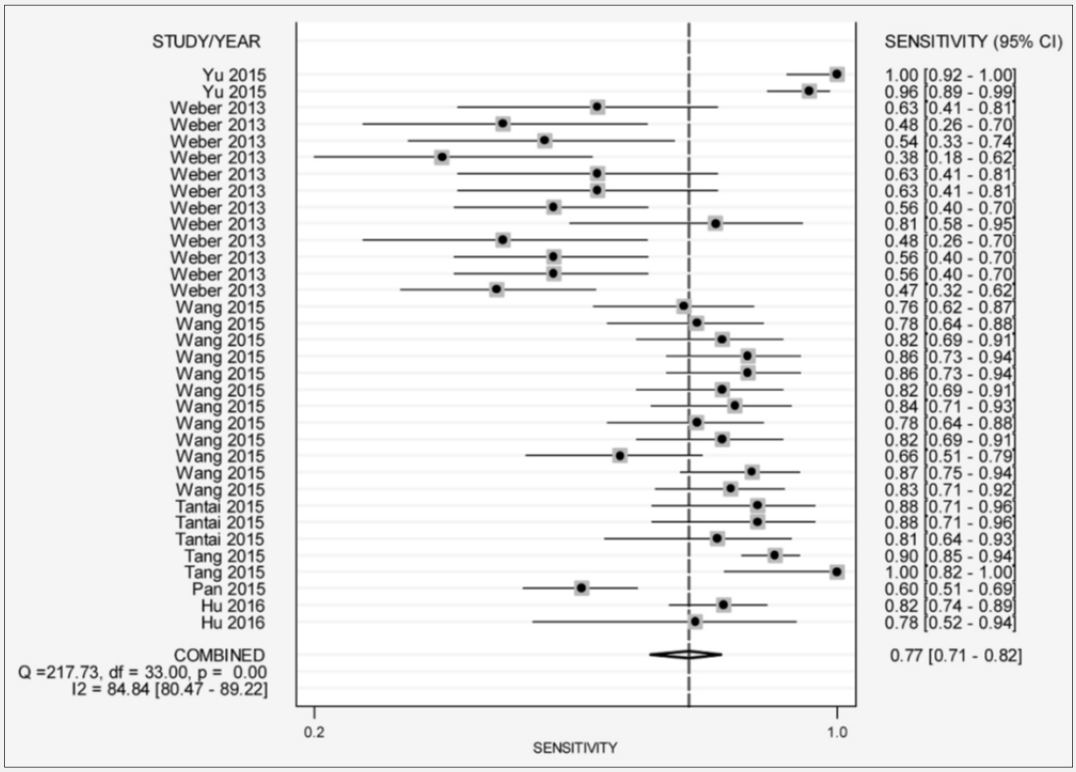
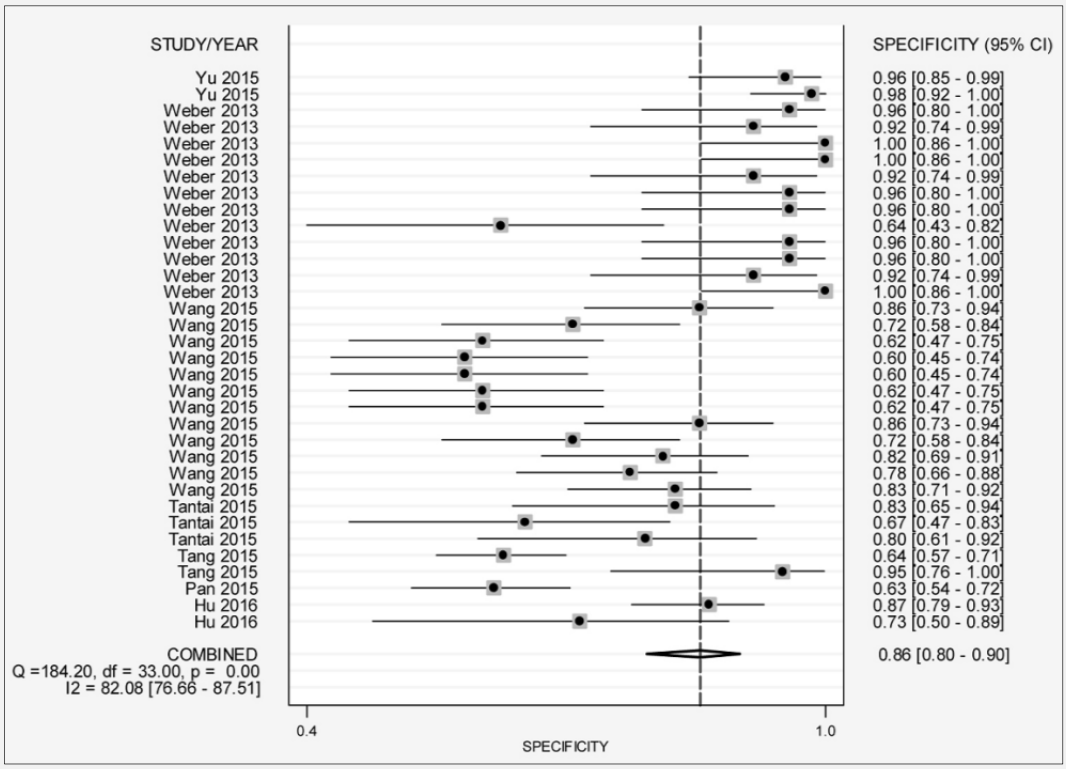


