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ORIGINAL ARTICLE

Diagnostic value of lncRNAs as potential biomarkers for oral squamous cell carcinoma diagnosis: a meta-analysis*

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Abstract

Objective Several studies have revealed the critical role of long non-coding RNAs (lncRNAs) as biomarkers for diagnosing oral squamous cell carcinoma (OSCC). However, the data remain inconsistent. This meta-analysis was performed to summarize the potential of lncRNAs as OSCC biomarkers.

Methods We searched PubMed, Cochrane Library, Web of Science, and China National Knowledge Infrastructure databases for literature published until December 10, 2020. Study quality was assessed using Quality Assessment for Studies of Diagnostic Accuracy-2, and sensitivity, specificity, and other measures regarding lncRNAs for OSCC diagnosis were pooled using bivariate meta-analysis models. Data analyses were performed using STATA 14.0.

Results Overall, 8 studies with 981 cases and 585 controls were included in the pooled analysis. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and area under the receiver operating characteristic curve values were as follows: 0.76 [95% confidence interval (CI), 0.65–0.84], 0.90 (95% CI, 0.82–0.95), 7.5 (95% CI, 4.20–13.40), 0.27 (95% CI, 0.18–0.39), 28 (95% CI, 13.00–58.00), 0.90 (95% CI, 0.87–0.93), respectively. Deeks' funnel plot asymmetry test (P = 0.56) indicated no potential publication bias.

Conclusion Our meta-analytical evidence suggests that lncRNAs could be employed as a potential non-invasive diagnostic tool for OSCC.

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Key words: lncRNA; oral squamous cell carcinoma; diagnosis; meta-analysis

Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide [1–2]. OSCC accounts for 95% of all oral malignancies, with a global five-year survival rate that ranges between 50% and 60%, especially in patients diagnosed at advanced stages [3–4]. Most clinical examinations and imaging techniques currently used to detect OSCC fail to reliably distinguish early OSCC [5–6]. Therefore, early diagnosis and monitoring of OSCC progression remain crucial.

Long non-coding RNAs (lncRNAs) are novel non-coding RNAs with a length of more than 200 nucleotides, which do not encode proteins [7–8]. LncRNAs are characterized by low expression, moderate sequence conservation, and high tissue specificity and play an important role in several fundamental biological processes, including transcriptional regulation, cell differentiation, and chromatin modification [9]. Notably, growing evidence implicates a close relationship between tumor occurrence and development [10–13]. Additionally, dysregulated lncRNAs promote the occurrence and metastasis of malignancies [14–15]. Reportedly, altered lncRNA expression can be detected in biological fluids in different cancer types [16–17], including OSCC [18–19]; however, no consensus has been reached regarding the clinical value of lncRNAs in OSCC diagnosis. Therefore, we conducted a bivariate meta-analysis to summarize the

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diagnostic accuracy of lncRNAs for detecting OSCC.

Materials and methods

Literature search strategy and selection criteria

Two investigators searched PubMed, the Cochrane Library, Web of Science, and China National Knowledge Infrastructure (CNKI) databases for literature published until December 10, 2020, to identify relevant studies by employing the following terms: "lncRNAs" or "long ncRNAs" or "lincRNAs" or "long non-coding RNAs" or "long non-translated RNAs" or "long untranslated RNAs" or "long non-protein-coding RNAs" or "long intergenic non-protein coding RNAs" or "diagnostic value" or "diagnoses" or "receiver operating characteristics" or "ROC curve" or "sensitivity" or "specificity" and "OSCC" or "oral squamous cell carcinoma" or "oral cancer" or "oral tumor." Two investigators (WYX and LXQ) assessed study titles and abstracts and scanned full texts to eliminate irrelevant studies, using the following inclusion criteria: (1) Diagnostic data, including sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC), were provided or could be calculated from the studies; (2) Studies included patients with OSCC and healthy controls; (3) Original research articles published in English or Chinese.

Data extraction and quality assessment

Two investigators (WYX, LXQ) independently reviewed and extracted the following information from each eligible study: first author, year, country, sample size, gender, mean age, tumor-node-metastasis (TNM) stage, type of sample, detection method, cut-off value, true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The quality of included studies was assessed using Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).

Statistical analysis

Statistical analyses were conducted using Stata 14.0 (Stata, College Station, TX, USA). The number of subjects with a sensitivity, specificity, diagnostic odds ratio (DOR), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and their corresponding 95% confidence intervals (CIs) was calculated using a bivariate meta-analysis model. Then, summary receiver operator characteristic (SROC) curve analysis and AUC were calculated to evaluate the pooled diagnostic value of lncRNAs in OSCC detection. These data were confirmed using a hierarchical summary receiver operating characteristic (HSROC) model. The heterogeneity of eligible studies induced by the threshold effect was evaluated using Spearman correlation coefficients and ROC plane analyses. Heterogeneity of non-threshold effects was tested using Cochran's Q and inconsistency index (I^2) tests. For the Q test, $P < 0.05$ or $I^2 > 50\%$ indicated significant heterogeneity among identified studies. Moreover, Fagan's nomogram was used to assess relationships between prior-test probability, likelihood ratio, and post-test probability. Publication bias was assessed using Deeks' funnel plots.

