

# Abnormally expressed microRNA as auxiliary biomarkers for nasopharyngeal carcinoma: A meta-analysis

ZHENZHOU XIAO, WEI PENG, XUSHENG ZHOU, XIAOLI LUO, YAN CHEN and ZHAOLEI CUI

Laboratory of Biochemistry and Molecular Biology Research, Department of Clinical Laboratory,  
Fujian Provincial Key Laboratory of Tumor Biotherapy, Fujian Cancer Hospital and Fujian  
Medical University Cancer Hospital, Fuzhou, Fujian 350014, P.R. China

Received November 1, 2017; Accepted November 20, 2017

DOI: 10.3892/br.2017.1026

**Abstract.** Aberrant expression of microRNA (miRNA) has been highlighted as a helpful indicator to aid in nasopharyngeal carcinoma (NPC) diagnosis. The present meta-analysis aimed to validate the efficacy of miRNA as potential biomarkers for NPC detection. Publication searches were conducted on the online PubMed and EMBASE databases from inception to June 2016. A bivariate meta-analysis was performed to generate the diagnostic parameters based on Meta-Disc 1.4 and Stata 12.0 programs. Sensitivity analysis and meta-regression tests were applied to trace heterogeneity sources among eligible studies. A total of six studies comprising 528 patients with NPC and 252 matched controls were enrolled. Results from the present meta-analysis demonstrated that miRNA testing achieved a pooled sensitivity of 0.78 [95% confidence interval (CI), 0.70-0.84] and specificity of 0.79 (95% CI, 0.73-0.84) in confirming NPC, corresponding to an area under the curve (AUC) value of 0.85. Additionally, the pooled diagnostic odds ratio was estimated to be 9.01 (95% CI, 5.62-14.44), along with a positive likelihood ratio of 2.81 (95% CI, 2.19-3.61) and negative likelihood ratio of 0.35 (95% CI, 0.28-0.44). Additionally, the stratified analyses revealed that paralleled testing of miRNA sustained a pooled accuracy superior compared with that of single miRNA testing (sensitivity, 0.88 vs. 0.70; specificity, 0.85 vs. 0.69; AUC, 0.95 vs. 0.75). Testing of miRNA harbors a

moderate diagnostic efficacy and is acceptable as an auxiliary biomarker for NPC diagnosis.

## Introduction

Nasopharyngeal carcinoma (NPC) is one of the most malignant head and neck carcinomas with unique epidemiological features (1). Each year, >50,000 mortalities of patients with cancer are due to NPC in China (2). Epidemiologically, the regions of South China and South East Asia sustain the incidence peaks (2). Advanced NPC yields high lethality due to late stage diagnosis and metastasis, which remains to be the leading cause of therapeutic failure in the clinic (3). Due to the unique location and a lack of specific symptoms, NPC is rarely detected during regular medical examinations (1-3). In this regard, early and accurate diagnosis continues to be a key approach to obtain an optimal prognosis for patients with NPC.

The discovery of microRNA (miRNA) has offered novel perspectives for cancer research. miRNA are classically defined as a type of RNA transcript (20-22 nucleotides in length), which negatively regulate the expression of protein-coding genes at the transcriptional or translational level (4,5). Basal expression of miRNA in cells or tissues is essential for various somatic processes, including cell proliferation, differentiation, the cell cycle and apoptosis (4-6). Numerous studies have documented that deregulated miRNA expression is strongly linked to the occurrence of various carcinomas (7,8). It has become increasingly apparent that miRNA are of crucial importance for the carcinogenesis and progression of NPC, and therefore they have been highlighted as powerful screening predictors in confirming or monitoring NPC (9-15). Unfortunately, the clinical utility of miRNA signatures remain unpopular in the clinic. Additionally, it appears that different studies have presented inconsistent results with regard to miRNA profiling in identifying NPC. For example, a previous study has demonstrated that miRNA testing is adequately sensitive and specific for NPC confirmation, with a diagnostic sensitivity and specificity of up to 96% (13). However, some research has reported that miRNA testing achieved an efficacy <60% (10,14,15). Therefore, the present study conducted a comprehensive meta-analysis and

---

*Correspondence to:* Dr Zhaolei Cui or Professor Yan Chen, Laboratory of Biochemistry and Molecular Biology Research, Department of Clinical Laboratory, Fujian Provincial Key Laboratory of Tumor Biotherapy, Fujian Cancer Hospital and Fujian Medical University Cancer Hospital, 420 Fuma Road, Fuzhou, Fujian 350014, P.R. China

E-mail: cuileidizi@163.com

E-mail: yanc2015@163.com

**Key words:** microRNA, nasopharyngeal carcinoma, diagnosis, meta-analysis

aimed to assess the overall diagnostic performance of miRNA as biomarkers for NPC identification.

