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# Diagnostic and Prognostic Potential of Let-7 miRNAs in Breast Cancer: A Meta-Analysis

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**Abstract**

MicroRNAs (miRNAs), particularly those in the let-7 family, show significant promise as diagnostic and prognostic biomarkers in breast cancer (BC). This meta-analysis systematically evaluates their diagnostic accuracy and prognostic value, addressing inconsistencies across previous studies. We searched PubMed, PMC, ScienceDirect and EMBASE databases for studies assessing let-7 miRNAs in BC. A total of 23 studies involving 8,249 participants were included. For diagnosis, the let-7 miRNA family demonstrated a pooled sensitivity of 0.87 (95 % CI: 0.80 - 0.92) and specificity of 0.76 (95 % CI: 0.62 - 0.86), with a diagnostic odds ratio (DOR) of 36.05 (95 % CI: 17.26 - 75.26) and an area under the curve (AUC) of 0.92 (95 % CI: 0.86 - 0.98). Subgroup analysis revealed that miR-202 in liquid biopsy samples from Asian populations showed the highest diagnostic accuracy (AUC: 0.98). For prognosis, low expression of let-7 miRNAs correlated with poorer survival outcomes, with a pooled hazard ratio (HR) for overall survival (OS) of 0.76 (95 % CI: 0.59 - 0.99). Notably, let-7a and let-7c were associated with aggressive BC subtypes. These findings underscore the let-7 family's potential for BC diagnosis and prognosis, particularly miR-202 for non-invasive detection. However, high heterogeneity across studies was noted due to variations in methods and populations. Future research should validate these findings in diverse populations and standardize detection methods to explore their clinical utility.

**Keywords:** Let-7 miRNAs, Breast cancer, Diagnosis, Prognosis, Meta-analysis, miR-202, Biomarker

## Introduction

Breast cancer (BC) remains the most common cancer among women worldwide and a leading cause of cancer-related mortality. According to the Global Cancer Observatory (GLOBOCAN), statistics show BC accounted for over 680,000 deaths globally in 2020 [1]. Early detection and accurate prognostic evaluation are critical for improving patient outcomes; however, existing diagnostic and prognostic methods often fall short in sensitivity and specificity. Consequently, there is a growing interest is in identifying novel molecular biomarkers to enhance BC management.

MicroRNAs (miRNAs) have emerged as promising candidates due to their critical roles in gene regulation and cancer pathogenesis [2]. Several miRNAs have been explored for their diagnostic and prognostic potential in BC. For instance, miR-21 and miR-155 are well-known oncogenic miRNAs, often overexpressed in BC, and have been studied as potential diagnostic markers. In contrast, the miR-200 family and miR-145 are tumor-suppressor miRNAs, typically downregulated in cancerous tissues [3]. This study emphasizes that the deficiency of the let-7 microRNA family is associated with poor prognosis. The let-7

family stands out among the mentioned miRNAs due to its dual role as both an accurate diagnostic marker in liquid biopsy samples and as a prognostic factor for various invasive cancers [3]. Specifically, numerous studies have shown that the let-7 family acts as a tumor suppressor in cancer biology by targeting oncogenes such as RAS, HMGA2 and LIN28 [3-10]. Some studies have shown elevated levels of let-7 in breast cancer patients [14,16], while others have reported reduced expression in malignant tissues compared to normal controls [11-13]. Besides that, miR-202 is a member of the let-7 family, which has been identified as a promising marker in cancer [15]. The study by Kim *et al.* [17] demonstrated that miR-202 exhibits a dual role as both a tumor suppressor and an oncogene in previous studies. Notably, their research identified miR-202 as a potential biomarker in the Korean population, showing significantly higher expression levels in patients than in healthy individuals. Furthermore, the study revealed a high positive detection rate of miR-202 in early-stage BC. However, a study by Gao *et al.* [18] demonstrated that overexpression of miR-202 has the potential to suppress cell proliferation and metastasis. Additionally, their findings revealed that low expression of miR-202 in breast cancer cells highlights the tumor-suppressive function of this miRNA. These discrepancies emphasize the diverse roles and expression patterns of the let-7 family, depending on the population being studied.

However, most of the previous studies focused on the assessment of individual members of the let-7 family with small sample sizes. As a result, the diagnostic and prognostic roles of the miRNA-let-7 family in breast cancer require validation through a quantitative approach that combines data from multiple studies. This meta-analysis aims to evaluate the diagnostic accuracy and prognostic value of the let-7 miRNA family in BC, contributing to more effective clinical management strategies. Additionally, we will explore miR-202, a member of the let-7 family that has shown particular promise in recent studies as a reliable marker for BC detection, especially in liquid biopsy samples.