Figure 2. Study quality assessment using the QUADAS II checklist.

A


STUDY/YEAR
SENSITIVITY (95% CI)
Yu 2015 1.00 [0.92 - 1.00]
Yu 2015 0.96 [0.89 - 0.99]
Weber 2013 0.63 [0.41 - 0.81]
Weber 2013 0.48 [0.26 - 0.70]
Weber 2013 0.54 [0.33 - 0.74]
Weber 2013 0.38 [0.18 - 0.62]
Weber 2013 0.63 [0.41 - 0.81]
Weber 2013 0.63 [0.41 - 0.81]
Weber 2013 0.56 [0.40 - 0.70]
Weber 2013 0.81 [0.58 - 0.95]
Weber 2013 0.48 [0.26 - 0.70]
Weber 2013 0.56 [0.40 - 0.70]
Weber 2013 0.56 [0.40 - 0.70]
Weber 2013 0.47 [0.32 - 0.62]
Wang 2015 0.76 [0.62 - 0.87]
Wang 2015 0.78 [0.64 - 0.88]
Wang 2015 0.82 [0.69 - 0.91]
Wang 2015 0.86 [0.73 - 0.94]
Wang 2015 0.86 [0.73 - 0.94]
Wang 2015 0.82 [0.69 - 0.91]
Wang 2015 0.84 [0.71 - 0.93]
Wang 2015 0.78 [0.64 - 0.88]
Wang 2015 0.82 [0.69 - 0.91]
Wang 2015 0.66 [0.51 - 0.79]
Wang 2015 0.87 [0.75 - 0.94]
Wang 2015 0.83 [0.71 - 0.92]
Tantai 2015 0.88 [0.71 - 0.96]
Tantai 2015 0.88 [0.71 - 0.96]
Tantai 2015 0.81 [0.64 - 0.93]
Tang 2015 0.90 [0.85 - 0.94]
Tang 2015 1.00 [0.82 - 1.00]
Pan 2015 0.60 [0.51 - 0.69]
Hu 2016 0.82 [0.74 - 0.89]
Hu 2016 0.78 [0.52 - 0.94]
COMBINED 0.77 [0.71 - 0.82]
Q =217.73, df = 33.00, p = 0.00
I2 = 84.84 [80.47 - 89.22]
0.2
1.0
SENSITIVITY

