

Original Article

Prognostic value of miR-21 in patients with breast cancer: a meta-analysis

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Abstract: More and more studies investigated the effects of miR-21 expression level on the prognostic role in breast cancer. However, the available results have been controversial. The objective of this study was to perform a meta-analysis and evaluate the association between miR-21 expression and survival outcomes of breast cancer patients. Relevant studies were identified by a comprehensive search from PubMed and EMBASE databases. The hazard ratio (HR) and its corresponding 95% confidence interval (CI) were used to assess the strength of relationships. A total of 14 studies involving 2,789 cases were included in this meta-analysis. We found that up-regulated miR-21 had an unfavorable impact on breast cancer patients' survival, with pooled HR of 2.66 (95% CI 1.61-4.39, $P < 0.001$) for overall survival (OS), 2.55 (95% CI 2.14-3.05, $P = 0.042$) for disease-free survival (DFS) and 3.25 (95% CI 2.00-5.29, $P < 0.001$) for relapse-free survival (RFS)/metastasis-free survival (MFS). Subgroup analysis suggested that high miR-21 expression was significantly correlated with lower OS in the Asian cohort (HR=4.05, 95% CI 2.04-8.04, $P < 0.001$), but not in the Caucasian cohort (HR=1.86, 95% CI 0.92-3.75, $P = 0.082$). Sensitivity analysis further validated the role of miR-21 as a predictor for prognosis. Our results demonstrate that miR-21 might have a critical prognostic value in patients with breast cancer.

Keywords: Breast cancer, miR-21, prognosis, meta-analysis

Introduction

Breast cancer is one of the major concerns for women health and is responsible for the leading cause of women death [1]. A variety of clinical and pathological factors are routinely used to classify breast cancer patients to decide on the appropriate therapy. However, it is difficult for clinicians to evaluate the determination of prognosis and the prediction of treatment response in patients with breast cancer [2, 3]. Therefore, to individualize treatment and to predict outcomes, novel predictive biomarkers that will lead to molecular diagnostic tests are required.

MicroRNAs (miRNAs) are a group of about 22-nt non-coding small RNAs, which regulate gene expression at post-transcription level. Studies have shown that miRNAs are involved in various biological processes, including cell proliferation, apoptosis, differentiation and so on

[4-6]. Quantitative and clinical data demonstrated that the expression levels of some miRNAs are associated with cancer survival outcomes [7-9]. Moreover, miRNAs have been widely exploited as novel candidate diagnostic and prognostic biomarkers in human tumors [10, 11]. Among these miRNAs, miR-21 is known to be overexpressed in breast cancer [12]. Studies have revealed that up-regulated miR-21 can increase tumor growth, metastasis and invasion, and reduce sensitivity to chemotherapy by its various targets [13, 14].

There is accumulating evidence suggesting that elevated miR-21 expression is associated with a worse prognosis in breast cancer patients [15, 16]. But some other studies represented inconsistent or even opposite results [17-19]. Therefore, we conducted a systematic review and meta-analysis to evaluate the prognosis value of miR-21 for survival in patients with breast cancer.

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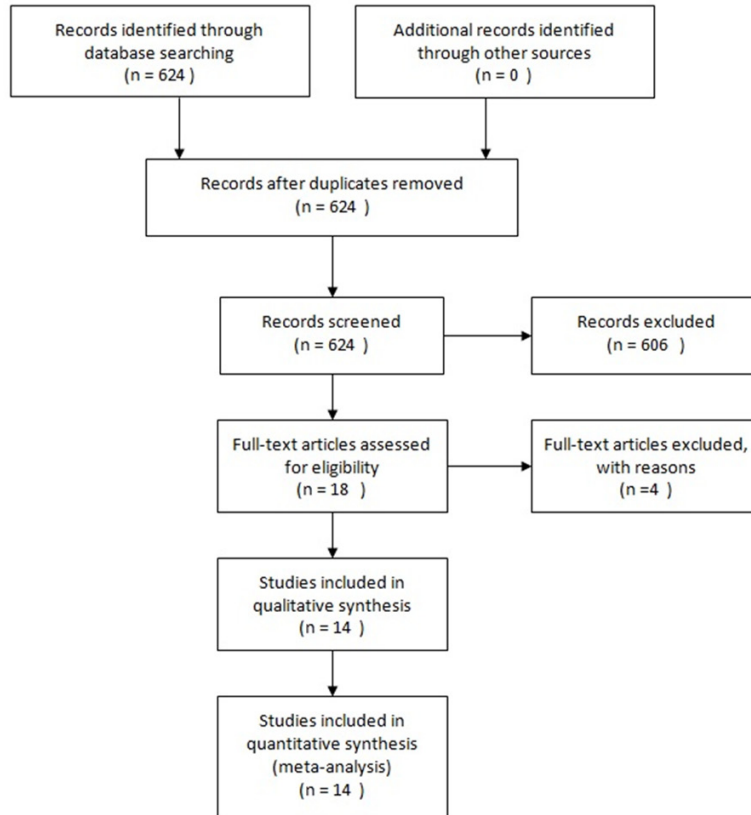


Figure 1. Flow chart of literature review and study selection process.