## Materials and methods

**Literature search strategy.** The entire contents of the present meta-analysis followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (16). Literatures were collected based on the online PubMed (ncbi.nlm.nih.gov/pubmed) and EMBASE (embase.com/#search) databases up to June 30th 2016, utilizing the following search words: 'Nasopharyngeal carcinoma,' 'microRNA,' 'miRNA' and 'diagnosis/sensitivity/specificity,' with limitations to 'human' and 'English.' The inclusion criteria were as follows: i) Studies that evaluated the diagnostic performance of miRNA for NPC; ii) the final diagnosis of NPC was confirmed by tissue-proven histopathology; iii) studies provided complete data to construct 2x2 contingency tables; and iv) studies explicitly addressed the control sources and size. Studies with the following criteria were excluded: i) Studies without complete data to construct 2x2 contingency tables; ii) studies failed to explicitly state the control group(s); and iii) non-English written papers, review articles, basic study, letters, commentaries and meta-analyses.

**Data extraction and quality grading.** Eligible data were retrieved by two authors, and the contents included the name of the first author, year of publication, origin, patient size, control sources and size, miRNA expression profiles, and sensitivity and specificity data. The included studies were further evaluated for the quality grading based on the diagnostic accuracy (Quality Assessment of Diagnostic Accuracy Studies; QUADAS) tool issued in 2003 (17). According to the QUADAS scoring criteria where 14 questions were included, a 'Yes' answer corresponded to a score of '1,' whereas a 'No' or 'Unclear' answer received a score of '0.'

**Statistical analysis.** Statistical analyses were performed based on the platforms of Stata 12.0 (StataCorp LP, College Station, TX, USA) and Meta-disc 1.4 (Unit of Clinical Biostatistics Team of the Ramón y Cajal Hospital, Barcelona, Spain) software. Heterogeneity from the threshold effect was evaluated by the Spearman's correlation coefficient, and that from the non-threshold effect was assessed by Cochran's Q test and inconsistency index ( $I^2$ ) test ( $P < 0.05$  or  $I^2 > 50\%$ ) (18). A random effects model was applied for the meta-analysis in the case of the existence of heterogeneity (19). The generated diagnostic parameters included pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and summary receiver operator characteristic (SROC) curve. The clinical utility (post-test probabilities) were assessed by Fagan's plot assays. Publication bias among studies was determined by Deeks' funnel plot asymmetry test with a significant level of  $P < 0.05$ .

## Results

**Literature search and enrollment.** The process of study selection is demonstrated in Fig. 1. According to the predefined

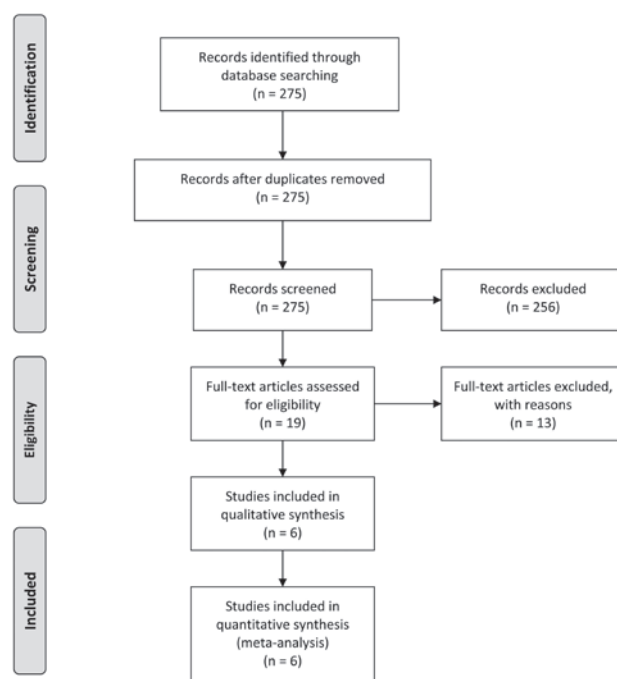


Figure 1. Flow diagram of literature selection process.