Results

Study selection and characteristics of included studies

As shown in the flowchart (Fig. 1), a total of eight eligible studies [20–27] (981 OSCC and 585 healthy controls) evaluating 20 different lncRNAs were included in the present meta-analysis. The expression of lncRNAs was detected by reverse transcription-polymerase chain reaction (PCR) (n = 7) and TaqMan quantitative PCR (n = 1), while plasma (n = 4) or serum (n = 4) was the most common specimen assessed; in the TNM classification, four articles included patients in stages I–IV and one article included patients in stages I–III, whereas the other three failed to mention the TNM stage. According to single lncRNA expression levels, eight lncRNAs were upregulated, and data for others were unavailable. Details regarding study participants are presented in Table 1.

Quality assessment

In the present study, the methodological quality of diagnosis was assessed using QUADAS-2. All included studies presented relatively moderate-to-high quality, with QUADAS-2 scores ranging between 5 and 7 (Table

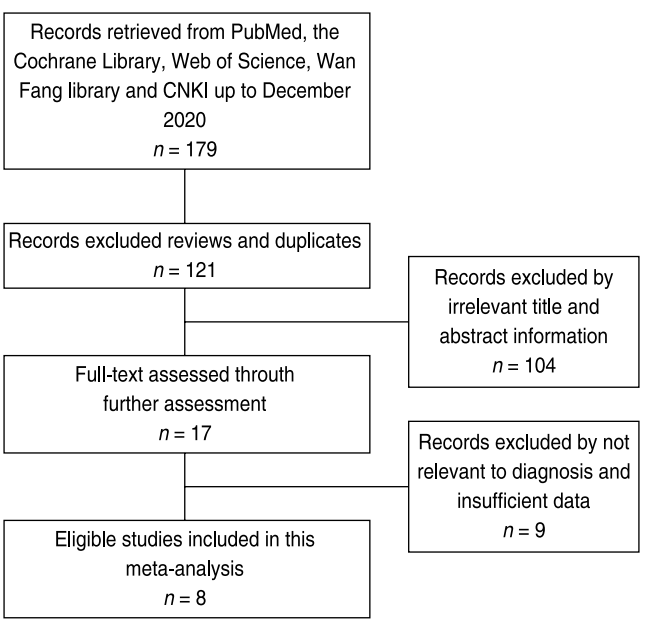

Fig. 1 Flow diagram of the study selection process

Table 1 Summary of main characteristics of included studies in this meta-analysis

First author	Year	Country	Case/ control	Sample type	Test method	Cutoff	TP	FP	FN	TN	LncRNAs Profiling	Up/Down	QUADAS-2
Zhao	2018	China	150/44	Plasma	qRT-PCR	NA	96	12	54	32	AC013268.5 RP11.65 L3.4 RP11.15A1.7	Up	5
Shao	2018	China	80/70	Serum	qRT-PCR	NA	62	11	18	59	AC007271.3	Up	6
Miao	2019	China	268/44	Plasma	qRT-PCR	1.44	157	1	111	43	LINC01629, AC083967.1, AC067863.1 AC022092.1, AC005532.1 LINC01629	NA	6
Zheng	2019	China	90/90	Serum	qRT-PCR	NA	55	7	35	83	SAMMSON	Up	7
Zhong	2019	China	116/116	Serum	qRT-PCR	NA	97	24	19	92	MIAT	Up	6
Le	2020	China	55/55	Plasma	qRT-PCR	NA	51	11	4	44	NCK-AS1	Up	7
Fan	2020	China	55/121	Serum	TaqMan-qPCR	3.29	36	5	19	116	LOC284454	Up	7
Li	2020	China	167/45	Plasma	qRT-PCR	NA	148	1	19	44	LINC01697, LINC02487 LOC105376575, AC005083.1 SLC8A1-AS1, U62317.1	NA	6

TP = true positive; FP = false positive; TN = true negative; FN = false negative; NA: Not available

