MicroRNA-21 as a potential diagnostic biomarker for breast cancer patients: a pooled analysis of individual studies

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ABSTRACT

MicroRNA-21 (miR-21) has been reported as the potential novel diagnostic biomarker for breast cancer in several studies, but their results were inconsistent. Therefore, we conducted a systematic analysis to evaluate the diagnostic value of miR-21 in detecting breast cancer. A comprehensive electronic and manual search was conducted for relevant literatures through several databases up to November 9, 2015. QUADAS-2 was used to assess the quality of the studies included in the study. All statistical analyses were performed using Meta-Disc 1.4 and Stata 12.0. Eleven studies with a total of 918 breast cancer patients and 613 controls were included. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with their 95% confidence intervals (CIs) were 0.72 (95% CI: 0.69–0.75), 0.80 (95% CI: 0.77–0.83), 3.37 (95% CI: 2.24–5.07), 0.30 (95% CI: 0.19–0.50), and 11.79 (95% CI: 5.23–26.57), respectively. The area under the curve of SROC was 0.8517. In conclusion, our analyses suggested that miR-21 is a promising biomarker in diagnosing breast cancer. For clinical purpose, further large-scale studies are warranted to validate its clinical application.

INTRODUCTION

Breast cancer is the most common cancer among women worldwide. Although breast cancer incidence rates still increase in many Western countries, mortality rates have been decreasing over the past two decades due to early detection and improved treatment [1]. The data from Surveillance, Epidemiology, and End Results Program (SEER) showed that the 5-year relative survival was 98.6% when diagnosed at localized stage as opposed to 23.3% when the disease at distant stage [2]. Thus, early detection and diagnosis has important clinical significances for breast cancer. The previous studies showed that the circulating tumor biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 153 (CA153) are already applied in clinic, but these biomarkers are not useful to detect early breast cancer due to their low sensitivity and they have long been used as prognostic markers to monitor disease progression or recurrence [3–5].

After the first report of elevated circulating levels of microRNA-21 (miR-21) in patients with diffuse large B-cell lymphoma [6], circulating miRNAs with their stability feature have been postulated as novel biomarkers for cancer processes, such as liver cancer, ovarian cancer, breast cancer [7–9]. Several studies have reported miR-21 as the potential novel diagnostic biomarker for breast cancer, but their results were inconsistent. A recent study suggested that the circulating miR-21 could serve as a potential serum-based biomarker for breast cancer detection in Chinese population, with 80.0% sensitivity and 87.7% specificity [10]. Another study investigated the diagnostic accuracy of single miR-21 and reported a much lower sensitivity with 25.8% [11]. In the Asaga's
study, significant up-regulation of miR-21 was detected, but it could not as candidate in the selection criteria at the microarray level [12]. Therefore, we conducted a systematic analysis to evaluate the diagnostic value of miR-21 in detecting breast cancer.

RESULTS

Included studies

A detailed flowchart of the review process was presented in Figure 1. A total of 504 articles were identified by initial search, with 503 records identified from database searching and 1 record by manual search. Two independent researchers reviewed articles for duplicates, excluding 169 records. After carefully reviewing titles and abstracts of 335 records, as a result, there were 277. Excluded: 248 were reviews, abstract and letters and 29 were not related to our topic, leaving 58 full-text articles for eligibility. Finally, 11 studies from 10 articles were included in this meta-analysis [10–19].

Study characteristics and quality assessment

The main characteristics of included studies were summarized in Table 1. Among the 11 studies, 7 studies were conducted in China [10, 11, 13–27], 1 in USA [12], 2 in Mexico [18], and 1 in Egypt [19]. The publication years ranged from 2011 to 2015. A total of 918 breast cancer patients and 613 controls were included. Circulation miR-21 expression levels were measured in serum (n = 8), tumor tissue (n = 2), and plasma (n = 1). In each study, the cutoff values of miR-21 appeared to be different. The quantitative real-time reverse transcription PCR method was used to measure the expression of miR-21. The sufficient data which were used to construct the 2 × 2 table, such as True positive (TP), false positive (FP), false negative (FN), and true negative (TN), were successfully extracted. The quality assessment of the QUADAS-2 tool was shown in Figure 2. Overall, most studies presented they were of high quality relatively.