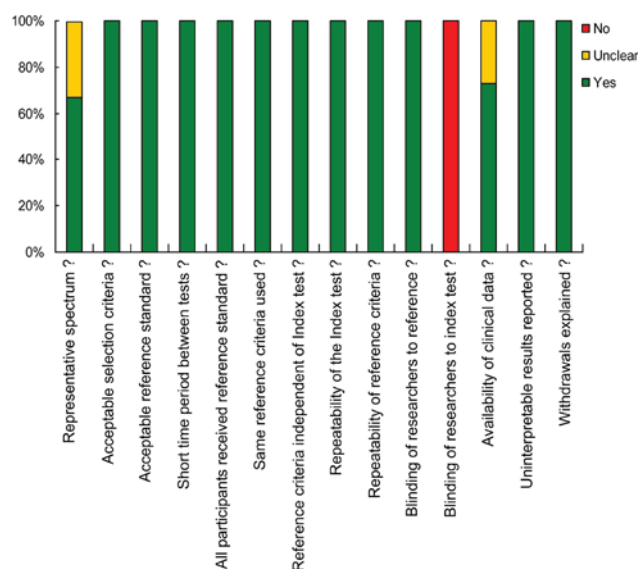


Figure 2. Proportions of studies with high, low or unclear risks determined by the 14-item Quality Assessment of Diagnostic Accuracy Studies checklist.

criteria, 275 relevant articles were obtained from online PubMed and EMBASE databases following elimination of the duplicated records. The retrieved papers received a detailed review of the study title and abstract, and 256 records were accordingly excluded. The possible eligible studies that received full text evaluation were restricted to 19, and 13 of them failed to meet the aim of the present study and were finally excluded. Eventually, six cohorts (including 21 individual studies) comprising 528 NPC cases and 252 matched controls were available for the meta-analysis (10-15).

Table I. Main features of included studies for miRNA signatures in the identification of nasopharyngeal carcinoma.

Author, year	Study population	Patient/control size	Specimen type	Test method	miRNA profile	Reference gene	Cut-off value	QUADAS score	(Refs.)
Liu <i>et al</i> , 2013	Chinese	217/73	Plasma	RT-qPCR	miR-16, miR-21, miR-24, miR-155, miR-378	U6	Unclear	11	(10)
Tang <i>et al</i> , 2014	Chinese	67/25	Tissue	RT-qPCR	miR-205, miR-135b	U6	Unclear	8	(11)
Wang <i>et al</i> , 2014	Chinese	50/50	Plasma	RT-qPCR	miR-483, miR-103, miR-29a, let-7c	Unclear	Unclear	10	(12)
Zeng <i>et al</i> , 2012	Chinese	74/57	Serum	RT-qPCR	miR-17, miR-20a, miR-29c and miR-223	miR-39 and miR-238	Unclear	10	(13)
Zhang <i>et al</i> , 2015	Chinese	89/28	Plasma	RT-qPCR	BART-13, BART-7	U6B	Unclear	12	(14)
Zheng <i>et al</i> , 2014	Chinese	31/19	Plasma	RT-qPCR	miR-548q, miR-483-5p	miR-454	Unclear	11	(15)

miRNA, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

**Study characteristics and quality assessments.** The main features for each included study are summarized in Table I. All six studies were conducted in China, and the evaluation methods for miRNA levels were all based on reverse transcription-quantitative polymerase chain reaction. The test matrices comprised serum, plasma and tissue, and the reference genes contained U6, miR-454, miR-39 and miR-238.

The article quality of each included publication was evaluated in terms of the 14-item QUADAS checklist (17). All of the studies yielded a QUADAS score  $\geq 8$ , suggesting a relatively high quality of the enrolled studies. The proportions of studies with low, high or unclear concerns are displayed in Fig. 2.