Material and methods

Search strategy

Published literatures were carefully searched from comprehensive databases including PubMed and EMBASE update to Jun, 1st 2015 by various combination of adopted key words: “microRNA-21 OR miR-21”, “survival”, “prognosis*”, “outcome”, “breast cancer”, “carcinoma” and “tumor”. In order to minimize the deviation caused by the search process, references in all relevant articles were scanned manually to identify other potentially applicable reports.

Study selection

Studies were considered eligible if they met the following criteria: (i) The object of study must be breast cancer patients. (ii) The correlation between miR-21 expression and survival outcome was investigated. (iii) Hazard ratio (HR) and 95% confidence interval (95% CI) could be obtained. Studies were excluded based on any of the following criteria: (i) review articles, laboratory articles, letters or experiment on animal models; (ii) not written in

English; (iii) investigated other tumors’ survival outcome. These identified articles were double-checked by two independent investigators (Zhu and Dong), another investigator (Yu) was invited to discuss until a consensus was reached when discrepancies existed between two investigators.

Quality assessment

We systematically evaluated the studies which included in this meta-analysis according to a critical review checklist of the Dutch Cochrane Centre proposed by MOOSE [20]. These following key points were contained in checklist: (i) clear information about the study population and origin of country; (ii) clear definition of the study design; (iii) clear description of outcome assessment; (iv) clear description of miR-21 measurement; (v) clear definition of the cut-off value of miR-21; (vi) sufficient period of follow-up. Otherwise,

we would exclude the studies in order to ensure the quality of the meta-analysis. Meanwhile, we assessed the quality of included studies by the representativeness of cases, detection methods, and follow-up of patients. The total scores ranged from 0 to 10, with higher scores indicating better quality.

Data extraction and conversion

Two reviewers performed the data extraction from all eligible studies independently. The key information included the following elements: first author, publication year, country, ethnicity, total number of cases, follow-up duration, sample size, cut-off value, survival outcomes, hazard ratio (HR), as well as their 95% CI and *P* value. When these data were not directly reported, we extracted and figured out them from Kaplan-Meier curves by using Digitizer Engauge 4.0 software according to the methods described by Tierney et al. [21].

Statistical methods

Cochran’s Q test and the Higgins’ I squared statistic were used to evaluate the heterogeneity

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Table 1. Main characteristics of the eligible studies included in the meta-analysis

Author	Year	Origin of population	No. of patients	Stage	Sample source	Assay	Cut-off	Survival analysis	HR estimation	HR (95%)	Follow-up (months)	Quality score
Yan	2008	China	113	I-IV	FFPE tissues	qRT-PCR	Mean	OS	Reported	4.133 (1.799-9.499)	66.2 (10.4-81.0)	8
Qian	2009	Italy	301	I-IV	Frozen tissues	qRT-PCR	Median	OS	Reported	1.21 (0.65-2.23)	86.2 (8-108)	8
Qian	2009	Italy	301	I-IV	Frozen tissues	qRT-PCR	Median	DFS	Reported	1.49 (0.86-2.57)	86.2 (8-108)	8
Walter	2011	USA	25	II-III	FFPE tissues	qRT-PCR	Median	OS	Reported	0.5 (0.07-3.73)	35.5	8
Ozgun	2013	Turkey	15	I-III	FFPE tissues	qRT-PCR	5.538	DFS	SC	8.22 (5.94-10.13)	NA	6
Lee	2011	Korea	109	I-III	FFPE tissues	qRT-PCR	Mean	OS	Reported	14.214 (1.338-15.096)	NA	7
Lee	2011	Korea	109	I-III	FFPE tissues	qRT-PCR	Mean	DFS	SC	1 (0.2-4.91)	NA	7
Dong	2014	China	72	I-III	Tissues	qRT-PCR	1.5 folds	OS	SC	2.79 (1.12-6.19)	NA	6
Gong	2014	China	268	NA	FFPE tissues	ISH	SI>4	RFS	SC	4.869 (1.527-15.524)	>140	7
Mackenzie	2013	USA	901	I-II	FFPE tissues	ISH	Scored 2, 3	RFS	Reported	1.96 (1.38-2.78)	124	8
Wang	2014	China	326	I-III	Serum	qRT-PCR	Median	RFS	Reported	3.942 (1.42-8.345)	>60	8
Wang	2014	China	326	I-III	Serum	qRT-PCR	Median	DFS	Reported	2.732 (1.038-7.273)	>60	8
Anastasov-1	2012	Germany	86	NA	FFPE tissues	qRT-PCR	1.8 folds	MFS	SC	3.1 (1.22-8.82)	113 (5-468)	8
Anastasov-2	2012	Germany	86	NA	FFPE tissues	qRT-PCR	1.8 folds	MFS	SC	6.27 (1.83-11.29)	113 (5-468)	8
Muller-1	2014	Germany	127	I-III	blood samples	qRT-PCR	Median	OS	SC	5.44 (3.21-15.21)	62.15 (5.56-66.28)	8
Muller-2	2014	Germany	127	I-III	blood samples	qRT-PCR	Median	OS	SC	4.36 (2.8-14.45)	62.15 (5.56-66.28)	8
Radojicic	2011	Greece	49	NA	FFPE tissues	qRT-PCR	Median	OS	SC	1.01 (0.1-9.79)	NA	6
Radojicic	2011	Greece	49	NA	FFPE tissues	qRT-PCR	Median	DFS	SC	0.41 (0.12-1.28)	NA	6
Ota	2011	Japan	291	NA	Bone marrow	qRT-PCR	5.84	OS	SC	3.13 (1.13-9.44)	61 (2-90)	8
Ota	2011	Japan	291	NA	Bone marrow	qRT-PCR	5.84	DFS	SC	6.22 (1.56-10.3)	61 (2-90)	8
Markou	2014	Greece	106	I-III	FFPE tissues	qRT-PCR	Median	OS	SC	1.19 (0.47-3.01)	84 (10-149)	8
Markou	2014	Greece	106	I-III	FFPE tissues	qRT-PCR	Median	DFS	Reported	2.494 (1.295-4.802)	68 (5-149)	8

