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Review

miRNAs in liquid biopsy for oral squamous cell carcinoma diagnosis: Systematic review and meta-analysis

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is often diagnosed at advanced stages and is associated with poor survival rates. Increasing evidence suggests that microRNAs (miRNAs) present in liquid biopsies could be potential biomarkers for non-invasive OSCC diagnosis. Here, we performed a comprehensive meta-analysis to evaluate the overall diagnostic accuracy of blood and salivary miRNAs in detecting OSCC. A literature search using PubMed EMBASE, Web of Science, LILACS, Scopus, and the Cochrane Library was undertaken up to February 2019. Study quality was assessed with the Quality Assessment for Studies of Diagnostic Accuracy-2, and sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and their corresponding 95% confidence intervals (CIs) were calculated using a bivariate random-effect meta-analysis model. Meta-regression and subgroup analyses were performed to assess the heterogeneity. Twenty-five study units from 16 articles with 2562 subjects were included in this meta-analysis. The pooled sensitivity and specificity of blood and salivary miRNAs in the diagnosis of OSCC were 0.78 (95% CI: 0.76–0.80) and 0.82 (95% CI: 0.79–0.84), respectively, and the pooled positive and negative likelihood ratios were 4.31 (95% CI: 3.38–5.51) and 0.25 (95% CI: 0.20–0.32), respectively. The overall area under the curve was 0.91 (95% CI: 0.88–0.93), with a diagnostic odds ratio of 21.46 (95% CI: 13.37–34.45). These findings provide evidence regarding the potential clinical application of blood and salivary miRNAs as a novel, non-invasive, and accurate diagnostic tool for OSCC.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of head and neck cancer, accounting for > 90% of all oral malignancies [1]. According to GLOBOCAN, OSCC is one of the leading causes of morbidity and mortality worldwide, with an estimated 354,864 cases and 177,384 OSCC-related deaths occurring in 2018 [2]. Oral carcinogenesis has been associated with several genetic and/or lifestyle-associated risk factors, including smoking, alcohol consumption, poor oral hygiene, chronic mechanical trauma, and viral infections, such as those involving human papillomavirus or Epstein–Barr virus [1,3]. Despite progress in OSCC diagnosis and treatment, most patients are

Abbreviations: AUC, area under the SROC; CI, confidence interval; dOR, diagnostic odds ratio; FN, false negative; FP, false positive; OSCC, oral squamous cell carcinoma; miRNA, microRNA; NLR, negative likelihood ratio; PLR, positive likelihood ratio; PRISMA, preferred reporting items for systematic reviews and meta-analysis; SROC, summary receiver operator characteristic; TN, true negative; TP, true positive

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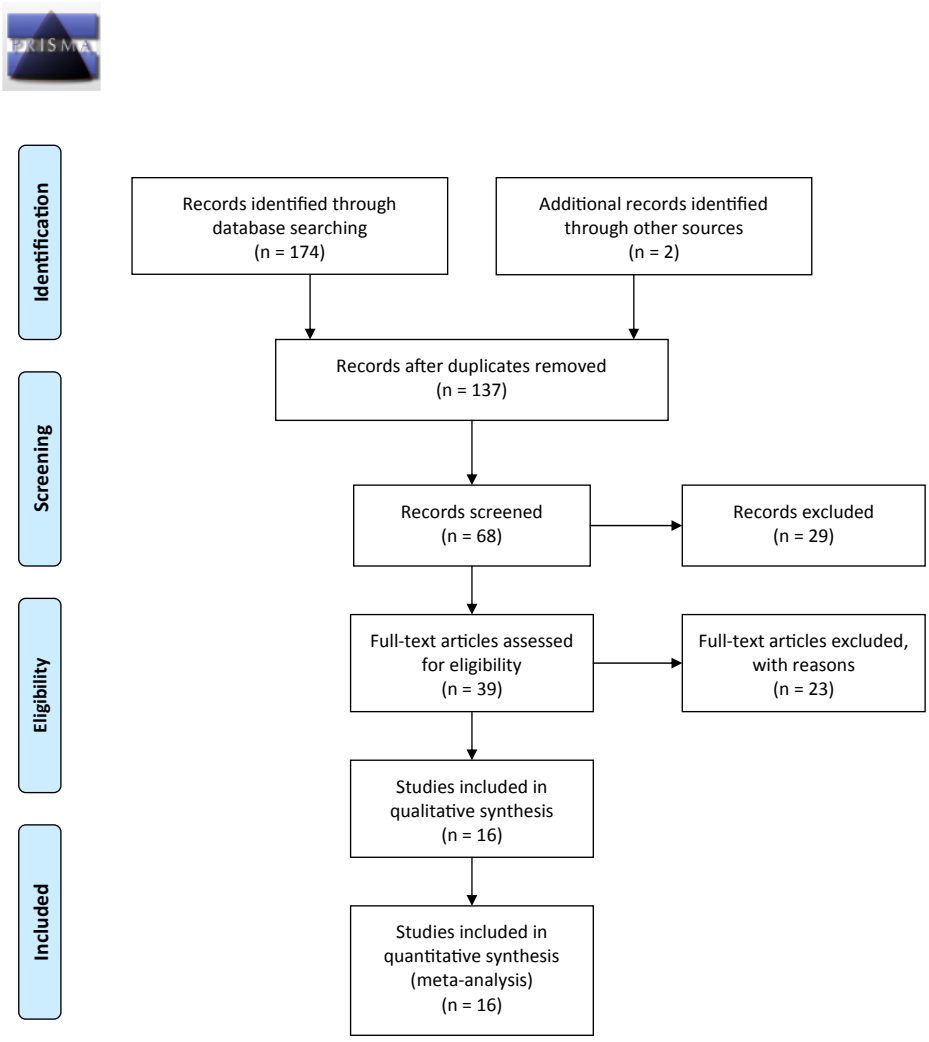