B


STUDY/YEAR
SPECIFICITY (95% CI)
Yu 2015 0.96 [0.85 - 0.99]
Yu 2015 0.98 [0.92 - 1.00]
Weber 2013 0.96 [0.80 - 1.00]
Weber 2013 0.92 [0.74 - 0.99]
Weber 2013 1.00 [0.86 - 1.00]
Weber 2013 1.00 [0.86 - 1.00]
Weber 2013 0.92 [0.74 - 0.99]
Weber 2013 0.96 [0.80 - 1.00]
Weber 2013 0.96 [0.80 - 1.00]
Weber 2013 0.64 [0.43 - 0.82]
Weber 2013 0.96 [0.80 - 1.00]
Weber 2013 0.96 [0.80 - 1.00]
Weber 2013 0.92 [0.74 - 0.99]
Weber 2013 1.00 [0.86 - 1.00]
Wang 2015 0.86 [0.73 - 0.94]
Wang 2015 0.72 [0.58 - 0.84]
Wang 2015 0.62 [0.47 - 0.75]
Wang 2015 0.60 [0.45 - 0.74]
Wang 2015 0.60 [0.45 - 0.74]
Wang 2015 0.62 [0.47 - 0.75]
Wang 2015 0.62 [0.47 - 0.75]
Wang 2015 0.86 [0.73 - 0.94]
Wang 2015 0.72 [0.58 - 0.84]
Wang 2015 0.82 [0.69 - 0.91]
Wang 2015 0.78 [0.66 - 0.88]
Wang 2015 0.83 [0.71 - 0.92]
Tantai 2015 0.83 [0.65 - 0.94]
Tantai 2015 0.67 [0.47 - 0.83]
Tantai 2015 0.80 [0.61 - 0.92]
Tang 2015 0.64 [0.57 - 0.71]
Tang 2015 0.95 [0.76 - 1.00]
Pan 2015 0.63 [0.54 - 0.72]
Hu 2016 0.87 [0.79 - 0.93]
Hu 2016 0.73 [0.50 - 0.89]
COMBINED 0.86 [0.80 - 0.90]
Q =184.20, df = 33.00, p = 0.00
I2 = 82.08 [76.66 - 87.51]
0.4
1.0
SPECIFICITY

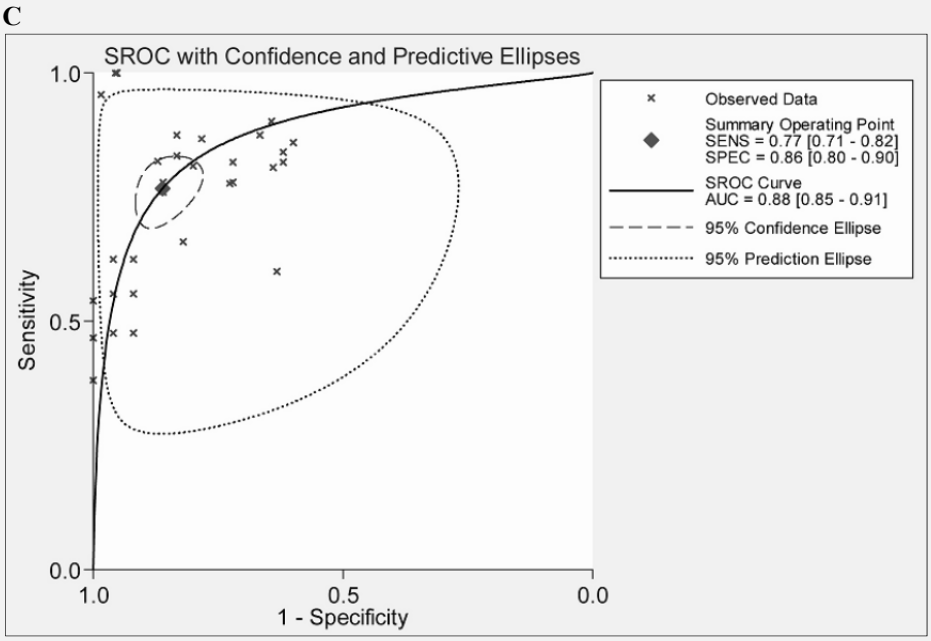


Figure 3. Forest plots of the overall pooled analysis for lncRNA signature in confirming NSCLC. (A) sensitivity; (B) specificity; and (C) SROC curve.