## Materials and methods

### Search strategy

We systematically searched 4 major databases - PubMed, PMC, ScienceDirect and EMBASE - using keywords related to the let-7 miRNA family and BC. The search terms included combinations of miRNAs "microRNA-98" or "microRNA-202" or "let-7a" or "miR-let-7b" or "miR-let-7c" or "miR-let-7d" or "miR-let-7e" or "miR-let-7f" or "miR-let-7g" or "miR-let-7i" and "BC" or "breast neoplasm" or "breast carcinoma" or "breast tumor" and "diagnostic" or "prognostic." We limited the search to studies published up to June 2024. We manually searched Google Scholar for additional relevant studies, ensuring comprehensive coverage of the diagnostic and prognostic potential of let-7 miRNAs in BC.

### Inclusion and exclusion criteria

Studies were included if they met the following criteria: (1) Focused on the diagnostic or prognostic value of the let-7 miRNA family in BC; (2) Used histopathology as the reference standard; (3) Employed case-control or cohort designs; (4) Provided data sufficient to calculate sensitivity (SEN), specificity (SPE), diagnostic odds ratio (DOR), or hazard ratio (HR) with confidence intervals (CIs); and (5) Used validated molecular detection methods such as reverse transcription-quantitative PCR (RT-qPCR), next-generation sequencing (NGS), or microarray. Exclusion criteria encompassed review articles, conference abstracts, animal or cell line studies, and studies with incomplete data.

### Data extraction and quality assessment

Two independent reviewers performed data extraction. For diagnostic studies, information was gathered on study characteristics, methods, sample type and diagnostic outcomes (SEN, SPE, positive likelihood ratio (PLR), negative likelihood ratio (NLR, DOR). Prognostic studies focused on HRs for overall survival (OS), and disease-free survival (DFS)/ progression-free survival (PFS), along with sample size and outcome methods. There are 4 key domains in the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) platform: Patient selection, index test, reference standard and flow and timing (**Figure S1**). A study's risk of bias was judged as "yes," "no," or "unclear" answers. The "yes" answer indicates a low risk of bias. If any signaling question is answered "no," it may flag the potential for bias. The "unclear" should only be applied if studies reported insufficient data [19]. Prognostic studies, otherwise, were assessed for risk of

bias by QUIPS (Quality in Prognostic Studies) [20]. The tool was based on many questions in 6 domains: Study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding and statistical analysis and reporting. In a similar way to QUADAS-2, QUIPS was also conducted by answering "yes," "no," or "unclear" for the specific questions of each domain and finally evaluated the quality of prognostic studies (**Figure S2**). All the results for quality assessment were illustrated by Revman.4.1.

### Statistical methods

The diagnostic SEN, SPE, PLR, NLR and DOR estimates with a 95 % CI in each study were captured in a paired forest plot to investigate variance between studies. To assess the overall diagnostic accuracy of the let-7 family in BC, the summary receiver operating characteristic (SROC) curve was used to visualize data points of true-positive rate and false-positive rate in each study, and the value of area under the curve (AUC) was calculated to evaluate the diagnostic efficacy: AUC > 0.90 is excellent, AUC > 0.80 is very good, and AUC < 0.80 is medium [21,22]. For prognostic studies, HR with a 95 % CI was utilized to estimate the association between let-7 and indicators such as OS, PFS, relapse-free survivals (RFS), DFS and event-free survival (EFS).

Statistical heterogeneity is examined based on Cochrane's Q test and inconsistency index $I^2$ values statistic. $I^{2.}$ values range, typically fluctuating from 0 (unobserved heterogeneity) to 100 % (considerable heterogeneity). The existence of significant heterogeneity across studies is indicated if $p < 0.05$ for the Q test and/or $I^2 \geq 50$ % [23], then the random-effect model should be applied for further analysis; otherwise, the fixed-effect model was used [24]. Subgroup analysis was conducted based on 4 categories, including member, ethnicity, sample type and genotyping method, to explore the source of heterogeneity presence. Finally, the Trim-and-fill funnel plots and Egger's regression test were used to assess the potential for publication bias by illustrating a funnel plot, a simple scatter plot using standard error as the y-axis, and log odds ratio as the x-axis. All statistical analyses were performed using R software 4.4.2 [25].