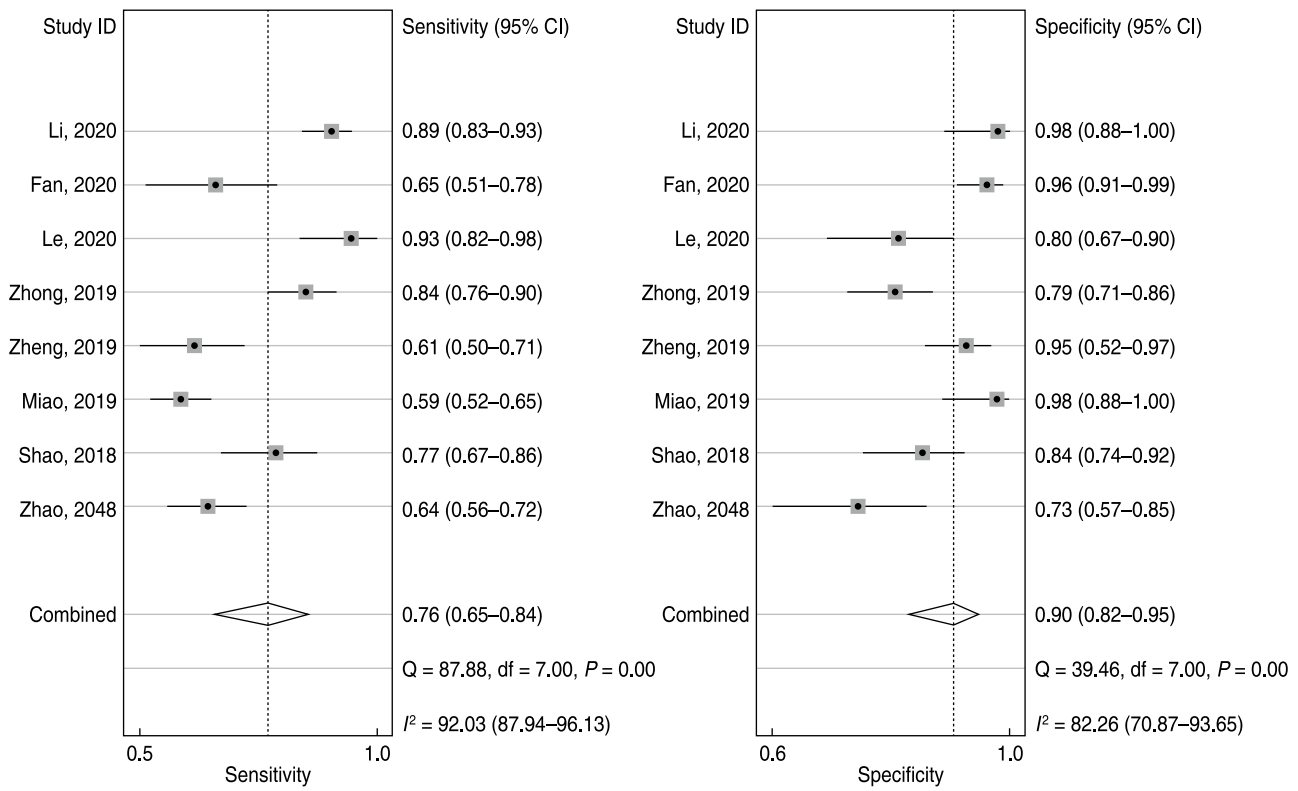

Fig. 2 Forest plots of lncRNA sensitivity and specificity for the diagnosis of OSCC

1); this indicated the reliable foundation of our meta-analysis.

Data analysis

For the eight included studies, forest plots of lncRNA sensitivity and specificity data for diagnosing OSCC are shown in Fig. 2. The pooled sensitivity and specificity of lncRNAs for diagnosing OSCC were 0.76 (95% CI, 0.65–0.84) and 0.90 (95% CI, 0.82–0.95), respectively. Additionally, the pooled PLR was 7.5 (95% CI, 4.20–13.40), NLR was 0.27 (95% CI, 0.18–0.39), and DOR was 28 (95% CI, 13.00–58.00) (Figs. 3 and 4). The overall