Diagnostic accuracy and threshold analysis

Firstly, we conducted analysis of diagnostic threshold to explore whether the threshold effect was existed in this study, which was an important source of heterogeneity. The results showed that there was no heterogeneity from threshold effect with the spearman correlation coefficient of sensitivity and 1-specificity of 0.178 (P = 0.601). Then Cochran-Q and inconsistency index (I^2) were used to measure whether there was heterogeneity from non-threshold effect in order to choose appropriate calculation model. We used the random effects model to calculate those pooled diagnostic parameters for breast cancer. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with their 95% confidence intervals (CIs) were 0.72 (95% CI: 0.69–0.75, Figure 3A), 0.80 (95% CI: 0.77–0.83, Figure 3B), 3.37 (95% CI: 2.24–5.07, Figure 3C), 0.30 (95% CI: 0.19–0.50, Figure 3D), and 11.79 (95% CI: 5.23–26.57, Figure 4), respectively. The area under the curve (AUC) of SROC was 0.8517 (Figure 5).

Meta-regression, subgroup analysis and publication bias

We also performed meta-regression analysis to explore source of heterogeneity based on ethnicity, sample size (≥ 100 vs. < 100), sample source, reference controls, RNA extraction, measurements (Table 2). The results showed that none of the above covariates contributed the heterogeneity (all P > 0.05). Then we conducted subgroup analysis based on those covariates. The results of different subgroups were relatively consistent with the major results, which suggested that our results were relatively credible (Table 3). Moreover, Egger’ test (P = 0.909) or Begg’s test (P = 0.488) was detected and the results showed that there was no significant publication bias in our study.

DISCUSSION

We performed a systematic review to evaluate the diagnostic value of miR-21 as a potential diagnostic biomarker for breast cancer patients. Our finding suggested that the pooled sensitivity, specificity, PLR, NLR and DOR were 0.72 (95% CI: 0.69–0.75), 0.80 (95% CI: 0.77–0.83), 3.37 (95% CI: 2.24–5.07), 0.30 (95% CI: 0.19–0.50) and 11.79 (95% CI: 5.23–26.57), respectively. The AUC of SROC was 0.8517.

Currently, a number of convenient and novel biomarkers have been established in the routine evaluation of breast cancer. Although estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) for predicting the response to endocrine and biological therapies are already available, their performances are far from perfect. For example, there were still some non-responding patients in the assessment of ER and HER2 status [20, 21]. In addition, other molecular biomarkers, such as CEA, cytokeratin fragment (CYFRA 21-1), and neuron specific enolase (NSE), were limited in the clinic with their low sensitivity and specificity [22].

Recently, various studies showed that abnormal expression of miRNAs played an important role in the pathogenesis, metastasis and prognosis for breast cancer [23, 24]. Some studies reported that miR-21 might be as a potential biomarker for breast cancer diagnosis because breast cancer patients had higher serum miR-21 expression than healthy women [25, 26]. In our meta-analysis, the
### Table 1: Main characteristics of included studies