## Results and discussion

### Literature screening

Initially, 1,676 articles were identified, with 535 duplicates removed. After reviewing titles and abstracts, 963 articles were considered, leaving 178 for full-text assessment. Of these, 155 were excluded for various reasons, leaving 23 studies, including 11 for diagnostic accuracy and 12 for prognostic analysis (**Figure 1**). These studies collectively involved 8,249 participants and provided data in the prognostic analysis, including pooled HR for outcomes such as OS and DFS for let-7 miRNAs in breast cancer [5,16,17,26-45].

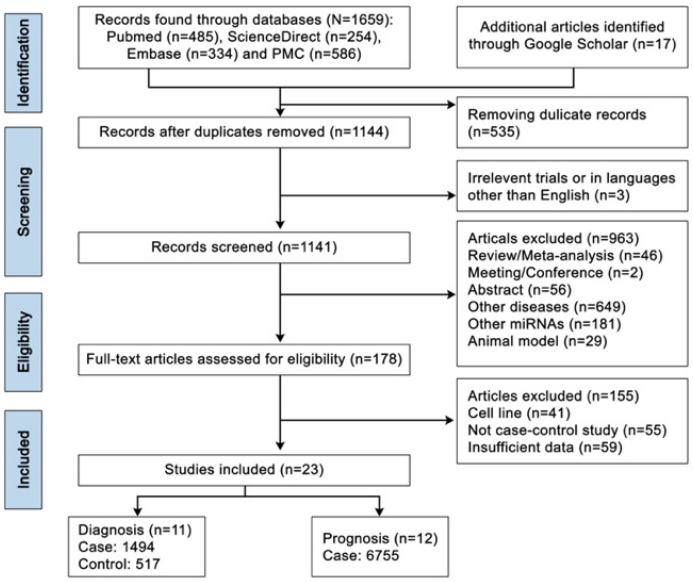


**Figure 1** Flowchart of study selection process for the meta-analysis.

The diagnostic studies varied in sample types and detection methods. Blood samples (serum or plasma) were used in eight studies, while 3 studies analyzed tissue samples. Pooled data for diagnosis accuracy from 11 studies involving patient specimens from 1,494 patients from China, Ireland, Germany, Egypt, Turkey and Korea were collected (**Table 1**). The primary detection method was RT-qPCR, used in 5 studies, with 6 studies employing NGS or microarray. Selected BC patients had different cancer stages (I-IV) at various ages. For prognostic analysis, combined data from 12 studies with 6,755 BC samples from China, the United States, the United Kingdom, Italy, Norway, Egypt, Canada and Greece were included (**Table 2**). All tissue samples were detected by different methods (NGS, RT-PCR and microarray). The differences in methodology may lead to variations in diagnostic and prognostic outcomes, which will be further examined in the subgroup analysis section, where the statistical significance of each contributing factor for heterogeneity will be presented.

**Table 1** Characteristics of studies included in the diagnostic accuracy analysis for Let-7 miRNAs in breast cancer.

| First author (Ref.) | Year | Country | miRNA | Sample size | | Stage | Sample | Test method | Cut-off | Reference gene |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Case | Control | | | | | |
| Heneghan *et al.* [16] | 2010 | Ireland | Let-7a | 83 | 44 | I-IV | Blood | RT-qPCR | Median | miR-16 |
| Lee *et al.* [26] | 2013 | China | Let-7c | 101 | 15 | I-IV | Tissue | Microarray | Median | NA |
| Joosse *et al.* [27] | 2014 | Germany | miR-202 | 68 | 27 | I-III | Blood | RT-qPCR | Median | miR-16 |
| Deng *et al.* [28] | 2014 | China | miR-98 | 98 | 40 | I-III | Tissue | RT-qPCR | 0.084 | U6 |
| Li *et al.* [29] | 2015 | China | Let-7c | 90 | 64 | NR | Blood | RT-qPCR | 374 | 5S rRNA |
| Huang *et al.* [30] | 2018 | China | Let-7a | 128 | 77 | NR | Blood | RT-qPCR | 1.6405 | cel-miR-39 |
| | | | | 30 | 30 | | | | 1.8865 | |
| Ibrahim *et al.* [31] | 2020 | Egypt | Let-7a | 30 | 20 | III | Blood | RT-qPCR | 0.52 | MiR-16 |
| Aksan *et al.* [32] | 2020 | Turkey | Let-7c | 45 | 48 | NR | Blood | RT-qPCR | 1.155 | RNU44 |
| Kim *et al.* [17] | 2020 | Korea | miR-202 | 30 | 30 | I-III | Blood | RT-qPCR | >2.1 | miR-16 |
| Kim [33] | 2021 | Korea | Let-7c | 755 | 86 | I-IV | Tissue | NGS | <4359 | NA |
| Zou *et al.* [34] | 2021 | China | Let-7b | 36 | 36 | I-III | Blood | RT-qPCR | 0.56 | cel-miR-39 |

NA is not available.