SC: survival curve; NA: not available; Anastasov-1 and Muller-1: results before therapy; Anastasov-2 and Muller-2: results after therapy.

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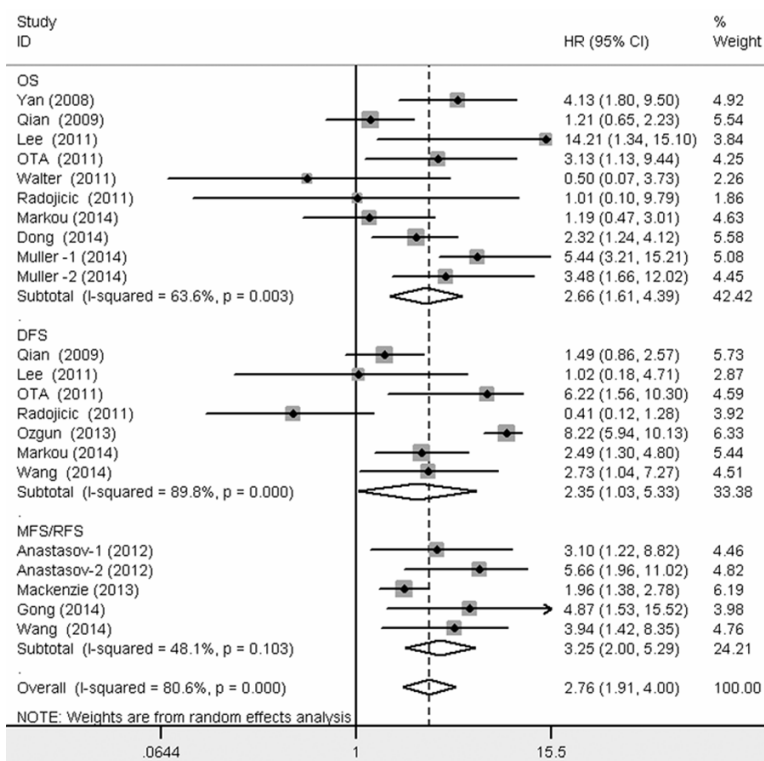


Figure 2. Forrest plots of studies evaluating HRs of miR-21 expression. The pooled HR=2.76 (95% CI: 1.91-4.00) indicates that Elevated miR-21 expression is significantly associated with poor prognosis according to the random effect estimations.

of combined HRs. A P value < 0.05 and/or $I^2 > 50\%$ indicated significant heterogeneous among studies, a random-effects model [22, 23] was used to calculate the pooled HR. Otherwise, the fixed-effects model was used [24]. Generally, pooled $HR > 1$ was assumed to indicate a significant association with worse prognosis. Publication bias was tested by Begg's funnel plots. Funnel plot symmetry was further assessed using Egger's linear regression method [25]. To validate the credibility of the summarized results, sensitivity analysis was performed by the successive omission of individual studies. For all analyses, a two-sided P value less than 0.05 was considered as statistically significant. All analyses were carried out using the statistical software STATA version 12.0 (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

As shown in **Figure 1**. A total of 624 potentially relevant records were retrieved after the initial literature search. After carefully reviewing the

articles, 606 studies were excluded because they were letters, reviews, abstracts, duplications or laboratory studies. Of the remaining 18 candidate articles, four articles were further removed due to the absence of the essential data. As a result, 14 eligible articles with 22 studies were enrolled in this meta-analysis [26-34]. All the included studies obtained scores of 6 or more (**Table 1**), implying that they were all of high quality.

The major characteristics of the eligible studies were summarized in **Table 1**. A total of 2789 participants from China, Germany, Italy, Korea, Japan, Greece, USA and Turkey respectively were admitted in the 22 studies. Among these studies, miR-21 level was measured by quantitative real-time polymerase chain reaction (qRT-PCR) in 20 studies and by in situ hybridization (ISH) assay in two studies.