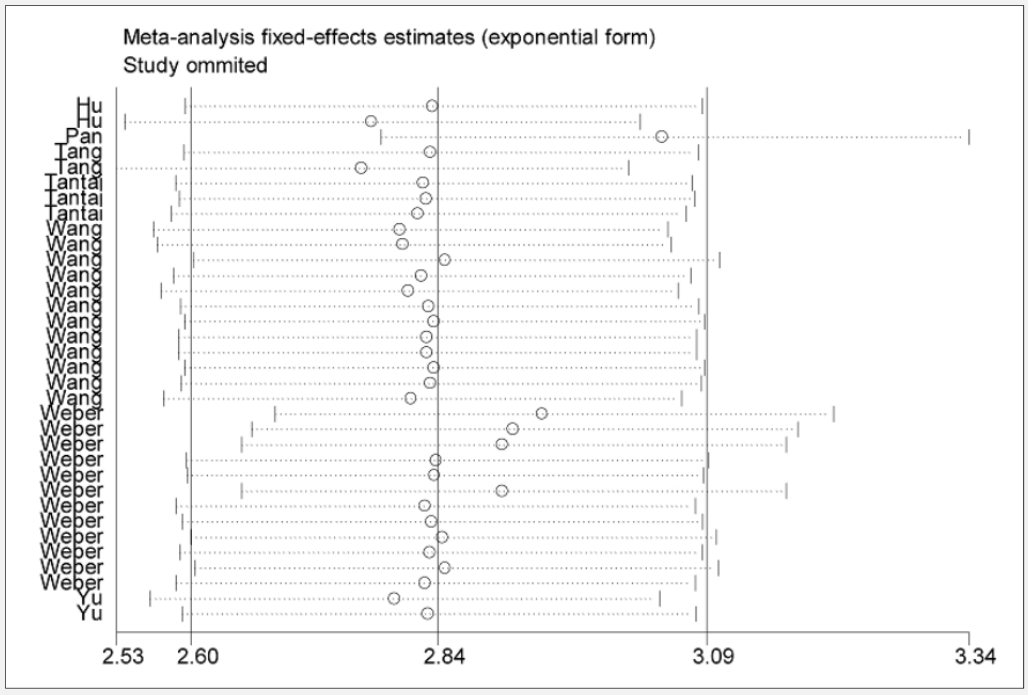


Figure 4. Sensitivity analysis of the overall pooled study.

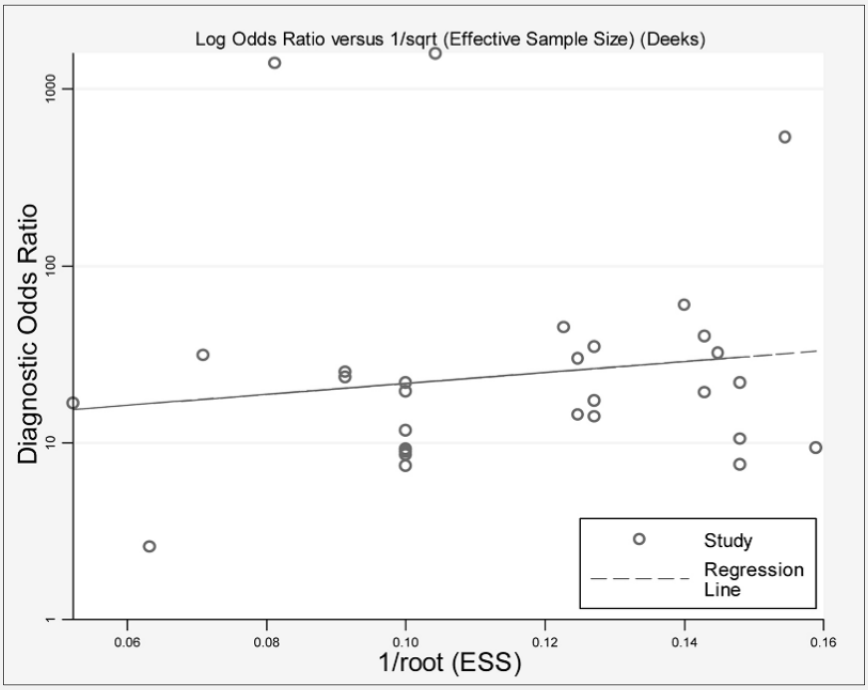


Figure 5. Publication bias assessed by Deek's funnel plot asymmetry test.

tors unrelated to cancer [22,23]. In recent years, the lncRNA expression signature has been highlighted as potential biomarker(s) for NSCLC by an increasing number of studies [8-17]. In the present study, we made a comprehensive evaluation of the diagnostic utilities of lncRNA expression profiling for NSCLC.