**Table 2** Characteristics of studies included in the prognostic value analysis for Let-7 miRNAs in breast cancer.

| First author (ref) | Year | Country | miRNA | N | Subtype | Stage | Sample | Test method | Cut-off | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| Quesne *et al.* [35] | 2012 | UK | Let-7b | 1342 | Luminal | I-IV | Tissue | microarray | Median | PFS |
| Volinia *et al.* [36] | 2012 | Italy | Let-7b/7i/7c | 80 | NA | I-IV | Tissue | NGS | Median | OS |
| Jonsdottir *et al.* [37] | 2012 | Norway | Let-7b | 204 | NA | I-II | Tissue | RT-PCR | > 3.2793 | MFS |
| Markou *et al.* [38] | 2014 | Greece | Let-7a | 84 | NA | I-III | Tissue | RT-PCR | Median | OS/DFS |
| Turashvili *et al.* [39] | 2018 | Canada | Let-7d | 51 | TNBC | I-IV | Tissue | RT-PCR | Median | OS/DFS |
| Guo *et al.* [40] | 2018 | China | Let-7a | 79 | NA | I-IV | Tissue | RT-PCR | Median | PFS |

| First author (ref) | Year | Country | miRNA | N | Subtype | Stage | Sample | Test method | Cut-off | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| Geng *et al.* [41] | 2019 | China | Let-7a/7b/7c/7e/7f/7g/7i/miR-98/202 | 1262 | NA | I-III | Tissue | microarray | Median | OS |
| Fahim *et al.* [42] | 2020 | Egypt | Let-7b | 157 | Luminal | | | microarray | Median | OS |
| Zaka *et al.* [43] | 2020 | UK | Let-7b/7c | 94 | TNBC | I-III | | microarray | Median | MFS |
| Song *et al.* [5] | 2020 | China | Let-7a/c/f | | NA | I-III | Tissue | microarray | Median | OS |
| Zhang *et al.* [44] | 2021 | China | miR-98 | 1097 | NA | I-IV | Tissue | RT-PCR | Median | OS |
| Fuso *et al.* [45] | 2021 | Italy | Let-7a | 200 | NA | I-IV | Tissue | NGS | Median | OS/EFS |
| | | | | 21 | HER2+ | | | | Median | OS/EFS |

NA not available, OS overall survival, EFS Event-free survival, MFS Metastasis-free survival, DFS Disease-free survival, PFS Progression-free survival.

The quality of diagnostic studies was assessed using the QUADAS-2 tool in Revman.4.1 with 72 % of studies showing a low risk of bias in patient selection and 100 % showing a low risk in the index test domain. For prognostic studies, the QUIPS tool was used, with 75 % of studies showing low bias. The quality ratings emphasize the importance of standardized methods and minimizing bias in future research to strengthen the evidence on the diagnostic and prognostic value of let-7 miRNAs in breast cancer (**Figures S1** and **S2**).

**Diagnostic accuracy**

The diagnostic efficacy of the let-7 miRNA family was assessed in 11 studies with 1,494 BC patients and 517 controls. $I^2$ values for SEN and SPE were 89 and 76 %, indicating significant heterogeneity among the studies. A random-effects model was used to account for this. The pooled SEN was 0.82 (95 % CI: 0.72 - 0.89), meaning that the test correctly identified 82 % of BC patients, demonstrating high effectiveness in detecting true positives. The pooled SPE was 0.90 (95 % CI: 0.79 - 0.95), indicating that 90 % of non-cancer individuals were correctly identified as disease-free (**Figure 2**). In this meta-analysis, the area under SROC was 0.92 (95 % CI: 0.86 - 0.96) (**Figure 3**), suggesting an excellent diagnostic accuracy of let-7 miRNAs for BC diagnosis and the ability to discriminate between BC and non-cancer individuals.