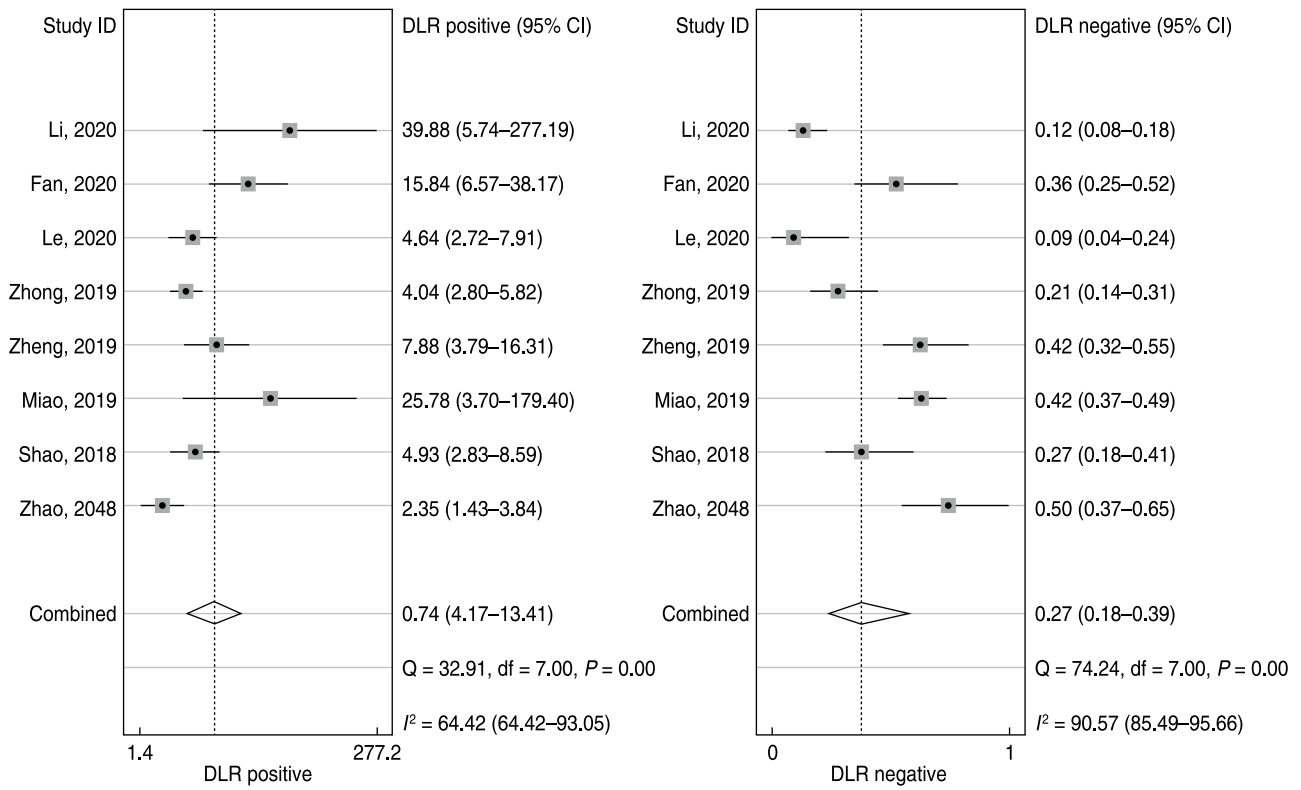

Fig. 3 Forest plots of the positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of lncRNAs for the diagnosis of OSCC