Tissue samples with formalin-fixed and paraffin-embedded (FFPE) tissues were used in most of studies, except for two studies used in serum and one in bone marrow [30]. The cut-off values of miR-21 expression appeared to be different, the most frequently used were the median or mean in eight studies. All of the studies were retrospective in design. 9 of the studies presented HRs and 95% CIs directly; In the remaining 13 studies, the HRs and 95% CIs were calculated by Digitizer Engauge 4.0 software. 10 studies reported HRs for OS, 7 for DFS, 5 for RFS/MFS.

Meta-analysis results

As illustrated in **Figure 2**, miR-21 high expression was a prognostic factor for poor survival in breast cancer patients, with the pooled HRs of 2.66 (95% CI 1.61-4.39, $P=0.003$) for OS and 2.55 (95% CI 2.14-3.05, $P=0.042$) for DFS, with a random model because of the significant heterogeneity ($I^2=63.6\%$, $P=0.003$; $I^2=89.80\%$, $P<0.001$, respectively). In addition, elevated miR-21 expression was significantly correlated with poor MFS/RFS in patients with breast can-

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Table 2. Results of meta-analysis for miR-21 on prognostic effect in patients with breast cancer for OS and DFS

Outcome	Variables	No. of studies	Model	Pooled HR (95%)	P value	Heterogeneity	
						I ² (%)	P value
Overall		22	Random	2.76 (1.91-4.00)	0.000	80.60%	0.000
OS	ALL	10	Random	2.66 (1.61-4.39)	0.000	63.60%	0.003
	Ethnicity						
	Asian	4	Random	4.05 (2.04-8.04)	0.000	58.00%	0.068
	Caucasian	6	Random	1.86 (0.92-3.75)	0.082	63.00%	0.019
	Sample source						
	FFPE	5	Random	2.42 (0.81-7.22)	0.114	72.70%	0.005
	Frozen	2	Random	1.68 (0.89-3.18)	0.110	54.50%	0.138
	Blood	2	Fixed	4.59 (2.49-8.46)	0.000	0%	0.487
	Bone marrow	1	Random	3.13 (1.08-9.05)	0.035	-	-
	Cut-off Value						
	Median	6	Random	1.86 (0.92-3.75)	0.082	63.0%	0.019
	Mean	2	Random	7.06 (2.13-23.45)	0.001	63.20%	0.099
	Other	2	Fixed	2.50 (1.48-4.21)	0.001	0%	0.630
DFS	ALL	7	Random	2.55 (2.14-3.05)	0.042	89.80%	0.000
	Ethnicity						
	Asian	3	Fixed	3.07 (1.24-7.61)	0.016	48.20%	0.145
	Caucasian	4	Random	2.04 (0.63-6.66)	0.237	94.40%	0.000
	Sample source						
	FFPE	4	Random	1.92 (0.52-7.05)	0.324	91.80%	0.000
	Frozen	1	Random	1.49 (0.86-2.58)	0.153	-	-
	Serum	1	Random	2.73 (1.03-7.22)	0.043	-	-
	Bone marrow	1	Random	6.22 (2.42-15.98)	0.000	-	-
	Cut-off Value						
	Median	5	Random	2.17 (0.80-5.92)	0.129	92.70%	0.000
	Mean	1	Random	1.02 (0.20-5.22)	0.981	-	-
	Other	1	Random	6.22 (2.42-15.98)	0.000	-	-
MFS/RFS	ALL	5	Fixed	3.25 (2.00-5.29)	0.000	48.10%	0.103

Random-effects model was used when *P*-value for heterogeneity test <0.05; otherwise, fixed-model was used. *I*² the percentage of variability in HR attributable to heterogeneity.

cer, with the pooled HR of 3.25 (95% CI 2.00-5.29, *P*<0.001) calculated by a fixed model because of the absence of heterogeneity (*I*²=48.10%, *P*=0.103).

To explore the sources of heterogeneity, we also conducted subgroup analysis for OS and DFS by the ethnicity, sample source and cut-off value. The main results of this subgroup analysis are summarized in **Table 2**. In ethnicity subgroup analysis, the significant association between miR-21 over expression and poor OS was found in Asian (HR=4.05, 95% CI 2.04-8.04, *P*=0.000) (**Table 2**), but not in Caucasian (HR=1.86, 95% CI 0.92-3.75, *P*=0.082). In addition, further analysis of studies on sample source also indicated that up-regulated miR-21

remained to be a promising prognostic biomarker in FFPE tissue (HR=2.42, 95% CI 0.81-7.22, *P*=0.114) and serum sample (HR=4.59, 95% CI 2.49-8.46, *P*<0.001), except for frozen tissue (HR=1.68, 95% CI 0.89-3.18, *P*=0.110). Moreover, stratified by the positive threshold, we found that high miR-21 expression had a worse prognosis in mean cut-off value (HR=7.06, 95% CI 2.13-23.45, *P*=0.001), but not in the median cut-off group (HR=1.86, 95% CI 0.92-3.75, *P*=0.082).