**Heterogeneity.** Table II summarizes the evaluated study heterogeneity from threshold and non-threshold effects. As indicated in Table II, the P-value obtained from Spearman's correlation coefficient in the overall pooled studies was estimated to be 0.012, indicating a significant heterogeneity generated from threshold effect. Additionally, the Cochran's Q test for the overall pooled analysis yielded a P-value of  $P < 0.0001$ , along with an  $I^2$  value of 83.8%, suggesting that the non-threshold effect is likely to be a source of heterogeneity as well. As a result, a random analysis model was selected for the final meta-analysis.

**Diagnostic performance and clinical utility.** As indicated in Table III, the pooled sensitivity, specificity, PLR, NLR, DOR and area under the curve (AUC) for miRNA profiling were 0.76 [95% confidence interval (CI), 0.70-0.81], 0.76 (95% CI, 0.69-0.82), 2.81 (95% CI, 2.19-3.61), 0.35 (95% CI, 0.28-0.44), 9.01 (95% CI, 5.62-14.44) and 0.83, respectively. Following adjustment of the outlier studies, the combined sensitivity, specificity, PLR, NLR, DOR and AUC for miRNA profiling were estimated to be 0.78 (95% CI, 0.76-0.80), 0.78 (95% CI, 0.75-0.81), 3.19 (95% CI, 2.48-4.11), 0.32 (95% CI, 0.25-0.40), 11.43 (95% CI, 7.02-18.61) and 0.84, respectively. Forest plots of pooled sensitivity, specificity as well as the SROC curve are demonstrated in Fig. 3A-C.

For the clinical utility assessed by Fagan's plot assay, apparent improvements of post-test probabilities were displayed in the pooled analysis, with a post-test probability of a positive result of 45% and negative result of 7% (Fig. 3D).

**Subgroup analysis.** Subgroup analysis was stratified by miRNA profiling (single or in parallel) and the test matrix. As exemplified in Table III, paralleled testing of miRNA achieved better diagnostic efficacy than single miRNA analysis: The pooled sensitivity was 0.88 (95% CI, 0.85-0.90) vs. 0.70 (95% CI, 0.68-0.72), specificity was 0.85 (95% CI, 0.81-0.89) vs. 0.69 (95% CI, 0.65-0.72) and AUC was 0.95 vs. 0.75. When the studies were stratified by the test matrix, the data manifested that serum/tissue-based tests conferred higher accuracy than plasma-based miRNA analysis in confirming NPC (sensitivity, 0.91 vs. 0.73; specificity, 0.94 vs. 0.71; AUC, 0.97 vs. 0.78).

**Influence assay and meta-regression.** Influence analysis and meta-regression tests were performed to deeply trace the heterogeneity sources. As demonstrated in Fig. 4, three individual studies were evaluated to undergo a deviation status. Following adjustment of the data by eliminating the outliers,

Table II. Analyses of study heterogeneity of all pooled studies.

Analysis	Spearman's correlation coefficient	Cochran's Q test	I <sup>2</sup> (%)	Heterogeneity	
				Threshold effect	Non-threshold effect
Single miRNA	-0.165, P=0.557	37.16, P=0.0007	62.3	No	Yes
Multiple miRNA	-0.928, P=0.008	27.01, P=0.0001	81.5	Yes	Yes
Plasma	-0.275, P=0.285	80.95, P<0.0001	80.2	No	Yes
Serum/tissue	-0.600, P=0.400	17.63, P=0.0005	83	No	Yes
Outlier excluded	-0.539, P=0.021	75.76, P<0.0001	77.6	Yes	Yes
Overall	-0.535, P=0.012	123.43, P<0.0001	83.8	Yes	Yes

miRNA, microRNA.