| First author | Year | Country | Ethnicity | Sample size | Cases | Controls | TP | FP | FN | TN | Cut-off value | Sample types | Reference controls | RNA extraction | Measurements |
|--------------|------|---------|-----------|-------------|-------|----------|----|----|----|----|--------------|--------------|------------------|----------------|--------------|
| Li           | 2011 | China   | Asian     | 33          | 49    |           | 17 | 3  | 16 | 46 | 18.32        | Serum        | miR-16           | TRIzol         | SYBR         |
| Asaga        | 2011 | USA     | Caucasian | 102         | 20    |           | 72 | 3  | 30 | 17 | 3.3-dCq      | Serum        | miR-16           | TRIzol         | SYBR         |
| Sun          | 2012 | China   | Asian     | 103         | 55    |           | 77 | 18 | 26 | 37 | 1.358 2^{-\Delta \Delta Ct} | Serum | cel-miR-39 | Filter cartridge | Taqman         |              |
| Wang         | 2012 | China   | Asian     | 50          | 39    |           | 40 | 5  | 10 | 34 | 4.58 2^{-\Delta \Delta Ct} | Serum | miR-16 | TRIzol | SYBR         |
| Mar-Aguilar  | 2013 | Mexico  | Caucasian | 61          | 10    |           | 58 | 2  | 3  | 8  | 6.48 2^{-\Delta \Delta Ct} | Serum | 18S RNA | miRNAeasy kit | Taqman         |              |
| Gao          | 2013 | China   | Asian     | 89          | 55    |           | 78 | 7  | 11 | 48 | 13.22        | Serum        | CA153, CEA | TRIzol | SYBR         |
| Lee          | 2013 | China   | Asian     | 110         | 15    |           | 99 | 4  | 11 | 6  | 2.5 2^{-\Delta \Delta Ct} | Tissue | 18S RNA | TRIzol | SYBR         |
| Ng           | 2013 | China   | Asian     | 170         | 100   |           | 128| 22 | 42 | 78 | 2.34 2^{-\Delta \Delta Ct} | Plasma | miR-145 | TRIzol | Taqman       |
| Li           | 2013 | China   | Asian     | 120         | 200   |           | 31 | 46 | 89 | 154| NA’          | Serum        | CA153, CEA | Roche Elecsys | Taqman         |              |
| Toraih       | 2015 | Egypt   | Caucasian | 30          | 60    |           | 20 | 8  | 10 | 52 | 7.02 2^{-\Delta \Delta Ct} | Serum | RNU6B   | miRNAeasy kit | Taqman         |              |

*Data unavailable.*

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**Figure 1: A detailed flowchart of the review process.**
Table 2: Results of the multivariable meta-regression model for the characteristics with backward regression analysis (Inverse variance weighs)

| Variables          | Coefficient | Standard Error | P      | RDOR | 95% CI       |
|--------------------|-------------|----------------|--------|------|--------------|
| Cte                | 5.451       | 0.7895         | 0.0023 | ---  | ---          |
| S                  | 0.205       | 0.1766         | 0.3095 | ---  | ---          |
| Ethnic             | 0.424       | 0.4500         | 0.3992 | 1.53 | 0.44–5.33    |
| Sample size        | −1.304      | 0.5388         | 0.0519 | 0.27 | 0.08–1.04    |
| Sample types       | −0.243      | 0.2440         | 0.3568 | 0.78 | 0.43–1.42    |
| Reference controls | −0.056      | 0.2244         | 0.8178 | 0.95 | 0.46–1.93    |
| RNA extraction     | −1.131      | 0.5786         | 0.0863 | 0.32 | 0.08–1.23    |
| Measurements       | 2.768       | 1.2297         | 0.0742 | 15.92| 0.67–375.70  |

Figure 2: Risk of bias and applicability concerns graph a review of authors’ judgments about each domain presented as percentages across included studies.