Publication bias and sensitivity analysis

Begg's funnel plot and the Egger's linear regression test were used to assess publication bias of the included studies in the meta-analysis. As

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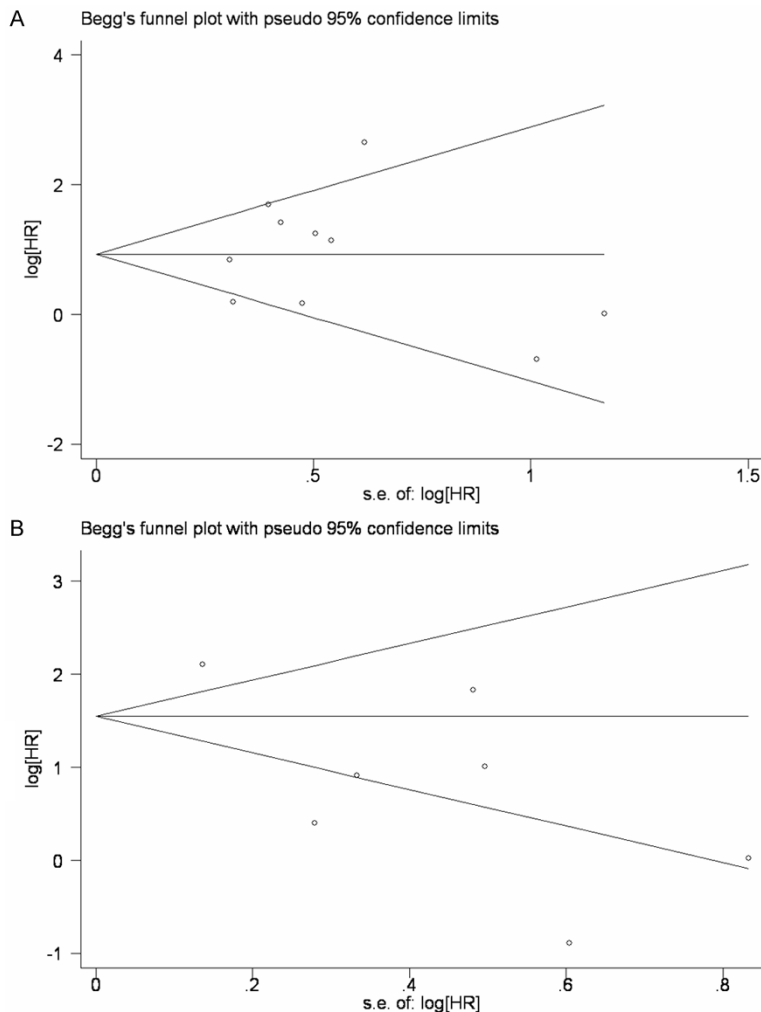


Figure 3. Begg's funnel plot for publication bias test of OS (A) and DFS (B). Each point represents a separate study for the indicated association.

shown in **Figure 3A** and **3B**, the funnel plots were almost symmetric for both OS and DFS, and *P* values of Egger's test were 0.929 and 0.652, respectively, indicating no evidence of significant publication bias in present meta-analysis. Meanwhile, the results of the sensitivity analysis demonstrated that no individual study significantly influenced the overall HR, as shown in **Figure 4A** and **4B**.

Discussion

Breast cancer is the most frequently diagnosed malignancy and is the leading cause of cancer-related deaths among females. Breast cancer is also known to be fairly complex and heterogeneous in tumor development, progression, and response to treatment [1, 35, 36]. Recently,

both tissue and serum-based tumor biomarkers are widely used to screen early-stage breast cancer and predict its progression in advance. Conventional biomarkers available such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 receptors (HER2) and P53, could not effectively reflect the integral prognostic significance for breast cancer patients. Therefore, researchers are supportive to explore novel biomarkers for the optimization of breast cancer prognosis and treatment. Previous studies have evidenced that microRNAs are involved in many crucial processes such as cellular proliferation, differentiation, cell cycle control, development, and apoptosis [37-39]. Recently, more and more findings have demonstrated that miRNAs are important roles in predicting the prognostic value of the breast cancer patients, acting as oncogenic or tumor suppressive miRNAs [37, 40, 41]. Despite early studies on miRNAs expression only has been completed on tissue samples, the

use of miRNAs as biomarkers for cancer shows a great promise. MiR-21, known as a potential oncogenic player, is one of the most ordinarily observed aberrant miRNAs in breast cancer [15-19, 26-34]. Some articles proved that miR-21 was significantly connected with breast cancer patients' survival [16, 31]. On the other hand, the opposite results were also observed in other studies [18, 19]. Thus, we performed a quantitative meta-analysis to determine the relationship between the expression of miR-21 and survival prognosis in breast cancer patients.

This meta-analysis showed that elevated miR-21 expression could predict poor survival in patients with breast cancer, the pooled HR for OS was 2.66 (95% CI 1.61-4.39, *P*<0.001).