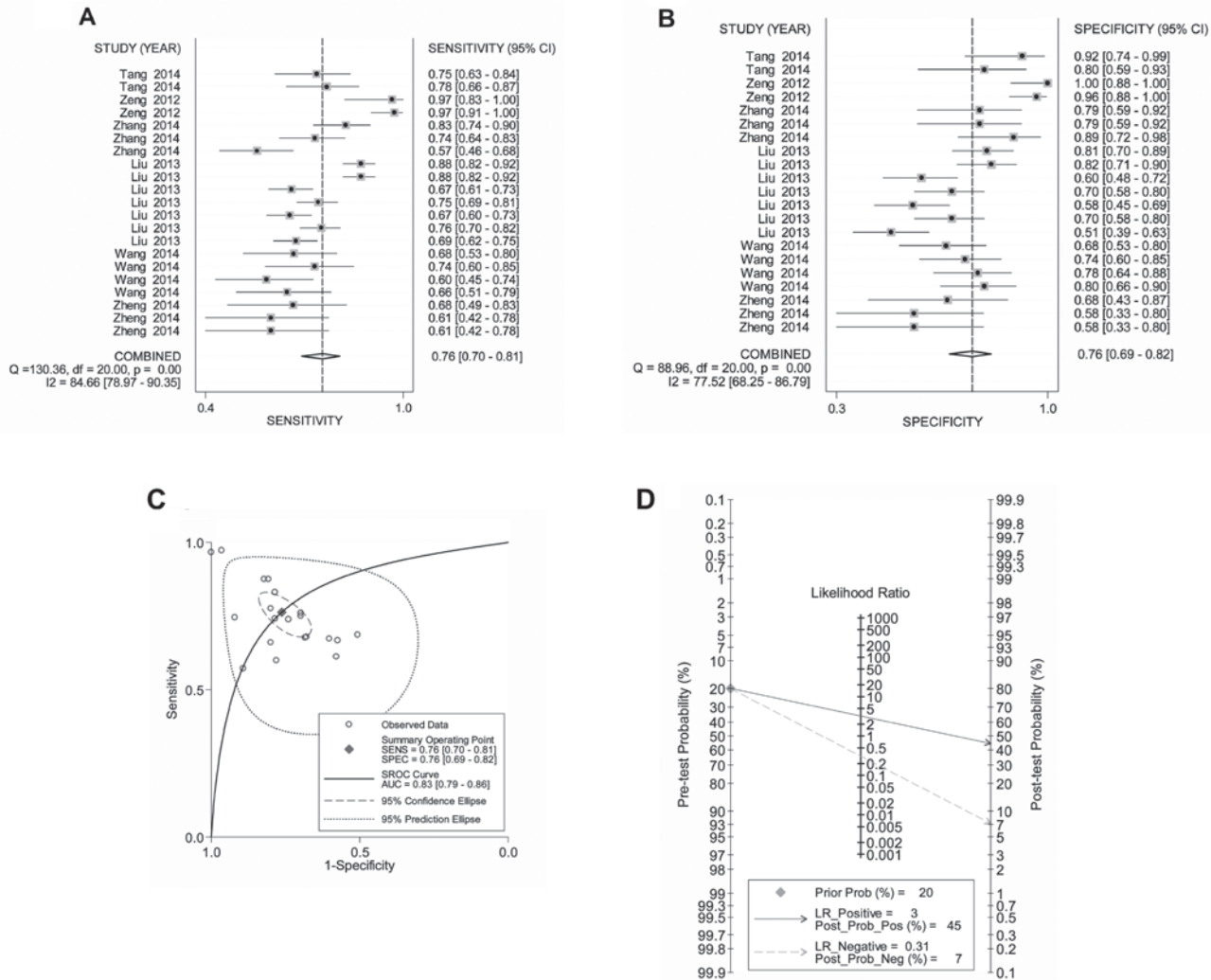


Figure 3. Evaluation of diagnostic performance and clinical utility. Forest plots of pooled (A) sensitivity, (B) specificity, (C) SROC curve and (D) post-test probability analyzed by Fagan's plot. CI, confidence interval; df, degrees of freedom; SROC, summary receiver operator characteristic; AUC, area under the curve.

the P-value of Spearman's correlation coefficient was altered from 0.012 to 0.021, and the I<sup>2</sup> value declined from 83.8 to 77.6%, hinting that the deviation data is likely a source of heterogeneity (Table II).

The meta-regression test was conducted based on five pre-specified covariates: Test matrix (plasma, serum or tissue), reference gene (U6 or other), test pattern (panel or single), NPC cases (<100 or ≥100), control size (<50 or ≥50) and article

Table III. Pooled diagnostic indices of miRNA profiling in confirming nasopharyngeal carcinoma.