Figure 3: Forest plots of pooled sensitivity (A), specificity (B), positive likelihood ratio (C), and negative likelihood ratio (D) for miR-21 in the diagnosis of breast cancer.
| Subgroup                  | No. of studies (No. of cases) | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | AUC           |
|--------------------------|-------------------------------|----------------------|----------------------|--------------|--------------|--------------|---------------|
| Ethnicity                |                               |                      |                      |              |              |              |               |
| Asian                    | 7 (675)                       | 0.70 (0.66–0.73)     | 0.79 (0.76–0.83)     | 3.27 (1.92–5.56) | 0.33 (0.17–0.62) | 10.64 (3.66–30.97) | 0.8472       |
| Caucasian                | 4 (243)                       | 0.77 (0.72–0.82)     | 0.83 (0.74–0.90)     | 3.65 (2.18–6.11) | 0.29 (0.17–0.49) | 13.73 (5.54–34.04) | 0.8625       |
| Sample size              |                               |                      |                      |              |              |              |               |
| ≥ 100                    | 5 (605)                       | 0.67 (0.63–0.71)     | 0.76 (0.71–0.80)     | 2.32 (1.41–3.82) | 0.38 (0.19–0.75) | 6.31 (2.09–19.00) | 0.7935       |
| < 100                    | 6 (313)                       | 0.80 (0.75–0.84)     | 0.87 (0.82–0.91)     | 4.89 (3.13–7.62) | 0.26 (0.15–0.44) | 20.89 (10.14–43.06) | 0.8948       |
| Sample types             |                               |                      |                      |              |              |              |               |
| Serum                    | 8 (588)                       | 0.67 (0.63–0.71)     | 0.81 (0.77–0.85)     | 3.95 (2.19–7.12) | 0.31 (0.17–0.57) | 13.46 (4.37–41.41) | 0.8865       |
| Tissue                   | 2 (160)                       | 0.86 (0.79–0.91)     | 0.60 (0.36–0.81)     | 2.07 (1.20–3.56) | 0.26 (0.11–0.62) | 8.04 (2.86–22.58) | —            |
| Plasma                   | 1 (170)                       | —                    | —                    | —            | —            | —            | —            |
| Reference control        |                               |                      |                      |              |              |              |               |
| miR-16                   | 3 (185)                       | 0.70 (0.63–0.76)     | 0.90 (0.83–0.95)     | 6.18 (3.51–10.89) | 0.36 (0.24–0.55) | 18.81 (9.06–39.06) | 0.8954       |
| 18S RNA                  | 3 (221)                       | 0.88 (0.83–0.92)     | 0.67 (0.47–0.83)     | 2.37 (1.44–3.89) | 0.17 (0.06–0.46) | 15.09 (3.49–65.19) | 0.5981       |
| CA153, CEA               | 2 (209)                       | 0.82 (0.74–0.89)     | 0.87 (0.79–0.93)     | 5.86 (3.59–9.58) | 0.23 (0.09–0.63) | 15.40 (6.97–92.54) | —            |
| RNA extraction           |                               |                      |                      |              |              |              |               |
| TRIzol                   | 6 (554)                       | 0.78 (0.75–0.82)     | 0.84 (0.79–0.88)     | 4.45 (3.02–6.54) | 0.28 (0.19–0.40) | 17.90 (10.63–30.15) | 0.8800       |
| Others                   | 5 (364)                       | 0.62 (0.56–0.67)     | 0.77 (0.72–0.81)     | 2.36 (1.35–4.13) | 0.36 (0.17–0.77) | 6.84 (1.95–23.97) | 0.8058       |
| Measurements             |                               |                      |                      |              |              |              |               |
| SYBR                     | 5 (384)                       | 0.80 (0.75–0.84)     | 0.87 (0.81–0.92)     | 5.02 (3.09–8.16) | 0.26 (0.16–0.43) | 23.59 (13.66–40.73) | 0.8974       |
| Taqman                   | 6 (534)                       | 0.78 (0.73–0.81)     | 0.77 (0.71–0.82)     | 3.02 (2.17–4.19) | 0.32 (0.22–0.45) | 10.03 (5.50–18.26) | 0.8289       |