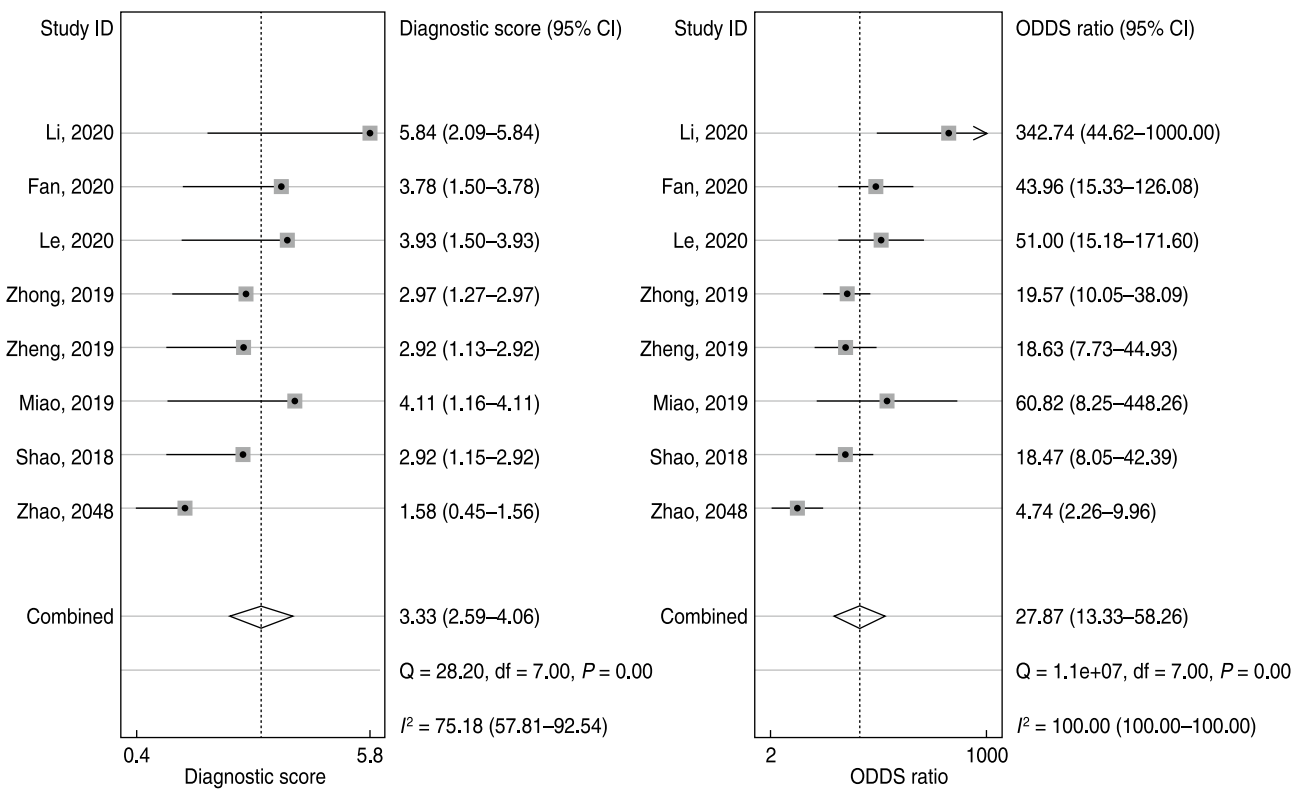

Fig. 4 Forest plots of diagnostic odds ratio (DOR) of lncRNAs for the diagnosis of OSCC

SROC curve for the eight included studies is shown in Fig. 5. The AUC of lncRNAs was 0.90 (95% CI, 0.87–0.93), which indicates that the lncRNAs were highly accurate in differentiating patients with OSCC from controls. Next, to assess the clinical utility of the index test, Fagan's nomogram was used to predict the increasing inerrability of a positive diagnosis by using the test value, which was employed for estimating post-test probabilities. As presented in Fig. 6, the pre-test probability was 20% and the positive post-test probability was increased to 65%, while the negative post-test probability was decreased to 6%. All pooled estimates indicated that lncRNAs in this analysis presented a relatively moderate-to-high accuracy in distinguishing patients with OSCC from control individuals.

Heterogeneity and publication bias

The I^2 value of the heterogeneity test was 95.95%, indicating apparent heterogeneity. Nevertheless, only eight articles were included, and subgroup and meta-regression analyses could not be undertaken in this meta-analysis. Moreover, we selected Deeks' funnel plot asymmetry test to assess publication bias (Fig. 7), which suggested no significant publication bias ($P = 0.56$).

Discussion

In OSCC, the initial stage is typically asymptomatic; therefore, early diagnosis remains a priority to improve

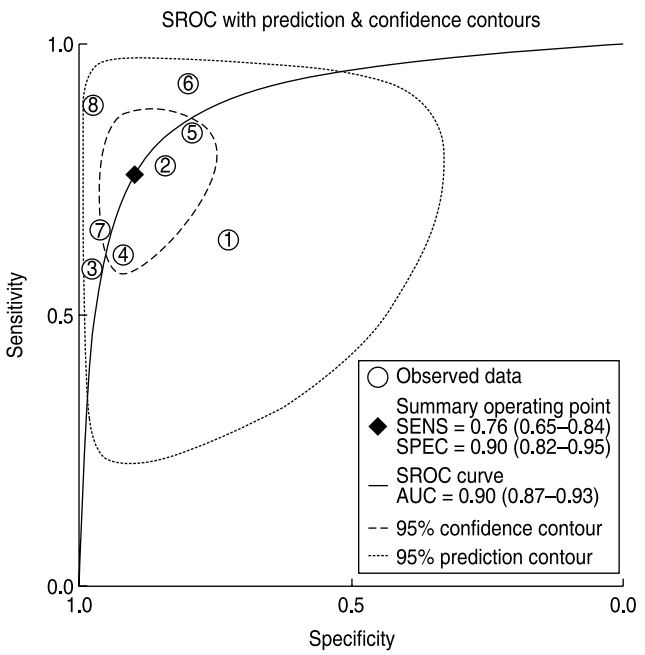

Fig. 5 Summary receiver operating characteristic (SROC) curve with pooled estimates of sensitivity, specificity, and area under the ROC curve (AUC)