Fig. 1. PRISMA flow diagram. *Abbreviations:* PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analysis.

diagnosed in advanced stages, which are associated with poor prognosis and high rates of local recurrence and distant metastasis [4]. Overall, the 5-year survival rate is ~80% when diagnosed at early stages and decreases to 20% at later stages [5]. Therefore, there is an urgent need to develop novel approaches that improve OSCC diagnosis.

MicroRNAs (miRNAs) are a large family of single-stranded non-coding RNAs comprising 19–25 nucleotides and that regulate gene expression at the post-transcriptional level. They usually bind to complementary sequences in the 3′ untranslated region of mRNAs, resulting in their degradation and inhibited translation [6]. miRNAs can target hundreds or thousands of different mRNAs, resulting in potential regulation of individual mRNAs by multiple different miRNAs [7]. Importantly, growing evidence implicates miRNAs in cancer hallmarks by acting as both tumor suppressors and promoters [7,8]. Additionally, dysregulated miRNAs have been detected in different body fluids [9], including plasma, serum, saliva, or urine, resulting in their classification as circulating miRNAs. Circulating miRNAs are highly stable under harsh conditions, such as high temperature, extreme pH, and in the presence of RNase activity, suggesting their potential as non-invasive biomarkers for cancer diagnosis [10]. Furthermore, several studies report altered expression of blood and salivary miRNAs in different tumor types [11,12], including OSCC [13,14]; however, there is not a clear consensus regarding the clinical value of miRNAs in liquid biopsy for OSCC diagnosis.

Therefore, the purpose of this systematic review and meta-analysis was to summarize the clinical results of published studies regarding the use of miRNAs as liquid biopsy biomarkers for OSCC diagnosis and assess their overall diagnostic accuracy in discriminating OSCC.

Methods

Protocol and registration

This study was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [15], and the protocol was registered with the International Prospective Register of Systematic Reviews (reference No. CRD42019122960).

Search strategy and study selection

The search strategy was designed to identify studies describing the diagnostic value of miRNAs present in liquid biopsies for identifying patients with OSCC. In this sense, a detailed literature search was performed in PubMed, EMBASE, Web of Science, LILACS, Scopus, and the Cochrane Library for eligible articles published up to 31 February 2019, without language restrictions. The following combinations of keywords and medical subject headings were used: (microRNA OR microRNAs OR miRNAs) AND (OSCC OR oral squamous cell carcinoma

Table 1
Summary of descriptive characteristics of included studies.

Author	Country	Healthy controls			OSCC patients				Type of sample	Reference gene	miRNA profiling	TP	FP	TN	FN	Cut-off	Expression Level
		N	Sex (male/ female)	Mean age (SD, range)	*N*	Sex (male/ female)	Mean age (SD, range)	TNM stage (*N*)									
Lin et al. (2010)	Taiwan	10	…	…	33	…	…	I–III (10) IV (33)	Plasma	RNU6B and let-7a	miR-24	23	1	10	9	1.09	▲
Lu et al. (2012)	Taiwan	36	31/5	50.5 (± 12.6)	54	51/3	52.0 (± 12.8)	I/II (20) III/ IV (34)	Plasma	RNU6B	miR-10b	51	7	3	29	…	▲
Liu et al. (2012)	Taiwan	24	23/1	51.1 (± 1.7)	45	43/2	53.7 (± 1.4)	I/II (21) III/ IV (24)	Saliva	miR-16	miR-31	36	8	9	16	…	▲
MacLellan et al. (2012)	Canada	26	13/13	62 (50–75)	30	21/9	CIS: 64 (50–84) OSCC: 62 (51–93)	…	Serum	global mean	miR-338-3p	24	5	6	21	…	▼
											miR-29a	23	6	7	20	…	▼
											miR-223	29	10	1	16	…	▼
											miR-16	18	2	12	24	…	▲
											let-7b	24	5	6	21	…	▲
Hung et al. (2013)	Taiwan	12	…	…	51	…	…	I-III (19) IV (40)	Plasma	miR-16	miR-146a	40	1	10	11		▲
Ren et al. (2014)	China	32	…	…	58	39/19	61 (25–92)	…	Blood	RNU6B	miR-21	36	3	22	29	9.646	▲
Momen-Heravi et al. (2014)	USA	9	5/3	60.16 (± 9.57, 32–77)	9	8/1	60.66 (± 11.83, 41–78)	…	Saliva	miR-191	miR-27b	8	0	1	9	14.73	▲
											miR-136	8	0	1	9	10.44	▼
Gu et al. (2015)	China	46	38/8	63.80 (± 10.42)	85	56/29	…	…	Plasma	cel-miR-39	miR-125b	76	3	9	43	…	▲
Gu et al. (2015)	China	44	35/9	63.68 (± 10.41)	83	54/29	…	…	Plasma	cel-miR-39	miR-4677	66	4	17	40	0.625	▼
Xu et al. (2015)	China	103	75/28	52.4 (± 9.0)	101	77/ 24	53.2 (± 10.3)	I/II (51) III/ IV (50)	Serum	RNU6B	miR-483-5p	86	26	15	77	…	▲
Lu et al. (2015)	Taiwan	53	37/16	47.2 (± 11.8, 22–74)	90	82/8	54.0 (± 11.7, 25–77)	I/II (30) III/ IV (60)	Plasma	…	miR-196a	60	2	30	50	…	▲
											miR-196b	88	10	2	43	…	▲
											miR-196a and miR-196b	79	4	11	49	…	▲
Duz et al. (2016)	Turkey	25	21/4	46.88 (± 3.63)	25	19/6	54.08 (± 2.38)	…	Saliva	RNU6b	miR-139-5p	18	4	6	21	…	▼
Tachibana et al. (2016)	Japan	31	…	…	31	20/11	75.74 (± 8.96, 58–91)	I/II (12) III/ IV (19)	Plasma	let-7a	miR-223	21	12	10	19	40.67	▲
Sun et al. (2018)	China	80	36/44	53.9 (± 4.2)	80	35/45	54.8 (± 6.4)	T1-2 (43) T3-4 (37)	Plasma	cel-miR-39	miR-200b-3p	72	9	8	71	…	▲
Chen et al. (2018)	China	55	…	…	121	73/48	…	I/II (75) III/ IV (46)	Serum	cel-miR-39	miR-99a	97	9	24	46	…	▼
Chang et al. (2018)	Taiwan	70	68/2	…	114	111/3	…	I/II (61) III/ IV (53)	Plasma	miR-130b-3p and miR-221-3p	miR-150-5p	69	16	45	54	5.010	▲
											miR-423-5p	67	19	47	51	2.185	▲
											miR-150-5p and miR-423-5p	81	19	33	51	0.510	▲