Pooled sensitivity and specificity were 0.72 and 0.80, which indicated that the diagnostic accuracy may not be high enough as expected. The results were consistent with the recently published studies by Li et al. and Shen et al. [27, 28]. However, compared with some traditional biomarkers, such as CEA, NSE (with sensitivities of 0.48 and 0.39), miR-21 still had higher diagnostic value in detecting breast cancer. The PLR and NLR were used to estimate the diagnostic accuracy in clinical level. The pooled PLR of 3.37 suggested that breast cancer patients could have about 3.37-fold higher chance of being miR-21 positive compared to healthy controls. The pooled NLR of 0.30 indicated that the possibility of individuals having cancer was 30% if the miR-21 was negative. Moreover, the value of DOR ranged from 0 to infinity, with higher value meaning better test discrimination [29]. The area under curve is another parameter to evaluate the diagnostic value. The ideal SROC curve position is near the upper-left corner which would imply a perfect test [30]. Statistically, if the range of AUC was 0.97 or above which was considered to have excellent accuracy; the range of AUC 0.93–0.96 was considered to be very good; the range of AUC 0.75–0.92 was considered to be good; and a range of AUC less than 0.75 should be cautiously to evaluate the accuracy which might be a random test [31]. Our results of DOR and AUC was
Figure 4: Forest plots of pooled diagnostic odds ratio for miR-21 in the diagnosis of breast cancer.

Figure 5: Summary receiver operating characteristic (SROC) curve for miR-21 in the diagnosis of breast cancer.
miR-21 for breast cancer patients, up to November 9, relevant studies which evaluated the diagnostic value of Embase, Chinese National Knowledge Infrastructure (CNKI), Wan Fang Data, and VIP database to identify miRNA-21 or micro RNA 21 or miRNA-21 or miR-21) and (breast cancer or breast tumor or breast neoplasm or breast carcinoma). Only the most recent or the largest sample size study was included in the final analysis. Publication languages were limited to English and Chinese.

**Study selection**

Studies included in present meta-analyses should meet the following criteria: (1) diagnostic effect about miR-21 for breast cancer; (2) breast cancer was confirmed by pathological examination; (3) the levels of miR-21 in tissue or serum was determined; (4) sensitivity, specificity, and cut-off values can be found in identified studies or calculated from the provided data. While the exclusion criteria were listed as follow: (1) studies without sufficient data to construct the 2 × 2 table; (2) Meta-analyses, reviews, comments, letters, editorial articles, conference abstracts, meeting, and animal and cell studies; (3) publications were identified as duplicates.

**Data extraction**

Two researchers reviewed the abstract first independently and then summarized the full selected articles. Any disagreements were resolved by discussion or consulting the third reviewer. The relevant data were extracted as follow: first author, publication years, country of origin, ethnicity, number of patients and controls, true and false positive and negative, cut-off value, sample types, reference control, RNA extraction, measurements.

**Quality assessment**

Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) [32] was used to assess the quality of the studies included in this meta-analysis independently by the same two researchers. Each of the assessment has seven questions with the answered with “yes”, “no”, or “unclear”. The answer of “yes” means that a study’s risk bias can be judged as low, while “no” and “unclear” mean that the risk of bias can be judged as high.

**Statistical analysis**

Pooled sensitivity, pooled specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and corresponding 95% CIs were calculated to evaluate the diagnostic value of miR-21. Summary receiver operating characteristic which shows the relationship between sensitivity and specificity, was used to evaluate the consistency of results among all studies and the accuracy of the diagnostic test. The Spearman correlation coefficient was used to test the diagnostic threshold effect, which may produce significant heterogeneity ($P < 0.05$). Additionally, the chi-square, Q value and $I^2$ test were used to assess the heterogeneity.
from non-threshold effect. A value of $P$ less than 0.1 or an $I^2 \geq 50\%$ indicated the existence of significant heterogeneity. Meta-regression and subgroup analyses were conducted to explore sources of heterogeneity. Egger’s test [33] and Begg’s test [34] were performed to examine the potential publication bias. All statistical analyses were performed using Meta-Disc 1.4 and Stata 12.0 [35].

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

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