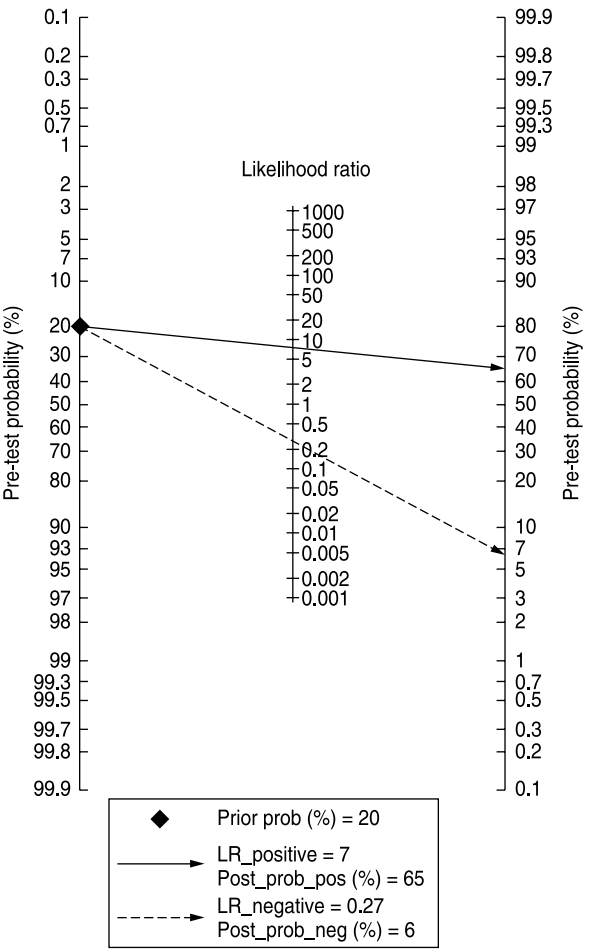

Fig. 6 Fagan's nomogram assessing the post-test probabilities

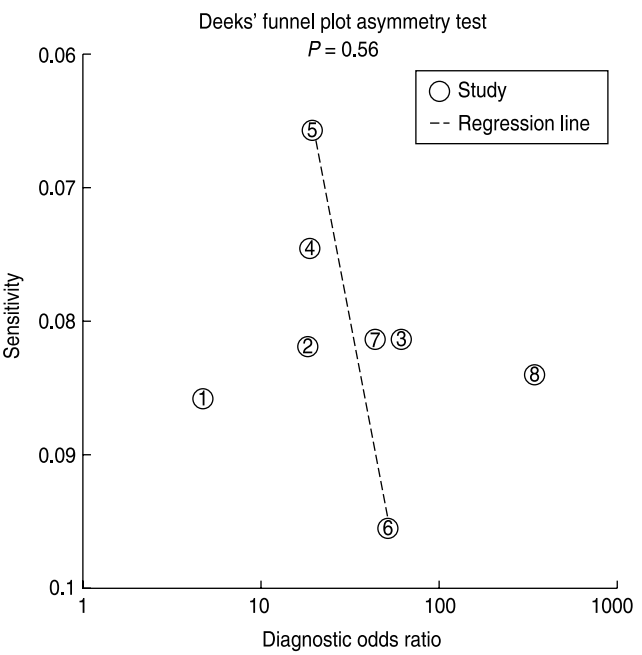

Fig. 7 Graph of Deeks' funnel plot asymmetry test

survival. Most patients with OSCC are diagnosed at late stages, resulting in a poor prognosis [19, 28]. Therefore, to optimize the survival rate and quality of life of patients with OSCC, it is essential to explore novel, practical, and non-invasive biomarkers for early detection of OSCC.

LncRNAs exert significant effects on the occurrence and development of several human diseases. Accumulating evidence suggests that altered lncRNA expression and its mutations can promote tumor occurrence and metastasis [14–15]. Furthermore, changes in lncRNA expression can be significantly associated with tumorigenesis, angiogenesis, and cell function [29]. Dysregulation of lncRNAs is reportedly involved in the occurrence, metastasis, and prognosis of cancer [8, 30]. Moreover, given their role in cancer biology and easy detection in biological fluids, lncRNAs could be potential and predictive cancer biomarkers. Circulating lncRNAs are promising new cancer biomarkers based on their role in cancer biology, and previous studies have demonstrated their feasibility as diagnostic and prognostic tools for different types of malignant tumors [30–32]. Tumor-related lncRNAs can be detected in various biological fluids, including blood [33–34].

The present review is the first evidence-based analysis to assess the diagnostic role of lncRNAs in OSCC detection. This meta-analysis included eight studies with 981 cancer cases and 585 controls, indicating that lncRNAs possess relatively high diagnostic accuracy with an overall pooled sensitivity of 0.76 (95% CI, 0.65–0.84), specificity of 0.90 (95% CI = 0.87–0.95), and AUC of 0.90 (95% CI, 0.87–0.93). Furthermore, the pooled DOR of 28 (95% CI, 13.00–58.00) suggested that lncRNAs are reliable for detecting OSCC.