Abbreviations: (▲) = upregulated; (▼) = downregulated; TP = true positive; FP = false positive; TN = true negative; FN = false negative; CIS = oral carcinoma in situ.

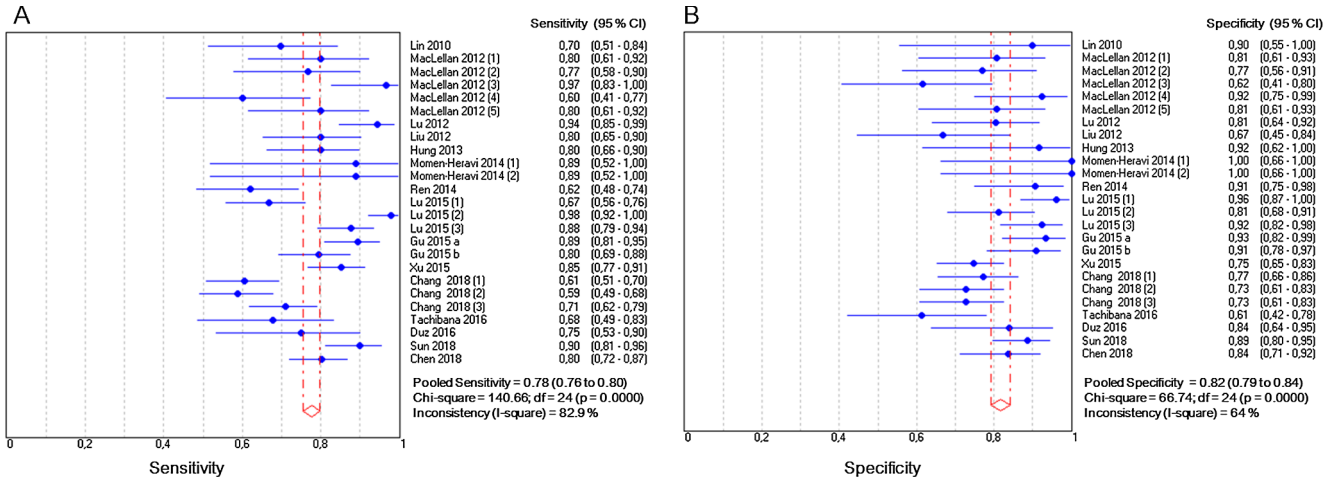

Fig. 2. Forest plot of (A) sensitivities and (B) specificities from test-accuracy studies of blood and salivary miRNAs for predicting OSCC diagnosis. *Abbreviations:* miRNA = microRNAs; OSCC = oral squamous cell carcinoma.