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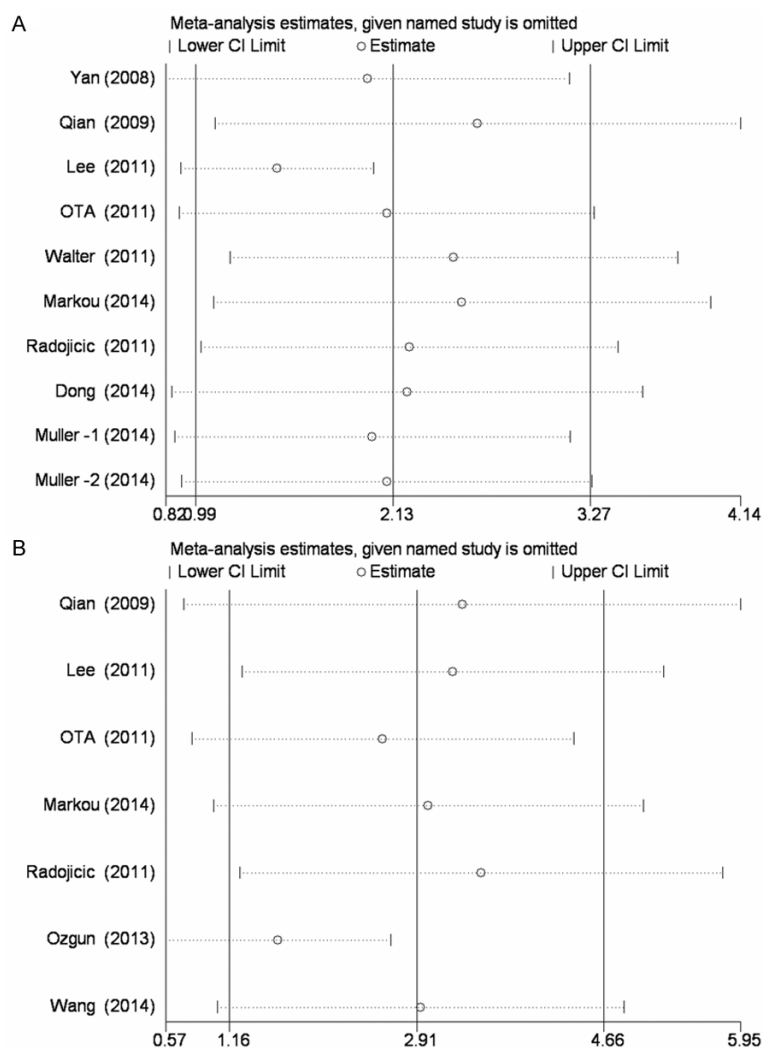


Figure 4. Sensitivity analysis of the influence of individual studies on the pooled HR for OS (A) and DFS (B). The middle vertical axis indicates the overall HR and the two vertical axes indicate its 95% CI. Every hollow round indicates the pooled HR when the left study was omitted in this meta-analysis. The two ends of every broken line represent the 95% CI.

Wang et al. [42] analyzed 10 studies and yielded a similar result (HR=2.57, 95% CI 1.37-4.81, $P=0.003$), however, the number of articles included in this analysis was relatively small, which made the results not powerful. Furthermore, the author wrongly assigned the HR for DFS to HR for OS in Dong et al.'s study [27]. Moreover, the RR value was mistaken as the HR for DFS in OTA et al.'s study [31]. Therefore, the conclusion by Wang et al. [42] was still uncertain. In view of this, we performed this updated meta-analysis including 14 articles with 22 studies. Because of existing significant heterogeneity among OS and DFS studies, we

then perform a subgroup analysis based on ethnicity, sample source and cut-off value. The results indicated that over-expression of miR-21 could predict a poor survival outcome in breast cancer. In Asian subgroup analysis, high expression level of miR-21 was a significantly negative prognostic biomarker both in OS and DFS. In addition, further analysis on sample source demonstrated that poor survival is positively correlated with FFPE tissues. In cut-off subgroup analysis, mean cut-off value significantly estimated poor OS. Publication bias is a major concern for meta-analysis. We conducted an abundant evaluation of the studies to avoid selection bias and ensure the quality and credibility of studies. Neither Egger's test nor Begg's test showed evidence of publication bias in these studies. Sensitivity analyses also contributed to the further strength of this meta-analysis. Therefore, our research design is relatively rigorous and the results are credibility.

Although the predictive effect of miR-21 was statistically proved, it had to be interpreted with caution since several limitations should be taken into account. First, there are only 14 studies included in this meta-analysis, with a relatively small sample size. Second, the publications retrieved in our study were limited in English, which might partially cause the publication bias. Third, no abundant miR-21 expression data in global population makes it difficult to set a standard value for the measurement of miR-21. The cut-off values of miR-21 were defined differently in eligible studies, leading to between-study heterogeneity. Fourth, the majority of published studies lacked required data regarding patients' treatment, and these sources of variability could contrib-

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ute to potential selection bias. Fifth, oncogenesis was a complicated process involving in many molecular pathways, we did not analyze the prognostic value of a combination of miR-21 and other miRNAs markers for breast cancer cases.

In conclusion, this meta-analysis clarified that miR-21 over-expression was significantly associated with poor survival in patients with breast cancer. miR-21 might be a novel prognostic biomarker. However, these findings should be considered with caution due to the limitations listed above. More multicenter clinical investigations with larger sample size are still needed to further validate these results.

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Disclosure of conflict of interest

None.