Analysis	Diagnostic indices				
	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled positive likelihood ratio (95% CI)	Pooled negative likelihood ratio (95% CI)	Pooled diagnostic odds ratio (95% CI)
miRNA profile					
Single miRNA	0.7 (0.68-0.72)	0.69 (0.65-0.72)	2.25 (1.83-2.76)	0.44 (0.38-0.51)	5.34 (3.78-7.52)
Multiple miRNA	0.88 (0.85-0.90)	0.85 (0.81-0.89)	5.23 (2.91-9.39)	0.16 (0.09-0.27)	41.29 (13.83-123.22)
Sample type					
Plasma	0.73 (0.68-0.77)	0.71 (0.66-0.76)	2.5 (2.02-3.08)	0.39 (0.32-0.49)	6.46 (4.31-9.68)
Serum/tissue	0.91 (0.73-0.97)	0.94 (0.82-0.98)	15.8 (4.33-57.70)	0.1 (0.03-0.33)	157.53 (15.34-1617.17)
Outlier eliminated	0.78 (0.76-0.80)	0.78 (0.75-0.81)	3.19 (2.48-4.11)	0.32 (0.25-0.40)	11.43 (7.02-18.61)
Overall	0.76 (0.70-0.81)	0.76 (0.69-0.82)	2.81 (2.19-3.61)	0.35 (0.28-0.44)	9.01 (5.62-14.44)
miRNA, microRNA; CI, confidence interval.					



Table IV. Inverse variance weights for the potential source of heterogeneity assessed by meta-regression tests.

Study characteristic	P-value	Relative diagnostic odds ratio (95% confidence interval)
Test pattern (panel vs. single)	0.0001	7.53 (3.27-17.32)
Test matrix (plasma vs. serum vs. tissue)	0.0763	0.28 (0.07-1.16)
Reference gene (U6 vs. other)	0.0026	0.61 (0.45-0.82)
Nasopharyngeal carcinoma cases (<100 vs. ≥100)	0.1735	0.51 (0.19-1.39)
Control size (<50 vs. ≥50)	0.1101	3.40 (0.73-15.88)
Article quality (QUADAS≥10 vs. QUADAS<10)	0.0703	0.71 (0.49-1.03)

QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

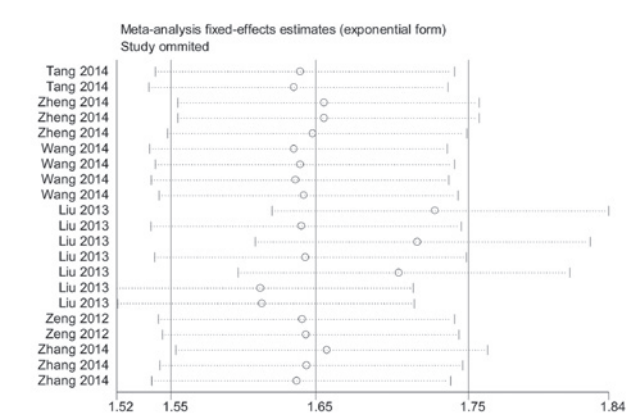


Figure 4. Influence analysis of the outliers for the overall pooled study.

quality (QUADAS≥10 or <10) (20). The results revealed that the test pattern (P=0.0001) and reference gene (P=0.0026) were the key factors that contributed to the heterogeneity sources (Table IV).

**Publication bias.** The funnel plots for publication bias demonstrated no asymmetry for the overall pooled analysis, along with a P-value of 0.702, indicating that there was no bias from the publications (Fig. 5).

Discussion

Advanced NPC sustains high lethality and delayed diagnosis remains to be the leading cause for therapeutic failure (1,2). The treatment outcomes, as well as NPC management, may be improved by the development of non-invasive biomarker assays that help to reinforce the overall diagnostic efficacy (3). Large quantities of research regarding the diagnostic value of miRNA profiling for NPC are available (9-15); however, there are currently no consistent results among these studies and the clinical utility of miRNA profiling for NPC management is hotly debated. In this regard, the present meta-analysis was conducted and a comprehensive evaluation of the predictive efficacy of miRNA signatures for NPC identification was performed.