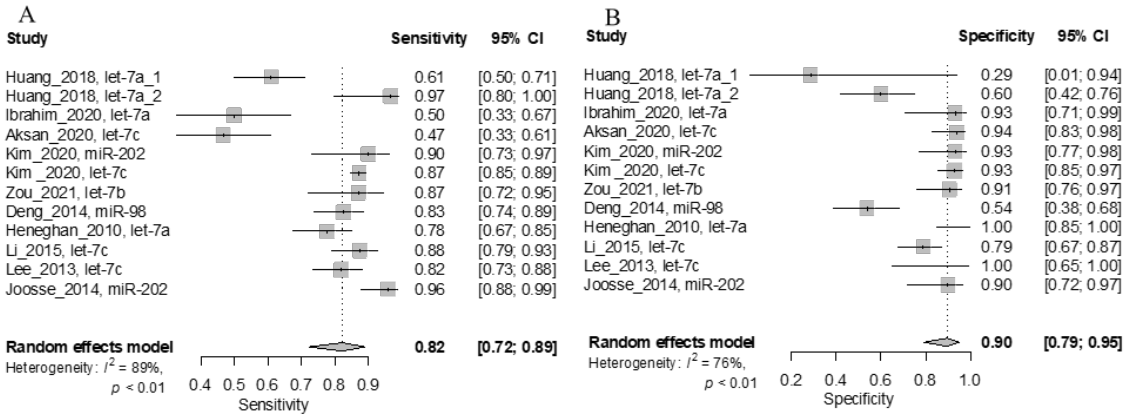


**Figure 2** Forest plots of sensitivity and specificity for Let-7 miRNAs in breast cancer diagnosis, (A) The pooled sensitivity and (B) The pooled specificity.

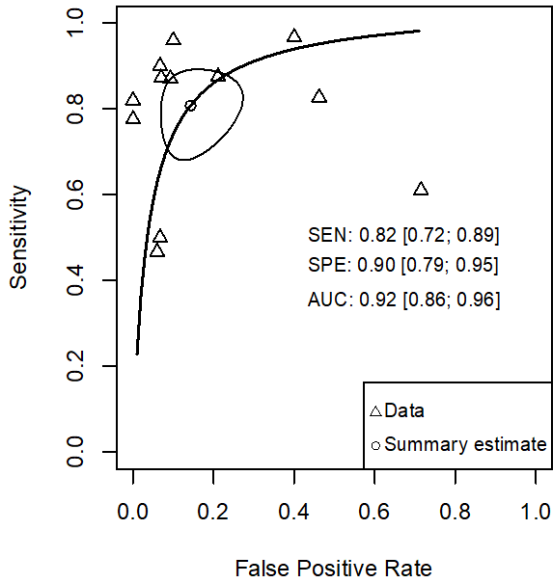


**Figure 3** Summary receiver operating characteristic (SROC) curve of Let-7 miRNAs in BC diagnosis. SEN: Sensitivity, SPE: Specificity, AUC: under the curve.

To further analyze the factors influencing the heterogeneity in the diagnostic value of let-7 miRNAs in BC. Subgroup analysis was conducted based on 4 categories, including member, ethnicity, sample type and genotyping method. The results from meta-regression based on the DOR demonstrated the varying expressions of miRNA members might be a factor contributing to heterogeneity ($p$ = 0.019). In addition, the data from ethnicity and method subgroups also showed that let-7 miRNAs being detected in Asians by RT-PCR technique had higher diagnostic performance than in Caucasians by other detection methods. The differences in these subgroups, however, were not significantly different ($p$ > 0.05). In the reporter dye group, Meta-regression showed that the Taqman probe was shown a higher value than SYBR green, which might contribute to heterogeneity ($p$ = 0.002) (**Table 3**).

Concerning miRNA members, miR-202 had the highest diagnosis accuracy value among others, with an AUC of 0.98 (95 % CI: 0.89 - 1.06) (**Table 3**). The diagnostic value of miR-202 is notably higher, with a specificity of 0.94, compared to the specificity (73.1 %) of biomarkers such as CA 15-3 and CEA in monitoring breast cancer recurrence [46]. Thus, its high diagnostic potential positions mi-202 as a valuable addition to the repertoire of molecular markers that could improve clinical outcomes, especially in cases where traditional biomarkers might not provide sufficient sensitivity or specificity.