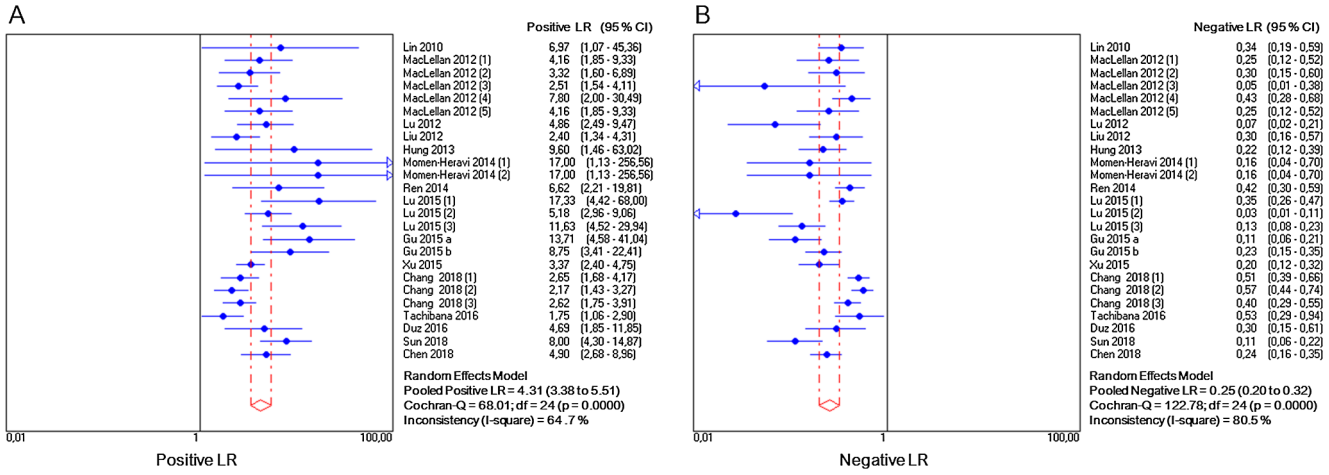

Fig. 3. Forest plot of likelihood ratios for (A) positive and (B) negative test results from blood and salivary miRNA studies for predicting OSCC diagnosis. *Abbreviations:* miRNA = microRNAs; OSCC = oral squamous cell carcinoma.

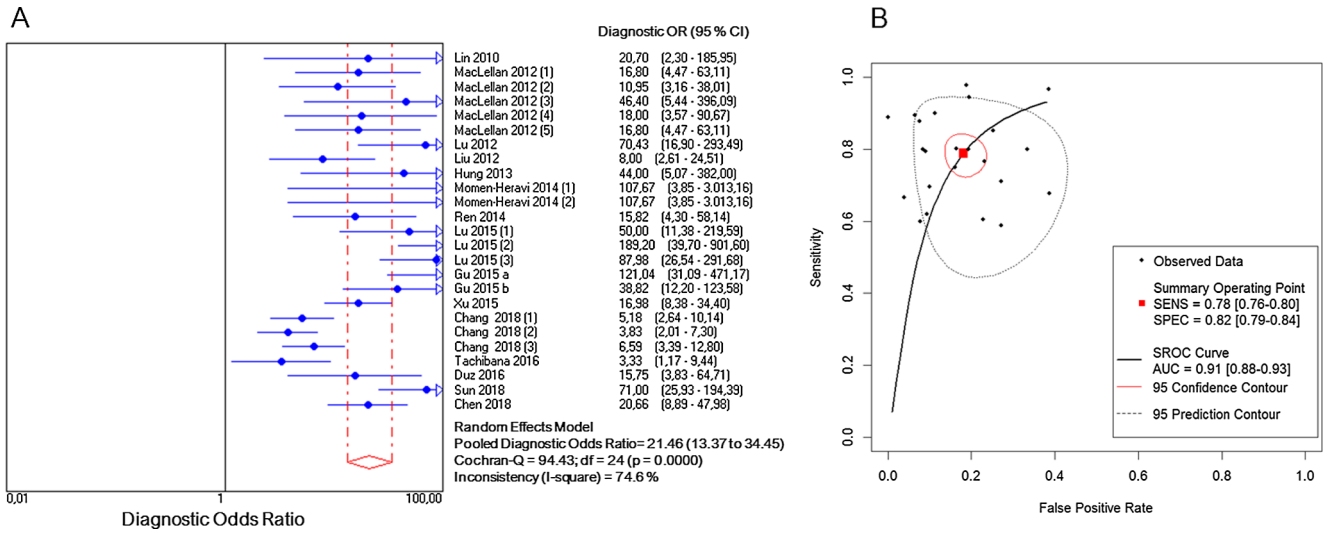

Fig. 4. Overall performance of blood and salivary miRNAs in diagnosing oral cancer. (A) Forest plot of the pooled dOR of miRNAs for diagnosing oral cancer. (B) SROC curve with pooled estimates of sensitivity, specificity, and the AUC for all included studies of miRNAs for detecting OSCC. *Abbreviations:* AUC = area under the SROC curve; miRNA = microRNAs; dOR = diagnostic odds ratio; OSCC = oral squamous cell carcinoma; SROC = summary receiver operator characteristic.