Results from the present meta-analysis demonstrated that miRNA profiling retained a pooled sensitivity of 0.76, speci-

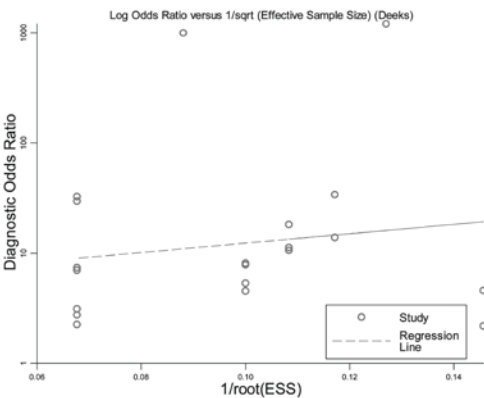


Figure 5. Funnel chart of the publication bias assessed by Deeks' funnel plot asymmetry test. ESS, effective sample size.

ficity of 0.76 and AUC of 0.83 for its capacity to discriminate the patients with NPC from cancer-free individuals. Another important indicator, termed the DOR (18), was estimated to be 9.01 in the present analysis, which indicated a relatively high discriminatory performance of miRNA testing in the management of NPC. Additionally, the pooled PLR of 2.81 suggested that miRNA profiling yielded a ratio of nearly 3 between the true positive rate and false positive rate. Correspondingly, the pooled NLR indicated that the probability of NPC cases that tested negative for miRNA vs. the probability of cases that tested positive for these miRNA achieved a ratio of 0.35. For the clinical utility, miRNA signatures raised the post-test probability of a positive result to 46% and lowered the post-test probability of a negative result to 7%. Overall, the present data demonstrated that miRNA signatures may be popularized as auxiliary biomarkers for NPC identification.

Stratified analysis was also conducted based on miRNA profiling (single or parallel) and test matrices. The data demonstrated that multiple testing of miRNA achieved higher diagnostic efficacy than single miRNA analysis. A study by Liu *et al* (10) evidenced that a combination of five miRNA (miR-16, -21, -24, -155 and -378) reached a diagnostic sensitivity of 0.88 and specificity of 0.82 for NPC, which is better than the single tests. On the other hand, the analysis of miRNA signature test matrix revealed that serum/tissue-based testing achieved a higher diagnostic accuracy than that of

plasma-based analysis. A study by Wang et al (21) indicated that the coagulation process is likely to affect the spectrum of extracellular molecules in the blood, hinting that different matrices, including serum or plasma, may sustain altered diagnostic efficacy. Nevertheless, the analysis stratified by the matrix yielded a small study size and displayed high heterogeneity among studies. Hence, more investigations are warranted to reinforce this preliminary evidence.

The present pooled analysis demonstrated significant heterogeneity among studies. Heterogeneity mainly derives from threshold and non-threshold effects (18-20). The threshold effect is predominantly generated by the different cut-off value settings or thresholds used in different studies, whereas the non-threshold effect could be caused by different ethnicities, testing methods, sample types, as well as the severity of disease conditions (18). In the overall pooled analysis, the Spearman's correlation coefficient, Cochran's Q and  $I^2$  tests all presented significant results, indicating that heterogeneity came from both threshold and non-threshold effects. As a result, sensitivity analysis and meta-regression tests were conducted in the present study to deeply trace the heterogeneity sources. The results revealed that different miRNA test patterns, as well as the non-unified reference gene, appeared to be a contributor of study heterogeneity, whereas the control types, study size and article quality demonstrated a low likelihood of influencing the heterogeneity sources.

In conclusion, the present analyses evaluated the diagnostic value of miRNA profiling for NPC identification, in particular elucidating that parallel testing and non-plasma based miRNA signatures yield improved efficacy. Nevertheless, due to the small number of studies, obvious heterogeneity, as well as complicated control sources in the present study, the overall pooled accuracy is compromised. More investigations are warranted to further testify the present preliminary evidence.

## Acknowledgements

The present study was supported by the National Clinical Key Specialty Construction Program of China (2013) and Provincial Natural Science Fund of Fujian (grant no. 2016J01511).