**Table 3** Subgroup analysis of diagnostic accuracy for let-7 miRNAs in breast cancer.

| Subgroup | | Diagnostic accuracy | | | | | | Meta-regression | |
|---|---|---|---|---|---|---|---|---|---|
| | | SEN (95 % CI) | SPE (95 % CI) | PLR (95 % CI) | NLR (95 % CI) | DOR (95 % CI) | AUC (95 % CI) | $R^2$ (%) | *p*-value |
| Member | Let-7a | 0.71 (0.64 - 0.76) | 0.89 (0.42 - 0.99) | 2.66 (1.4 - 5.06) | 0.2 (0.08 - 0.46) | 26.45 (9.82 - 71.19) | 0.87 (0.42 - 2.66) | 65.6 | 0.019 |
| | Let-7c | 0.85 (0.83 - 0.87) | 0.92 (0.81 - 0.96) | 7.07 (3.53 - 14.16) | 0.22 (0.10 - 0.48) | 36.04 (13.16 - 98.75) | 0.77 (0.5 - 1.22) | | |
| | miR-202 | 0.94 (0.87 - 0.97) | 0.92 (0.81 - 0.97) | 12.50 (4.87 - 32.10) | 0.06 (0.02 - 0.20) | 202.98 (51.62 - 798.08) | 0.98 (0.89 - 1.06) | | |
| Sample | Blood | 0.82 (0.68 - 0.91) | 0.85 (0.72 - 0.92) | 4.97 (2.81 - 8.79) | 0.18 (0.11 - 0.32) | 34.69 (18.26 - 65.88) | 0.92 (0.86 - 0.99) | 0 | 0.613 |
| | Tissue | 0.81 (0.75 - 0.86) | 0.99 (0.64 - 1.00) | 6.29 (1.19 - 33.20) | 0.2 (0.12 - 0.32) | 33.63 (3.53 - 320.64) | 0.91 (0.48 - 7.66) | | |

| Subgroup | | Diagnostic accuracy | | | | | | Meta-regression | |
|---|---|---|---|---|---|---|---|---|---|
| | | SEN (95 % CI) | SPE (95 % CI) | PLR (95 % CI) | NLR (95 % CI) | DOR (95 % CI) | AUC (95 % CI) | $R^2$ (%) | *p*-value |
| Test method | RT-PCR | 0.82 (0.69 - 0.90) | 0.87 (0.75 - 0.94) | 3.80 (2.50 - 5.75) | 0.17 (0.10 - 0.30) | 29.64 (13.69 - 64.18) | 0.91 (0.85 - 0.98) | 8.24 | 0.265 |
| | NonRT-PCR | 0.87 (0.84 - 0.89) | 0.94 (0.87 - 0.97) | 12.33 (6.04 - 25.2) | 0.15 (0.11 - 0.2) | 87.87 (39.69 - 194.52) | 0.83 (65.28 - 0.04) | | |
| Measurement method | SYBR green | 0.83 (0.73 - 0.91) | 0.69 (0.54 - 0.8) | 3.09 (1.99 - 4.8) | 0.24 (0.13 - 0.41) | 19.2 (9.51 - 38.75) | 0.89 (0.81 - 0.98) | 65.89 | 0.002 |
| | Taqman | 0.86 (0.76 - 0.93) | 0.95 (0.82 - 0.99) | 12.06 (5.41 - 26.89) | 0.12 (0.05 - 0.31) | 159.052 (50.96 - 496.44) | 0.97 (0.94 - 0.99) | | |
| Ethnicity | Asian | 0.80 (0.71 - 0.87) | 0.91 (0.79 - 0.97) | 3.98 (2.38 - 6.67) | 0.16 (0.12 - 0.22) | 33.14 (14.30 - 76.79) | 0.97 (0.94 - 0.99) | 0 | 0.751 |
| | Caucasian | 0.90 (0.55 - 0.98) | 0.84 (0.60 - 0.95) | 5.27 (3.45 - 8.04) | 0.22 (0.09 - 0.55) | 50.22 (8.02 - 314.56) | 0.87 (0.79 - 0.98) | | |

SEN Sensitivity, SPE Specificity, PLR positive likelihood ratio, NLR negative likelihood ratio, DOR diagnostic odds ratio, AUC area under the curve.

**Prognostic value**

In 12 studies involving 6,755 BC patients, higher let-7 miRNA expression was associated with improved survival (**Table 4**). The pooled HR for OS was 0.76, indicating a 24 % reduced risk of death (**Figure 4**). For DFS/PFS, the pooled HR was 0.87, showing that increased let-7 expression correlates with a lower risk of recurrence or progression. High heterogeneity across studies was observed ($I^2$ values of 98.66 % for OS and 96.35 % for DFS/PFS), suggesting variability due to factors such as study populations and detection methods (**Figure 5**).