The overall pooled analysis showed the lncRNA expression signature hallmarked an estimated sensitivity of 0.77 and specificity of 0.86 in discerning NSCLC cases from cancer-free individuals with an AUC of 0.88, suggesting that lncRNA profiling confers a relatively high efficacy for NSCLC detection. In addition, the PLR of 5.52 indicated that patients with NSCLC held nearly 6-fold higher chance of being tested lncRNA positive (or reveal marked alteration in expression levels) versus cancer-free individuals. The estimated NLR of 0.27 also implied that analysis of lncRNAs retained a false-negative rate of 27% in a negative result, which is low enough to rule out NSCLC. Another important indicator of diagnostic accuracy was the diagnostic odds ratio (DOR), for which a value less than 1.0 hints at a low discriminating ability for a diagnostic test [24]. Our analysis obtained a DOR value of 20.43, hinting at a high discriminatory performance for the lncRNA's testing. Taken together, our results showed promising accuracy for lncRNA expression signature in confirming NSCLC.

We further conducted a subgroup study stratified by lncRNA testing pattern (single or in parallel), ethnicity, histology type, and test matrix. Our results indicated that paralleled testing of lncRNAs substantially increased the diagnostic efficacy as compared with single lncRNA assay. A newly published study corroborates our findings: combined testing of two lncRNAs increased the AUC in gastric cancer [25]. Another study by Cui et al. also got the similar results [26]. Studies have documented that the clinical value of non-coding RNAs are racially diverse [27-29]. Similarly, our analysis stratified by ethnicity also presented robust results: Asian-based lncRNA signature conferred better diagnostic efficacy than that of the European-based analysis. Furthermore, a comparison of lncRNA expression patterns in tumor histology type manifested that testing of lncRNAs in adenocarcinoma hallmarked superior efficacy to that of squamous cell carcinoma. We finally stratified the study by test matrix, and the data showed that plasma-based lncRNA testing achieved higher accuracy than the tissue-based test, implying that plasma may be more suitable for the analysis of lncRNAs in conforming NSCLC. The matrix difference has been confirmed in a published meta-analysis regarding non-coding RNAs, which supported our findings [27]. However, our plasma-based analysis only included 3 individual studies and presented significant heterogeneity,

thus more evidence is still needed to reinforce this evidence.

The overall pooled analysis revealed significant heterogeneity which may potentially impact the study accuracy [30,31]. Based on these considerations, the sensitivity analysis and meta-regression test were carried out. The sensitivity analysis showed no outlier studies, suggesting a relatively homogeneous distribution of all included studies. Furthermore, the meta-regression test exhibited that histology type and control size were likely to be the underlying causes of heterogeneity among studies.

CONCLUSION

In summary, this meta-analysis suggests that lncRNA expression profiling may be suitable for use as diagnostic biomarker(s) in conforming NSCLC. Nevertheless, the current study still yielded some limitations: Firstly, the expression signature contained many types of lncRNA, yet the suitable lncRNA(s) or expression patterns for clinical applications still warranted further identification. Secondly, the control sources are complicated (included healthy, cancer-free, and para-carcinoma samples), which may further contribute to study heterogeneity. Last, there may be population bias among the combined assays, as only 3 individual studies conducted in Europeans were included and resulted in the small case sizes in our stratified analyses. More high quality clinical studies are required to further prove this preliminary evidence.

Declaration of Interest:

The authors stated that there are no conflicts of interest regarding the contents of this article.

References:

1. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016;5:288-300 (PMID: 27413711).
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32 (PMID: 26808342).
3. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14 (PMID: 17762336).
4. Brosnan CA, Voinnet O. The long and the short of noncoding RNAs. Curr Opin Cell Biol 2009;21:416-25 (PMID: 19447594).
5. Chen LL. Linking Long Noncoding RNA Localization and Function. Trends Biochem Sci 2016;41:761-72 (PMID: 27499234).
6. Dhamija S, Diederichs S. From junk to master regulators of invasion: lncRNA functions in migration, EMT and metastasis. Int J Cancer 2016;139:269-80 (PMID: 26875870).
7. Fang Y, Fullwood MJ. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. Genomics Proteomics Bioinformatics 2016;14:42-54 (PMID: 26883671).
8. Chen J, Wang R, Zhang K, Chen LB. Long non-coding RNAs in non-small cell lung cancer as biomarkers and therapeutic targets. J Cell Mol Med 2014;18:2425-36 (PMID: 25297942).
9. Hu X, Bao J, Wang Z, et al. The plasma lncRNA acting as fingerprint in non-small-cell lung cancer. Tumour Biol 2016;37:3497-504 (PMID: 26453113).
10. Pan LJ, Zhong TF, Tang RX, et al. Upregulation and clinicopathological significance of long non-coding NEAT1 RNA in NSCLC tissues. Asian Pac J Cancer Prev 2015;16:2851-5 (PMID: 25854373).
11. Tang Q, Ni Z, Cheng Z, Xu J, Yu H, Yin P. Three circulating long non-coding RNAs act as biomarkers for predicting NSCLC. Cell Physiol Biochem 2015;37:1002-9 (PMID: 26393913).
12. Tantai J, Hu D, Yang Y, Geng J. Combined identification of long non-coding RNA XIST and HIF1A-AS1 in serum as an effective screening for non-small cell lung cancer. Int J Clin Exp Pathol 2015;8:7887-95 (PMID: 26339353).
13. Wang HM, Lu JH, Chen WY, Gu AQ. Upregulated lncRNA-UCA1 contributes to progression of lung cancer and is closely related to clinical diagnosis as a predictive biomarker in plasma. Int J Clin Exp Med 2015;8:11824-30 (PMID: 26380024).
14. Wang P, Lu S, Mao H, et al. Identification of biomarkers for the detection of early stage lung adenocarcinoma by microarray profiling of long noncoding RNAs. Lung Cancer 2015;88:147-53 (PMID: 25758555).
15. Weber DG, Johnen G, Casjens S, et al. Evaluation of long noncoding RNA MALAT1 as a candidate blood-based biomarker for the diagnosis of non-small cell lung cancer. BMC Res Notes 2013;6:518 (PMID: 24313945).
16. Yu H, Xu Q, Liu F, Ye X, Wang J, Meng X. Identification and validation of long noncoding RNA biomarkers in human non-small-cell lung carcinomas. J Thorac Oncol 2015;10:645-54 (PMID: 25590602).
17. Yu X, Li Z. Long non-coding RNA HOTAIR: A novel oncogene (Review). Mol Med Rep 2015;12:5611-8 (PMID: 26238267).
18. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529-36 (PMID: 22007046).
19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006-12 (PMID: 19631508).
20. Luo Y, Zhang X, Tan Z, et al. Astrocyte Elevated Gene-1 as a Novel Clinicopathological and Prognostic Biomarker for Gastrointestinal Cancers: A Meta-Analysis with 2999 Patients. PLoS One 2015;10:e0145659 (PMID: 26710214).
21. Cui Z, Chen Y, Xiao Z, et al. Long noncoding RNAs as auxiliary biomarkers for gastric cancer screening: A pooled analysis of individual studies. Oncotarget 2016;7:25791-800 (PMID: 27015554).
22. Nakamura H, Nishimura T. History, molecular features, and clinical importance of conventional serum biomarkers in lung cancer. Surg Today 2017. doi: 10.1007/s00595-017-1477-y [Epub ahead of print] (PMID: 28229299).

23. Tang TT, Cheng HH, Zhang H, et al. Hypereosinophilic obliterative bronchiolitis with an elevated level of serum CEA: a case report and a review of the literature. Eur Rev Med Pharmacol Sci 2015;19:2634-40 (PMID: 26221894).

24. Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. Stat Med 1993;12:1293-316 (PMID: 8210827).

25. Sun W, Mo X, Li T, Xie Y, Guo J. Clinical significance of the long noncoding RNA RP11-19P22.6-001 in gastric cancer. Cancer Biomark 2017. doi: 10.3233/CBM-160264 [Epub ahead of print] (PMID: 28128738).

26. Cui X, Sun X, Niu W, et al. Long Non-Coding RNA: Potential Diagnostic and Therapeutic Biomarker for Major Depressive Disorder. Med Sci Monit 2016;22:5240-8 (PMID: 28039689).

27. Cui Z, Lin D, Song W, Chen M, Li D. Diagnostic value of circulating microRNAs as biomarkers for breast cancer: a meta-analysis study. Tumour Biol 2015;36:829-39 (PMID: 25296735).

28. Deng Q, Sun H, He B, et al. Prognostic value of long non-coding RNA HOTAIR in various cancers. PloS One 2014;9:e110059 (PMID: 25303230).