## References

- Petersson F: Nasopharyngeal carcinoma: A review. *Semin Diagn Pathol* 32: 54-73, 2015.
- Wei KR, Zheng RS, Zhang SW, Liang ZH, Ou ZX and Chen WQ: Nasopharyngeal carcinoma incidence and mortality in China in 2010. *Chin J Cancer* 33: 381-387, 2014.
- Cao SM, Simons MJ and Qian CN: The prevalence and prevention of nasopharyngeal carcinoma in China. *Chin J Cancer* 30: 114-119, 2011.
- Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, et al: MicroRNA expression profiles classify human cancers. *Nature* 435: 834-838, 2005.
- Pritchard CC, Cheng HH and Tewari M: MicroRNA profiling: Approaches and considerations. *Nat Rev Genet* 13: 358-369, 2012.
- Pileczki V, Cojocneanu-Petric R, Maralani M, Neagoe IB and Sandulescu R: MicroRNAs as regulators of apoptosis mechanisms in cancer. *Clujul medical* (1957) 89: 50-55, 2016. <https://doi.org/10.15386/cjmed-512>.
- Frixa T, Donzelli S and Blandino G: Oncogenic MicroRNAs: Key Players in Malignant Transformation. *Cancers (Basel)* 7: 2466-2485, 2015.
- Tuna M, Machado AS and Calin GA: Genetic and epigenetic alterations of microRNAs and implications for human cancers and other diseases. *Genes Chromosomes Cancer* 55: 193-214, 2016.
- Lee KT, Tan JK, Lam AK and Gan SY: MicroRNAs serving as potential biomarkers and therapeutic targets in nasopharyngeal carcinoma: A critical review. *Crit Rev Oncol Hematol* 103: 1-9, 2016.
- Liu X, Luo HN, Tian WD, Lu J, Li G, Wang L, Zhang B, Liang BJ, Peng XH, Lin SX, et al: Diagnostic and prognostic value of plasma microRNA deregulation in nasopharyngeal carcinoma. *Cancer Biol Ther* 14: 1133-1142, 2013.
- Tang JF, Yu ZH, Liu T, Lin ZY, Wang YH, Yang LW, He HJ, Cao J, Huang HL and Liu G: Five miRNAs as novel diagnostic biomarker candidates for primary nasopharyngeal carcinoma. *Asian Pac J Cancer Prev* 15: 7575-7581, 2014.
- Wang HY, Yan LX, Shao Q, Fu S, Zhang ZC, Ye W, Zeng YX and Shao JY: Profiling plasma microRNA in nasopharyngeal carcinoma with deep sequencing. *Clin Chem* 60: 773-782, 2014.
- Zeng X, Xiang J, Wu M, Xiong W, Tang H, Deng M, Li X, Liao Q, Su B, Luo Z, et al: Circulating miR-17, miR-20a, miR-29c, and miR-223 combined as non-invasive biomarkers in nasopharyngeal carcinoma. *PLoS One* 7: e46367, 2012.
- Zhang G, Zong J, Lin S, Verhoeven RJ, Tong S, Chen Y, Ji M, Cheng W, Tsao SW, Lung M, et al: Circulating Epstein-Barr virus microRNAs miR-BART7 and miR-BART13 as biomarkers for nasopharyngeal carcinoma diagnosis and treatment. *Int J Cancer* 136: E301-E312, 2015.
- Zheng XH, Cui C, Ruan HL, Xue WQ, Zhang SD, Hu YZ, Zhou XX and Jia WH: Plasma microRNA profiling in nasopharyngeal carcinoma patients reveals miR-548q and miR-483-5p as potential biomarkers. *Chin J Cancer* 33: 330-338, 2014.
- Moher D, Liberati A, Tetzlaff J and Altman DG: PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *J Clin Epidemiol* 62: 1006-1012, 2009.
- Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM and Kleijnen J: The development of QUADAS: A tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 3: 25, 2003.
- Cui ZL, Zheng DZ, Liu YH, Chen LY, Lin DH and Feng-Hua Lan: Diagnostic Accuracies of the TUNEL, SCD, and Comet Based Sperm DNA Fragmentation Assays for Male Infertility: A Meta-analysis Study. *Clin Lab* 61: 525-535, 2015.
- Chen Y, Cui Z, Xiao Z, Hu M, Jiang C, Lin Y and Chen Y: PAX1 and SOX1 methylation as an initial screening method for cervical cancer: A meta-analysis of individual studies in Asians. *Ann Transl Med* 4: 365, 2016.
- Cui Z, Chen Y, Xiao Z, Hu M, Lin Y, Chen Y and Zheng Y: Long noncoding RNAs as auxiliary biomarkers for gastric cancer screening: A pooled analysis of individual studies. *Oncotarget* 7: 25791-25800, 2016.
- Wang K, Yuan Y, Cho JH, McClarty S, Baxter D and Galas DJ: Comparing the MicroRNA spectrum between serum and plasma. *PLoS One* 7: e41561, 2012.