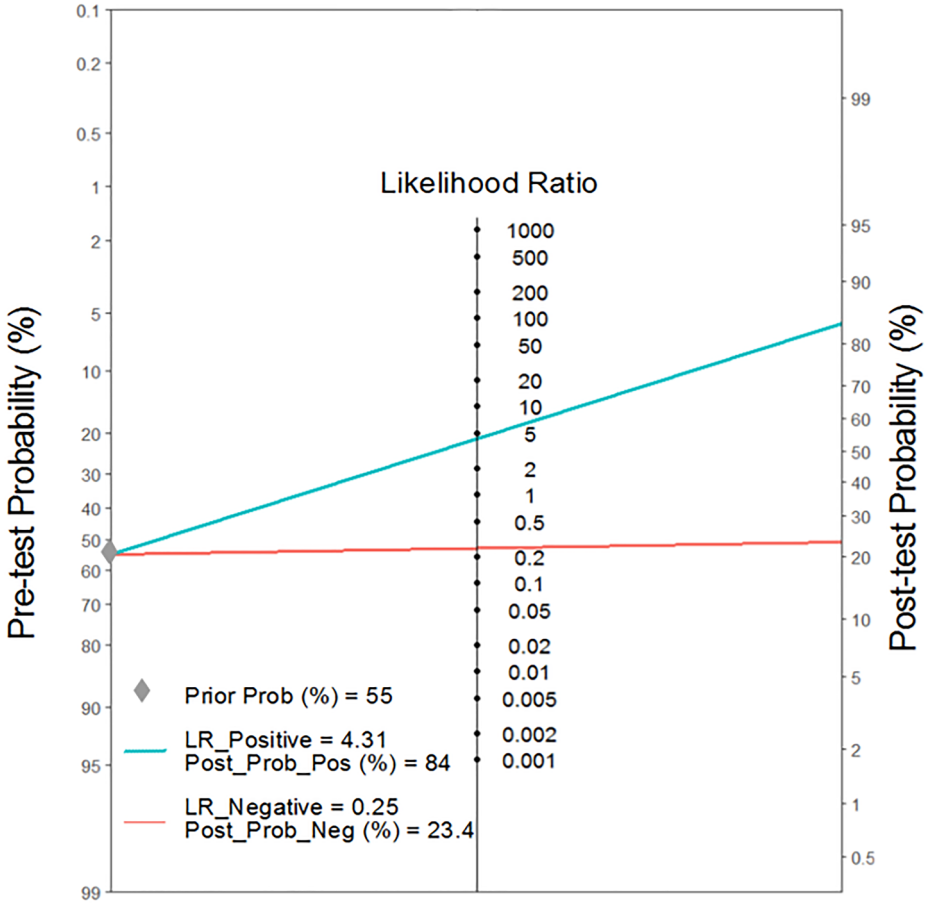

Fig. 5. Fagan's monogram evaluating the clinical utility of blood and salivary miRNAs for differentiating OSCC patients. *Abbreviations:* miRNA = microRNAs; OSCC = oral squamous cell carcinoma.

OR oral cancer OR oral tumor) AND (plasma OR blood OR whole blood OR serum OR saliva) AND (diagnosis OR sensitivity OR specificity OR ROC curve) AND (marker OR biomarker). All studies were screened based on the title and abstract, and eligible manuscripts were retrieved for full-text review. Additionally, we performed manual searches of reference lists in each original and review article in order to avoid missing potential studies. The literature search was performed independently by two investigators (ORG and MMSC), and disagreements during the selection process were resolved by consensus. The studies selected through the search strategy and other references were managed with RefWorks software (https://www.refworks.com/content/path_learn/faqs.asp), and duplicate items were removed using the associated tools.

Selection criteria

The inclusion criteria were as follows: (1) diagnostic studies with significantly dysregulated miRNAs from body fluids between OSCC and healthy controls; (2) study subjects included cancer patients and healthy controls; and (3) studies included enough data to generate a 2×2 contingency table containing true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) values. The exclusion criteria were as follows: (1) reviews, letters, personal opinions, book chapters, case reports, conference abstracts, and meetings; (2) duplicate publications; and (3) studies only reporting associations between saliva and oral cancer based on *in vitro* or *in vivo* animal experiments.

Data extraction

Two investigators (ORG and MMSC) independently assessed each eligible manuscript, extracted data using a pre-established form, and collated the data into a Microsoft Excel spreadsheet (Microsoft Corp. Redmond, WA, USA). Any disagreement among reviewers was resolved by consensus. The following information was extracted from each study: author, publication year, country, sample size, gender, mean age, TNM stage, type of fluid sample (blood, plasma, serum, or saliva), detection method, specific significantly dysregulated miRNAs, miRNA-expression pattern(s), and outcomes of statistical analysis, including the reference gene used for data normalization and diagnostic accuracy outcomes. If the required data were incomplete, attempts were made to contact the authors to obtain the missing information.

We defined a "study unit" as analysis of a relationship between miRNAs and OSCC. Because different miRNAs could be reported in the same manuscript, a single publication could then potentially report more than one study unit.

Quality assessment of individual studies

Quality assessment of the selected studies was separately conducted by two independent investigators (ORG and MMSC) using the Quality Assessment of Diagnostic Accuracy Studies-2 checklist (QUADAS-2) [16], as recommended by the Healthcare Research and Quality Agency. Discrepancies between the two investigators were resolved by a third reviewer (LMR). The QUADAS-2 checklist is a revised tool developed to assess the quality of studies of diagnostic tests and that analyzes four

Table 2
Subgroup analysis of blood and salivary miRNAs for OSCC detection based on different covariates.