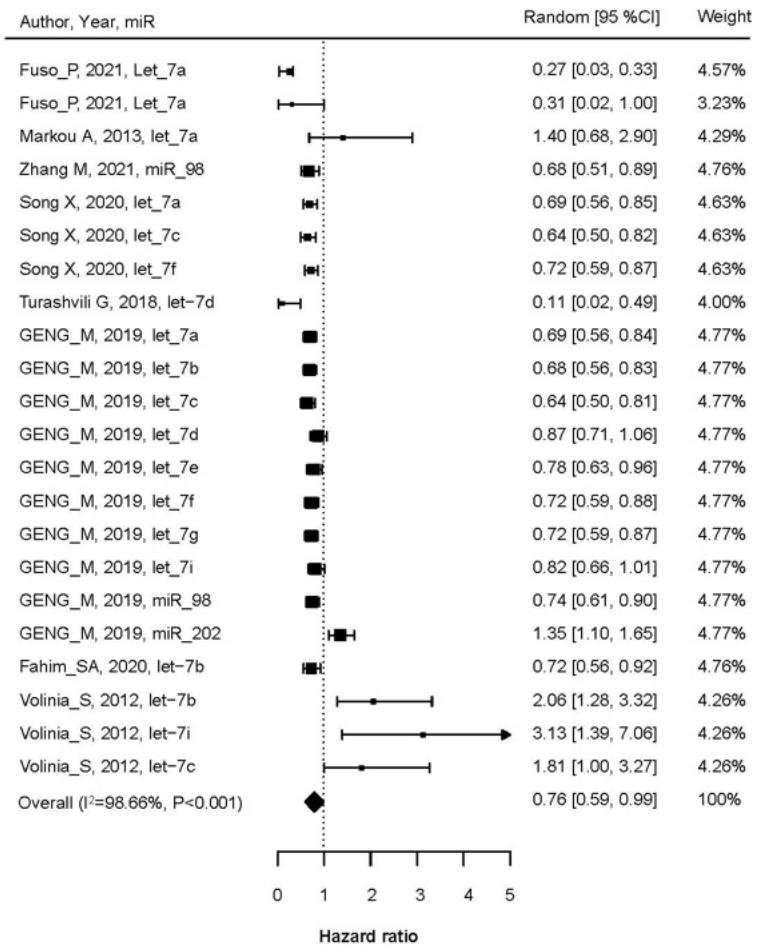


**Figure 4** Forest plot for overall survival (OS) outcomes of Let-7 family in breast cancer patients.

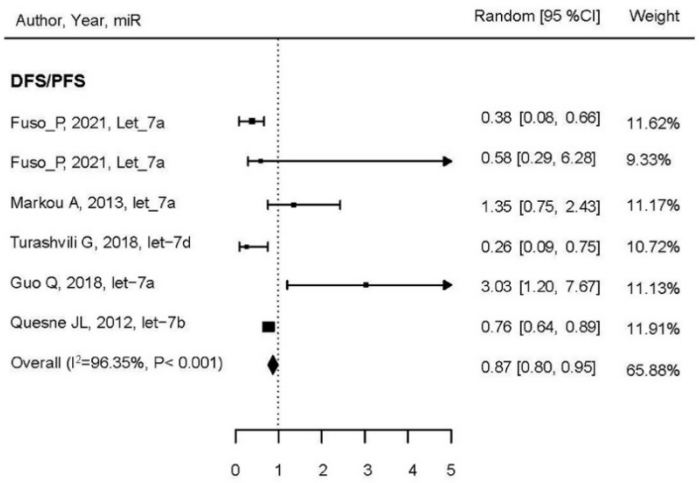


**Figure 5** Forest plot for disease-free survival (DFS)/ progression-free survival (PFS) outcomes of Let-7 family in breast cancer patients.

**Table 4** summarizes the subgroup analysis, showing that let-7a and let-7c were significantly associated with poorer OS and DFS/PFS, suggesting they could be prognostic markers for aggressive breast cancer. Let-7 miRNA's prognostic value also varied by BC subtype, with stronger associations observed in luminal and triple-negative BC subtypes. This supports the potential of using let-7 family members as specific prognostic markers, allowing for personalized treatment strategies based on miRNA expression profiles in different BC subtypes. The ethnicity-stratified analysis found significant differences in the prognostic value of let-7 miRNAs between Asian and Caucasian populations, possibly due to genetic or environmental factors (**Table 4**). While let-7 miRNAs show promise as prognostic markers globally, further validation in diverse populations is necessary.