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References

- [1] Van Gemert WA, Lanting CI, Goldbohm RA, van den Brandt PA, Grooters HG, Kampman E, Kiemeny LA, van Leeuwen FE, Monninkhof EM, de Vries E, Peeters PH and Elias SG. The proportion of postmenopausal breast cancer cases in the Netherlands attributable to lifestyle-related risk factors. *Breast Cancer Res Treat* 2015; 152: 155-162.
- [2] Porika M, Malotu N, Veldandi UK, Yadala N and Abbagani S. Evaluation of tumor markers in southern Indian breast cancer patients. *Asian Pac J Cancer Prev* 2010; 11: 157-159.
- [3] Guadagni F, Ferroni P, Carlini S, Mariotti S, Spila A, Aloe S, D'Alessandro R, Carone MD, Cicchetti A, Ricciotti A, Venturo I, Perri P, Di Filippo F, Cognetti F, Botti C and Roselli M. A re-evaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study. *Clin Cancer Res* 2001; 7: 2357-2362.
- [4] Jonas S and Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* 2015; 16: 421-433.
- [5] Kim SJ, Lee CH and Lee SW. Targeting the MicroRNA Passenger Strand for Regulating Therapeutic Transgenes. *Nucleic Acid Ther* 2015; 25: 209-218.
- [6] Gaynullina D, Dweep H, Gloe T, Tarasova OS, Sticht C, Gretz N and Schubert R. Alteration of mRNA and microRNA expression profiles in rat muscular type vasculature in early postnatal development. *Sci Rep* 2015; 5: 11106.
- [7] Chen QY, Jiao DM, Yan L, Wu YQ, Hu HZ, Song J, Yan J, Wu LJ, Xu LQ and Shi JG. Comprehensive gene and microRNA expression profiling reveals miR-206 inhibits MET in lung cancer metastasis. *Mol Biosyst* 2015; 11: 2290-2302.
- [8] Li X, Li D, Zhou W, Chai Y, Yuan R and Xiang Y. A microRNA-activated molecular machine for non-enzymatic target recycling amplification detection of microRNA from cancer cells. *Chem Commun (Camb)* 2015; 51: 11084-11087.
- [9] Liu Y, Cai Q, Bao PP, Su Y, Cai H, Wu J, Ye F, Guo X, Zheng W, Zheng Y and Shu XO. Tumor tissue microRNA expression in association with triple-negative breast cancer outcomes. *Breast Cancer Res Treat* 2015; 152: 183-191.
- [10] Wu J, Li G, Wang Z, Yao Y, Chen R, Pu X and Wang J. Circulating MicroRNA-21 Is a Potential Diagnostic Biomarker in Gastric Cancer. *Dis Markers* 2015; 2015: 435656.
- [11] Erbes T, Hirschfeld M, Rücker G, Jaeger M, Boas J, Iborra S, Mayer S, Gitsch G and Stickeler E. Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker. *BMC Cancer* 2015; 15: 193.
- [12] Marino AL, Evangelista AF, Vieira RA, Macedo T, Kerr LM, Abrahão-Machado LF, Longatto-Filho A, Silveira HC and Marques MM. MicroRNA expression as risk biomarker of breast cancer metastasis: a pilot retrospective case-cohort study. *BMC Cancer* 2014; 14: 739.
- [13] Kawakita A, Yanamoto S, Yamada S, Naruse T, Takahashi H, Kawasaki G and Umeda M. MicroRNA-21 Promotes Oral Cancer Invasion via the Wnt/ β -Catenin Pathway by Targeting DKK2. *Pathol Oncol Res* 2014; 20: 253-261.
- [14] Zaman MS, Shahryari V, Deng G, Thamminana S, Saini S, Majid S, Chang I, Hirata H, Ueno K, Yamamura S, Singh K, Tanaka Y, Tabatabai ZL and Dahiya R. Up-regulation of microRNA-21 correlates with lower kidney cancer survival. *PLoS One* 2012; 7: e31060.
- [15] Gong C, Nie Y, Qu SH, Liao JY, Cui XY, Yao HR, Zeng Y, Su F, Song E and Liu Q. MiR-21 Induces