Subgroups	No of study units	Sensitivity (95% CI)	I^2 (%)	Specificity (95% CI)	I^2 (%)	PLR (95% CI)	I^2 (%)	NLR (95% CI)	I^2 (%)	dOR (95% CI)	I^2 (95%)	AUC (95% CI)
Ethnicity												
Asian	17	0.78 (0.76–0.80)	87.22	0.82 (0.79–0.84)	68.50	4.47 (3.28–6.09)	73.63	0.25 (0.19–0.34)	85.93	21.83 (11.97–38.82)	23.23	0.91 (0.88–0.93)
Others	8	0.79 (0.73–0.85)	53.83	0.82 (0.75–0.87)	56.11	3.64 (2.66–4.98)	4.63	0.29 (0.21–0.39)	18.51	18.37 (10.38–23.50)	0	0.90 (0.86–0.94)
Samples size												
≥ 100	11	0.78 (0.75–0.80)	81.60	0.83 (0.80–0.85)	52.96	4.92 (3.39–7.16)	60.22	0.23 (0.16–0.34)	80.70	25.88 (12.09–55.41)	75.27	0.92 (0.87–0.96)
< 100	14	0.76 (0.74–0.81)	65.07	0.80 (0.75–0.84)	55.06	3.66 (2.69–4.98)	39.73	0.29 (0.23–0.38)	47.64	16.31 (9.89–29.91)	31.29	0.90 (0.86–0.93)
MiRNA expression												
Upregulated	18	0.77 (0.74–0.79)	86.88	0.82 (0.79–0.84)	68.37	4.41 (3.24–6)	70.83	0.26 (0.19–0.35)	84.76	21.48 (11.72–39.38)	80.60	0.91 (0.87–0.94)
Downregulated	7	0.81 (0.76–0.85)	27.27	0.82 (0.76–0.87)	53.78	4.15 (2.86–6.02)	35.48	0.24 (0.19–0.30)	0	21.32 (13.07–34.77)	0	0.91 (0.87–0.95)
MiRNA profiling												
Single-miRNA assay	23	0.78 (0.75–0.80)	83.32	0.82 (0.73–0.84)	30.34	4.28 (3.32–5.51)	62.14	0.26 (0.20–0.33)	80.12	21.46 (13.10–35.15)	72.56	0.91 (0.88–0.94)
Multiple-miRNA assay	2	0.78 (0.72–0.84)	88.52	0.81 (0.73–0.88)	87.97	5.23 (1.05–26.04)	89.81	0.24 (0.78–0.72)	91.66	22.91 (1.79–292.71)	92.78	0.71 (0.71–0.71)

Abbreviations: miRNAs = microRNAs; AUC = area under the SROC; dOR = diagnostic odds ratio; PLR = positive likelihood ratio; NLR = negative likelihood ratio; CI = confidence interval.

key domains: (1) patient selection, (2) index tests, (3) reference tests, and (4) flow and times. Each domain was evaluated in terms of its risk of bias, and the first three domains were also evaluated in terms of applicability concerns, with risk of bias and concerns of applicability for each domain considered "low", "high", or "unclear". If the response to the risk of bias and applicability questions were "low" risk or "low" concern, respectively, one point for each item was applied. The articles were grouped based on their score into high (6–7 points), moderate (4–5 points), and low (0–3 points) quality categories.

Statistical analysis

Statistical analysis was conducted using MetaDiSc software (v.1.4) [17], free R software (v.3.4.4; https://www.r-project.org), and STATA (v.14.0; https://www.stata.com). For the diagnostic meta-analysis, the number of subjects with a TP, FP, FN, and TN for each study unit was extracted to calculate the pooled sensitivity [TP/(TP + FN)], specificity [TN/(TN + FP)], positive likelihood ratio (PLR) [(sensitivity/(1 − sensitivity)], negative likelihood ratio (NLR), [(1 − specificity)/specificity)], diagnostic odds ratio (dOR) [PLR/NLR], and their corresponding 95% confidence intervals (CIs) using a bivariate random-effect meta-analysis model. The summary receiver operator characteristic (SROC) curve was plotted, and the area under the SROC curve (AUC) was calculated to evaluate the pooled diagnostic performance of blood and salivary miRNAs in OSCC detection. Heterogeneity in the meta-analysis was explored to understand the factors that influence accuracy indicators and evaluate the statistical model applied to the meta-analytic database [17]. The heterogeneity of included studies caused by a threshold effect was assessed by Spearman's correlation analysis and ROC plane plots. The heterogeneity of non-threshold effects was assessed using the Cochran's Q statistic test-based Chi-squared test and I^2 statistics. Heterogeneity was considered significant when $I^2 > 50\%$ and/or presence of a $p < 0.05$ for the Cochran's Q test. If significant heterogeneity was detected, the DerSimonian and Laird random-effects model was applied; otherwise, the Mantel–Haenszel fixed-effects model was used. Meta-regression and subgroup analyses were applied to analyze the potential sources of heterogeneity unrelated to the threshold effect among included studies. Additionally, Fagan's nomogram was used to assess the predictive value of the post-miRNAs test for OSCC diagnosis using likelihood ratios to calculate a post-test probability based on Bayes theorem. Publication bias was assessed using Deeks' funnel-plot asymmetry test [18], with statistical significance defined at a $p < 0.05$.