Additionally, PLR and NLR were used to evaluate diagnostic accuracy. In this analysis, the pooled PLR was 7.5 (95% CI, 4.20–13.40), and the NLR was 0.27 (95% CI, 0.18–0.38); this could be attributed to the fact that patients with OSCC have a 7.5-fold higher possibility of reporting lncRNAs positive for OSCC when compared with controls, and 27% of all individuals present a negative result. As shown in Fagan's nomogram, at a pre-test probability of 20%, the positive post-test probability would increase up to 65% with a PLR of 7, while the negative post-test probability would decline to 6% with an NLR of 0.27. These results indicate the potential value of lncRNAs in OSCC detection and diagnosis.

Notably, significant heterogeneity existed among included studies in the present meta-analysis. The ROC plane showed no "shoulder arm" pattern, suggesting that the threshold effect was not a major source of heterogeneity (Fig. 8). Moreover, the Spearman correlation coefficient was -0.30 with $P = 0.09$, indicating the absence of a threshold effect. However, we could not conduct meta-regression and subgroup analyses

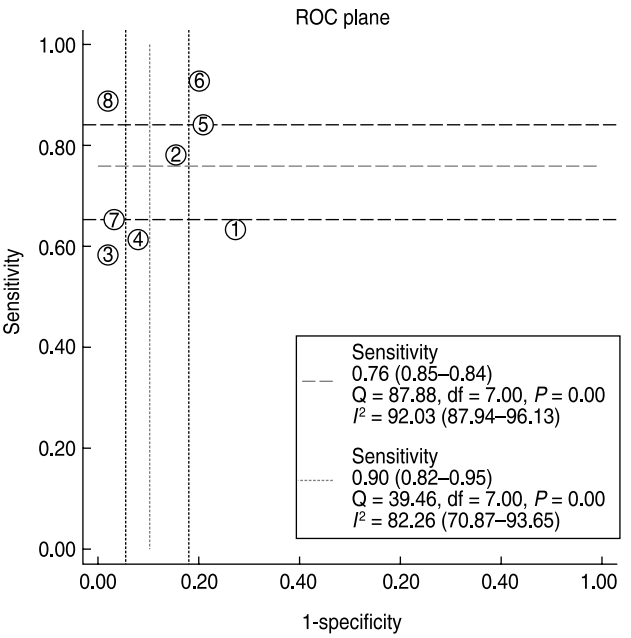

Fig. 8 Receiver operating characteristics (ROC) space to assess the threshold effect of lncRNAs

to detect the cause of heterogeneity, owing to a lack of eligible studies. Therefore, potential factors including age distribution, TNM stage, and gender proportion were not assessed as sources of heterogeneity.

However, the limitations of this study should be noted. First, our meta-analysis included a small sample size of lncRNAs in patients with OSCC. Second, the cut-off values for lncRNAs differed among included studies, which could be a potential source of heterogeneity. Third, this meta-analysis included a high proportion of Chinese populations, which may introduce unavoidable bias. Additionally, we did not determine differences in the diagnostic accuracy of lncRNAs presented in OSCCs, with respect to age distribution, TNM stage, and gender proportion. Finally, we identified the pooled lncRNA diagnostic value for OSCC but did not explore specific lncRNAs due to insufficient publications.