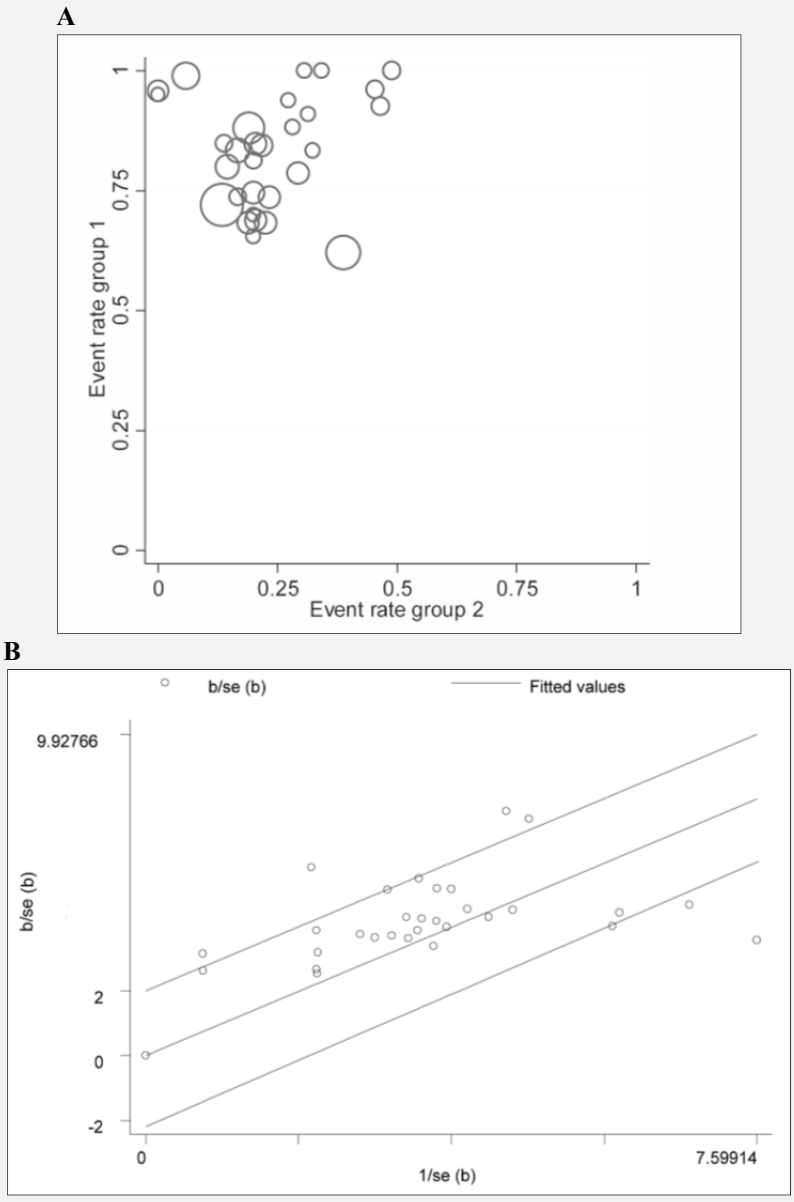
29. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol 2010; 28:1721-6 (PMID: 20194856).

30. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58 (PMID: 12111919).

31. Zhou Y, Dendukuri N. Statistics for quantifying heterogeneity in univariate and bivariate meta-analyses of binary data: the case of meta-analyses of diagnostic accuracy. Stat Med 2014;33:2701-17 (PMID: 24903142).

Supplement Table 1. Meta-regression test of the over pooled analysis based on different covariates.

Covariates	p-value	PDOR (95% CI)
Test matrix	0.24	0.66 (0.38 - 1.17)
Histology type	0.01	0.41 (0.22 - 0.75)
Ethnicity	0.70	1.19 (0.46 - 3.08)
Patient size	0.26	0.42 (0.09 - 1.98)
Control size	0.01	0.33 (0.16 - 0.70)
Reference gene	0.07	1.45 (0.97 - 2.16)
QUADAS score	0.51	0.92 (0.72 - 1.18)



Supplemental Figure 1. Study heterogeneity evaluated by (A) L'Abbe plot and (B) Galbraith plot.