Results

Study selection

The search strategy identified 174 records across the six electronic databases and two additional reports from the reference lists. After removing the duplicates, 137 records were screened, of which 29 records were excluded after title and abstract review. Therefore, full-text articles were retrieved for the remaining 68 articles. After a full-text review, 23 articles were excluded for the following reasons: not related to the diagnosis (2); reviews, letters, personal opinions, book chapters, case reports, conference abstracts, and meetings (7); not reporting sensitivity and specificity (13); and insufficient information for meta-analysis (1). Finally, 16 articles met the inclusion criteria and were included in the final analysis [13,14,19–32]. A flowchart detailing the selection process is shown in Fig. 1.

Characteristics of the included studies

A total of 25 study units from 16 articles (14 in English and 2 in Chinese) evaluating 22 different miRNAs were included in this meta-analysis. The sample size of the studies ranged from 18 to 204 and

comprised a total of 2562 subjects according to unit studies (1547 patients with OSCC and 1015 healthy individuals). Sixteen published studies (from 2010 to 2018) were performed in six countries, including six in Taiwan, six in China, one in the United States, one in Canada, one in Japan, and one in Turkey. Expression levels of single miRNAs ($n = 25$) and/or a panel of miRNAs ($n = 2$) were detected by real-time quantitative polymerase chain reaction. In terms of sample sources, miRNAs were analyzed in plasma ($n = 13$), serum ($n = 7$), saliva ($n = 4$), and whole blood ($n = 1$). According to single miRNA-expression levels, 16 miRNAs were upregulated and seven were downregulated. The main characteristics of the included studies are shown in Table 1.

Quality assessment of the included studies

Assessment of the methodological quality of the included studies was performed using the QUADAS-2 checklist (Fig. S1). A total of 10 studies showed a low risk of bias in three of four domains, four studies showed a low risk of bias in two of four domains, and two studies showed a low risk of bias in all domains. The patient selection and index-test domains represented most of the methodological concerns, showing unclear risk in nine studies and high risk in eight studies, respectively. Several studies did not report a detailed description of the inclusion/exclusion criteria or whether they used a blind method. Although the threshold was used, it was not pre-specified, which could indicate an overestimation of diagnostic value. All studies were of moderate-to-high quality, with QUADAS-2 scores between 5 and 7.

Synthesis of results

The pooled estimates for sensitivity and specificity of blood and salivary miRNAs to discriminate OSCC from healthy controls were 0.78 (95% CI: 0.76–0.80) and 0.82 (95% CI: 0.79–0.84), respectively (Fig. 2), corresponding to a PLR of 4.31 (95% CI: 3.38–5.51) and an NLR of 0.25 (95% CI: 0.20–0.32) (Fig. 3). The overall dOR was 21.46 (95% CI: 13.37–34.45), and the AUC was 0.91 (95% CI: 0.88–0.93) (Fig. 4). As shown in Fig. 5, when the pre-test probability of OSCC was 55%, a positive measurement improved the post-test probability of having cancer to 84%, whereas the post-test probability reduced to 23.4% when a negative measurement occurred. These results indicate the good discriminative ability of miRNAs as liquid biopsy biomarkers for OSCC detection.

Heterogeneity and subgroup analysis

The results of diagnostic meta-analysis indicated the existence of significant heterogeneity in the overall sensitivity ($I^2 = 82.9\%$; $p < 0.001$), specificity ($I^2 = 64\%$; $p < 0.001$), PLR ($I^2 = 64.7\%$; $p < 0.001$), NLR ($I^2 = 80.5\%$; $p < 0.001$), and dOR ($I^2 = 74.6\%$; $p < 0.001$). Further analysis of heterogeneity showed no “shoulder arm” pattern in the ROC plane, suggesting the absence of a threshold effect (Fig. S2). Moreover, Spearman’s correlation coefficient was -0.059 ($p = 0.779$), also indicating no obvious heterogeneity detected as a result of the threshold effect. To determine possible sources of heterogeneity, we performed meta-regression analysis with the following covariates as predictor variables: ethnicity (Asian *vs.* others), sample size ($n \geq 100$ *vs.* $n < 100$), miRNA-expression pattern (upregulated *vs.* downregulated), and miRNA profiling (single- *vs.* multiple-miRNA assay). Significant heterogeneity was not observed between the groups (Table S1). Although the meta-regression results were negative, we conducted subgroup analysis to further explore the potential diagnostic value of miRNAs in liquid biopsy (Table 2).

Evaluation of publication bias

Performance of Deeks’ funnel-plot asymmetry test to explore the potential publication bias from each miRNA liquid biopsy study revealed a slope coefficient p-value of 0.59 in the overall studies, indicating a symmetric pattern for the data and, therefore, absence of publication bias (Fig. S3)

Discussion

Despite easy accessibility to the oral cavity for exploring tumor lesions, OSCC is still diagnosed at late stages, which are associated with poor prognosis. To achieve better survival in OSCC patients, a non-invasive molecular test using saliva or blood could be applied for screening purposes in high-risk patients, in tumoral lesions with apparent benign appearance, in multifocal lesions, and in suspicious malignant lesions. There are currently no validated circulating biomarkers used in clinical practice in OSCC diagnosis, generally based on conventional clinical (visual and tactile) oral examination and tissue biopsy. Although tumor biopsy still represents the gold standard for clinical molecular analysis, it is an invasive procedure that is temporally and spatially limited and unable to reflect the complete molecular landscape of the tumor [33]. To overcome these limitations, circulating biomarkers might represent a new opportunity to understand the molecular profile and dynamic behavior of the tumor [34]. Accumulating evidence demonstrates that small non-coding RNAs (mainly miRNAs) play an important role in cancer pathogenesis [35], and recently, tumor-derived cell-free miRNAs obtained using liquid biopsies have emerged as potential biomarkers for oral cancer [36].