In conclusion, the results of this meta-analysis suggest that lncRNAs have a moderate-to-high diagnostic value in distinguishing patients with oral cancer from healthy controls, suggesting that lncRNAs can be utilized with high accuracy, particularly when performing multiple-lncRNA assays. However, large-scale prospective trials, as well as different ethnic groups, are necessary to confirm and extend our findings.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018, 68: 394–424.
2. Ferlay J, Colombet M, Soerjomataram I, *et al*. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer, 2019, 144: 1941–1953.
3. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol, 2009, 45: 309–316.
4. Dequanter D, Shahla M, Paulus P, *et al*. Long term results of sentinel lymph node biopsy in early oral squamous cell carcinoma. OncoTargets Ther, 2013, 6: 799–802.
5. Govers TM, Schreuder WH, Klop WM, *et al*. Quality of life after different procedures for regional control in oral cancer patients: cross-sectional survey. Clin Otolaryngol, 2016, 41: 228–233.
6. Govers TM, Takes RP, Karakullukcu MB, *et al*. Management of the N0 neck in early stage oral squamous cell cancer: a modeling study of the cost-effectiveness. Oral Oncol, 2013, 49: 771–777.
7. Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci, 2016, 73: 2491–2509.
8. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol, 2012, 9: 703–719.
9. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell, 2009, 136: 629–641.
10. Kim K, Jutooru I, Chadalapaka G, *et al*. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene, 2013, 32: 1616–1625.
11. Martens-Uzunova ES, Bottcher R, Croce CM, *et al*. Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol, 2014, 65: 1140–1151.
12. Salameh A, Lee AK, Cardo-Vila M, *et al*. PRUNE2 is a human prostate cancer suppressor regulated by the intronic long noncoding RNA PCA3. Proc Natl Acad Sci U S A, 2015, 112: 8403–8408.
13. Wang YQ, He HL, Li W, *et al*. MYH9 binds to lncRNA gene PTCSC2 and regulates FOXE1 in the 9q22 thyroid cancer risk locus. Proc Natl Acad Sci U S A, 2017, 114: 474–479.
14. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: A new paradigm. Cancer Res, 2017, 77: 3965–3981.
15. Huarte M. The emerging role of lncRNAs in cancer. Nat Med, 2015, 21: 1253–1261.
16. Rapado-Gonzalez O, Majem B, Muinelo-Romay L, *et al*. Human salivary microRNAs in Cancer. J Cancer, 2018, 9: 638–649.
17. Shen Y, Ding YJ, Ma Q, *et al*. Identification of novel circulating miRNA biomarkers for the diagnosis of esophageal squamous cell carcinoma and squamous dysplasia. Cancer Epidemiol Biomarkers Prev, 2019, 28: 1212–1220.
18. Chang YA, Weng SL, Yang SF, *et al*. A three-microRNA signature as a potential biomarker for the early detection of oral cancer. Int J Mol Sci, 2018, 19: 758.
19. Momen-Heravi F, Trachtenberg AJ, Kuo WP, *et al*. Genomewide study of salivary microRNAs for detection of oral cancer. J Dent Res, 2014, 93: 86S–93S.
20. Fan C, Wang JP, Tang YY, *et al*. Upregulation of long non-coding RNA LOC284454 may serve as a new serum diagnostic biomarker for head and neck cancers. BMC Cancer, 2020, 20: 917.
21. Le F, Ou YQ, Luo P, *et al*. LncRNA NCK1-AS1 in plasma distinguishes oral ulcer from early-stage oral squamous cell carcinoma. J Biol Res (Thessalon), 2020, 27: 16.
22. Li Y, Cao XF, Li H. Identification and validation of novel long non-coding RNA biomarkers for early diagnosis of oral squamous cell carcinoma. Front Bioeng Biotechnol, 2020, 8: 256.
23. Miao TT, Si QZ, Wei Y, *et al*. Identification and validation of seven prognostic long non-coding RNAs in oral squamous cell carcinoma. Oncol Lett, 2020, 20: 939–946.
24. Shao TR, Huang JX, Zheng ZN, *et al*. SCCA, TSGF, and the long non-coding RNA AC007271.3 are effective biomarkers for diagnosing oral squamous cell carcinoma. Cell Physiol Biochem, 2018, 47: 26–38.
25. Zhao CG, Zou HR, Wang JH, *et al*. A three long noncoding RNA-based signature for oral squamous cell carcinoma prognosis prediction. DNA Cell Biol, 2018, 37: 888–895.
26. Zheng XJ, Tian X, Zhang Q, *et al*. Long non-coding RNA SAMMSON as a novel potential diagnostic and prognostic biomarker for oral squamous cell carcinoma. J Dent Sci, 2020, 15: 329–335.
27. Zhong WS, Xu Z, Wen SL, *et al*. Long non-coding RNA myocardial infarction associated transcript promotes epithelial-mesenchymal transition and is an independent risk factor for poor prognosis of tongue squamous cell carcinoma. J Oral Pathol Med, 2019, 48: 720–727.
28. Eckert AW, Kappler M, Schubert J, *et al*. Correlation of expression of hypoxia-related proteins with prognosis in oral squamous cell carcinoma patients. Oral Maxillofac Surg, 2012, 16: 189–196.
29. Huang YK, Yu JC. Circulating microRNAs and long non-coding RNAs in gastric cancer diagnosis: An update and review. World J Gastroenterol, 2015, 21: 9863–9886.
30. Qi P, Zhou XY, Du X. Circulating long non-coding RNAs in cancer: current status and future perspectives. Mol Cancer, 2016, 17, 15: 39.
31. Ke D, Li HW, Zhang Y, *et al*. The combination of circulating long noncoding RNAs AK001058, INHBA-AS1, MIR4435-2HG, and CEBPA-AS1 fragments in plasma serve as diagnostic markers for gastric cancer. Oncotarget, 2017, 8: 21516–21525.
32. Xian HP, Zhuo ZL, Sun YJ, *et al*. Circulating long non-coding RNAs HULC and ZNFX1-AS1 are potential biomarkers in patients with gastric cancer. Oncol Lett, 2018, 16: 4689–4698.
33. Zhang KC, Shi HZ, Xi HQ, *et al*. Genome-wide lncRNA microarray profiling identifies novel circulating lncRNAs for detection of gastric cancer. Theranostics, 2017, 7: 213–227.
34. Yoruker EE, Keskin M, Kulle CB, *et al*. Diagnostic and prognostic value of circulating lncRNA H19 in gastric cancer. Biomed Rep, 2018, 9: 181–186.

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