Prognostic role of miR-21 expression in breast cancer

- Myofibroblast Differentiation and Promotes the Malignant Progression of Breast Phyllodes Tumors. *Mol Cell Pathobiol* 2014; 74: 4341-4352.
- [16] Lee JA, Lee HY, Lee ES, Kim I and Bae JW. Prognostic Implications of MicroRNA-21 Overexpression in Invasive Ductal Carcinomas of the Breast. *J Breast Cancer* 2011; 14: 269-275.
- [17] Qian BY, Katsaros D, Lu LG, Preti M, Durando A, Arisio R, Mu L and Yu H. High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF- β 1. *Breast Cancer Res Treat* 2009; 117: 131-140.
- [18] Radojicic J, Zaravinos A, Vrekoussis T, Kafousi M, Spandidos DA and Stathopoulos EN. MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. *Cell Cycle* 2011; 10: 507-517.
- [19] Beatriz AW, Gabriela GM, Vladimir AV, Mark S and Maria JM. MiR-21 Expression in Pregnancy-Associated Breast Cancer: A Possible Marker of Poor Prognosis. *J Cancer* 2011; 2: 67-75.
- [20] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA and Thacker SB. Meta-analysis of observational studies in epidemiology-A proposal for reporting. *JAMA* 2000; 283: 2008-2012.
- [21] Parmar MKB, Torri V and Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Statist Med* 1998; 17: 2815-2834.
- [22] DerSimonian R. Meta-analysis in the design and monitoring of clinical trials. *Stat Med* 1996; 15: 1237-1248.
- [23] DerSimonian R and Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
- [24] Mantel N and Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 719-748.
- [25] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629-634.
- [26] Anastasov N, Höfig I, Vasconcellos IG, Rappl K, Braselmann H, Ludyga N, Auer G, Aubele M and Atkinson MJ. Radiation resistance due to high expression of miR-21 and G2/M checkpoint arrest in breast cancer cells. *Radiat Oncol* 2012; 7: 206.
- [27] Dong G, Liang X, Wang D, Gao H, Wang L, Wang L, Liu J and Du Z. High expression of miR-21 in triple-negative breast cancers was correlated with a poor prognosis and promoted tumor cell in vitro proliferation. *Med Oncol* 2014; 31: 57.
- [28] MacKenzie TA, Schwartz GN, Calderone HM, Graveel CR, Winn ME, Hostetter G, Wells WA and Sempere LF. Stromal Expression of miR-21 Identifies High-Risk Group in Triple-Negative Breast Cancer. *Am J Pathol* 2014; 184: 3217-3225.
- [29] Athina M, George MY, Efstathios S, Vassilis G and Evi L. Prognostic Significance of Metastasis-Related microRNAs in Early Breast Cancer Patients with a Long Follow-up. *Clin Chem* 2014; 60: 197-205.
- [30] Müller V, Gade S, Steinbach B, Loibl S, von Minckwitz G, Untch M, Schwedler K, Lübke K, Schem C, Fasching PA, Mau C, Pantel K and Schwarzenbach H. Changes in serum levels of miR-21, miR-210, and miR-373 in HER2-positive breast cancer patients undergoing neoadjuvant therapy: a translational research project within the Geparquinto trial. *Breast Cancer Res Treat* 2014; 147: 61-68.
- [31] Ota D, Mimori K, Yokobori T, Iwatsuki M, Kataoka A, Masuda N, Ishii H, Ohno S and Mori M. Identification of recurrence-related microRNAs in the bone marrow of breast cancer patients. *Int J Oncol* 2011; 38: 955-962.
- [32] Alpaslan Özgüna, Bulent Karagoza, Oguz Bilgi, Tolga Tuncel, Huseyin Baloglub, Emin G and Kandemir. MicroRNA-21 as an Indicator of Aggressive Phenotype in Breast Cancer. *Onkologie* 2013; 36: 115-118.
- [33] Wang GN, Wang LZ, Sun SJ, Wu J and Wang QL. Quantitative Measurement of Serum MicroRNA-21 Expression in Relation to Breast Cancer Metastasis in Chinese Females. *Ann Lab Med* 2015; 35: 226-232.
- [34] Yan LY, Huang XF and Shao Q. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA* 2008; 14: 2348-2360.
- [35] Brédart A, Merdy O, Sigal-Zafrani B, Fiszer C, Dolbeault S and Hardouin JB. Identifying trajectory clusters in breast cancer survivors' supportive care needs, psychosocial difficulties, and resources from the completion of primary treatment to 8 months later. *Support Care Cancer* 2016; 24: 357-366.
- [36] Gorini A, Mazzocco K, Gandini S, Munzone S, McVie G and Pravettoni G. Development and psychometric testing of a breast cancer patient-profiling questionnaire. *Breast Cancer (Dove Med Press)* 2015; 7: 133-146.
- [37] Wong IO, Schooling CM, Cowling BJ and Leung GM. Breast cancer incidence and mortality in a transitioning Chinese population: current and future trends. *Br J Cancer* 2015; 112: 167-170.
- [38] Zhang K, Zhao S, Wang Q, Yang HS, Zhu J and Ma R. Identification of microRNAs in Nipple

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- Discharge as Potential Diagnostic Biomarkers for Breast Cancer. *Ann Surg Oncol* 2015; 22 Suppl 3: 536-544.
- [39] Zaharia M and Gómez H. Triple negative breast cancer: a difficult disease to diagnose and treat. *Rev Peru Med Exp Salud Publica* 2013; 30: 649-656.
- [40] Pimentel F, Bonilla P, Ravishankar YG, Contag A, Gopal N, LaCour S, Lee T and Niemz A. Technology in MicroRNA Profiling: Circulating MicroRNAs as Noninvasive Cancer Biomarkers in Breast Cancer. *J Lab Autom* 2015; 20: 574-578.
- [41] Shaker O, Maher M, Nassar Y, Morcos G and Gad Z. Role of microRNAs -29b-2, -155, -197 and -205 as diagnostic biomarkers in serum of breast cancer females. *Gene* 2015; 560: 77-82.
- [42] Wang Y, Zhang Y, Pan C, Ma F and Zhang S. Prediction of Poor Prognosis in Breast Cancer Patients Based on MicroRNA-21 Expression: A Meta-Analysis. *PLoS One* 2015; 10: e0118647.