To the best of our knowledge, this represents the first meta-analysis evaluating the diagnostic accuracy of miRNAs from body fluids, including blood and saliva, for discriminating OSCC patients. A previous systematic review by Troiano et al. [37] reported the potential of serum and plasma miRNAs as oral cancer biomarkers. The present study included a total of 16 studies (25 study units) comprising 1547 OSCC patients and 1015 healthy individuals, with study quality determined by the QUADAS-2 checklist for all included studies revealing moderate-to-high quality. According to the overall analysis, blood and salivary miRNAs demonstrated a high diagnostic accuracy to differentiate OSCC patients from healthy individuals, with sensitivity, specificity, and AUC values of 0.78, 0.82, and 0.91, respectively. Additionally, the pooled dOR was 21.46, which indicated high diagnostic effectiveness of miRNAs for detecting OSCC, and the pooled PLR value was 4.31, suggesting that the probability of cancer in a person with a positive miRNA test was ~4.31-fold higher than that in a healthy individual. By contrast, the pooled NLR indicated that the probability of not having cancer in a person with a negative test was 25%. Furthermore, Fagan’s nomogram results showed that when the pre-test probability was 55%, the correct diagnosis of OSCC increased to 84% after a positive miRNA test, whereas when the test was negative, OSCC was present in 23.4% of patients. Therefore, these results confirmed miRNAs as potentially useful diagnostic liquid biopsy biomarkers in OSCC patients.

Because heterogeneity is often present in meta-analysis of test-accuracy data, we explored possible causes that can potentially contribute to the existence of inconsistencies in accuracy estimates across studies. Given that we observed significant heterogeneity among the studies, we applied a bivariate random-effects model and assessed the threshold effect based on its being a primary cause of heterogeneity in test-accuracy studies. The results indicated no significant negative correlation between sensitivities and specificities in the included studies, and the ROC plane revealed a non-typical shoulder-arm pattern, which indicated the absence of a threshold effect. Because heterogeneity can arise from different factors in addition to the threshold effect, we performed meta-regression analysis to test the effect of ethnicity, sample size, miRNA-expression pattern, and miRNA profiling and found no significant differences. Therefore, it is possible that the heterogeneity present in the studies can be explained by other potential factors related to clinicopathological characteristics of subjects or the different approaches used for miRNA analysis, sample types, data normalization,

and the use of different cut-off values. Additionally, we observed a higher (but non-significant) accuracy between studies with large ($n \geq 100$) and small ($n < 100$) sample sizes and study populations ranging from nine to 121 OSCC patients and nine to 103 healthy individuals. Furthermore, a total of 22 different miRNAs were classified according to their expression patterns, with no significant differences found between upregulated and downregulated miRNAs. Interestingly, analysis of diagnostic accuracy between single- and multiple-miRNA assays revealed no differences. It is important to note that only two studies included in our meta-analysis [14,26] used miRNA panels. This indicates that future translational and clinical investigations should be focused on the combined use of multiple miRNAs to investigate their effect on diagnostic accuracy.

This meta-analysis revealed the potential of miRNAs as promising liquid biopsy biomarkers in OSCC; however, this study had some limitations. First, only a few studies investigated blood and/or salivary miRNAs in OSCC patients, with some of these involving a small sample size. Additionally, several studies focused on liquid biopsies described only miRNA-expression profiles in OSCC patients but did not analyze their diagnostic power. Moreover, there was a lack of clarity in the level of detail applied and/or the data-reporting techniques used by some of the studies, which hindered our analysis; therefore, specific miRNAs potentially useful for diagnosing OSCC were not included in our meta-analysis. Second, considerable heterogeneity was detected among the included studies. Although meta-regression and subgroup analyses were performed, we were unable to elucidate the potential sources contributing to this heterogeneity. Additionally, some variables, including TNM stage, fluid type, age distribution, gender proportion, and normalization strategy, were not specified by various studies, thereby precluding further subgroup analysis. Third, the studies included in the meta-analysis applied diverse cut-off values and different normalization methods, including global mean expression, exogenous synthetic miRNAs (i.e., cel-miR-39) and endogenous reference genes as miRNAs (i.e., miR-191) or small-nucleolar RNAs (i.e., RNU6B), as controls for miRNA quantification, which could substantially affect the results of our analysis and reports of potential clinical efficacy. This suggests that a consensus in the scientific community should be acquired to determine the optimal reference molecules for use as “gold standard” controls. Finally, several studies included in the meta-analysis were mainly developed in Asian population and only a few studies involved patients from a different ethnic population.

Conclusion

Overall, the results of this meta-analysis suggest that blood and salivary miRNAs represent potentially promising biomarkers for oral cancer diagnosis. Although these findings offer insight into possible clinical application of miRNAs present in liquid biopsies as a novel, non-invasive, and accurate diagnostic tools for OSCC detection, further research focusing on large-scale prospective, multicenter, and blinded studies involving both novel single- and combined-miRNA assays is necessary to validate the diagnostic efficacy of blood and salivary miRNAs in OSCC.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2019.104465.

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