**Table 4** Subgroup analysis of prognostic value for let-7 miRNAs in breast cancer.

| Subgroup | | OS | | | DFS/PFS | | |
|---|---|---|---|---|---|---|---|
| | | No. of studies | Pooled HR (95 % CI) | Meta-regression | No. of studies | Pooled HR (95 % CI) | Meta-regression |
| Member | Let-7a | 5 | 0.63 (0.58 - 0.70) | 0.32 | 4 | 0.55 (0.44 - 0.69) | 0.33 |
| | Let-7b | 3 | 0.72 (0.67 - 0.79) | | 1 | 0.76 (0.64 - 0.89) | |
| | Let-7c | 3 | 0.67 (0.61 - 0.74) | | 1 | 0.26 (0.09 - 0.75) | |
| | Let-7d | 1 | 0.87 (0.71 - 1.06) | | 0 | NA | |
| | Let-7e | 1 | 0.78 (0.63 - 0.96) | | 0 | NA | |
| | Let-7f | 2 | 0.72 (0.65 - 0.80) | | 0 | NA | |
| | Let-7g | 1 | 0.72 (0.59 - 0.87) | | 0 | NA | |
| | Let-7i | 2 | 0.89 (0.80 - 0.99) | | 0 | NA | |
| | miR-98 | 2 | 0.71 (0.66 - 0.77) | | 0 | NA | |
| | miR-202 | 1 | 1.35 (1.10 - 1.65) | | 0 | NA | |

| Subgroup | | OS | | | DFS/PFS | | |
|---|---|---|---|---|---|---|---|
| | | No. of studies | Pooled HR (95 % CI) | Meta-regression | No. of studies | Pooled HR (95 % CI) | Meta-regression |
| Subtype | NA | 19 | 0.77 (0.75 - 0.80) | 0.95 | 3 | 0.80 (0.65 - 0.98) | 0.98 |
| | Luminal | 1 | 0.72 (0.56 - 0.92) | | 1 | 0.76 (0.64 - 0.89) | |
| | HER2+ | 1 | 0.31 (0.02 - 1.00) | | 1 | 0.58 (0.29 - 6.28) | |
| | TNBC | 1 | 0.10 (0.23 - 0.49) | | 1 | 0.26 (0.09 - 0.75) | |
| Test method | RT-qPCR | 3 | 0.66 (0.59 - 0.74) | 0.35 | 3 | 0.76 (0.68 - 0.84) | 0.21 |
| | NGS | 2 | 0.82 (0.69 - 0.99) | | 1 | 0.39 (0.30 - 0.51) | |
| | Microarray | 3 | 0.77 (0.75 - 0.80) | | 1 | 3.03 (1.20 - 7.67) | |
| Ethnicity | Asian | 3 | 0.77 (0.74 - 0.79) | 0.91 | 1 | 3.03 (1.20 - 7.67) | 0.07 |
| | Caucasian | 5 | 0.73 (0.66 - 0.80) | | 4 | 0.70 (0.63 - 0.76) | |

NA not available, OS overall survival, DFS Disease-free survival, PFS Progression-free survival unduly influenced by selective reporting of positive results.

**Publication bias in diagnostic and prognostic studies**

We assessed publication bias using trim-and-fill funnel plots and Egger's regression test. In our analysis, the funnel plot appeared symmetrical before and after the trim-and-fill adjustments, Egger's test (*p*-value of 0.28) for prognostic studies, indicating no publication bias in these studies (**Figure 6(B)**). However, publication bias was observed in our diagnostic dataset with an Egger's test showing a *p*-value < 0.01 (**Figure 6(A)**). While prognostic findings are robust, the presence of publication bias in our diagnostic studies underscores the need for further validation in independent, high-quality cohorts. Several factors can lead to publication bias, including a preference for positive results, language and geographical bias and journal requirements. To reduce publication bias, strategies should include encouraging the publication of negative results, promoting open access to publicly available research, and increasing the capacity for publishing in multiple languages.

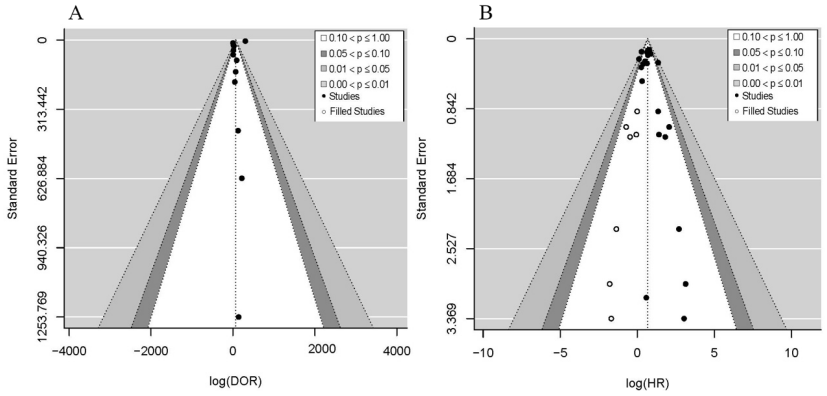


**Figure 6** Funnel plot for the detection of publication bias in diagnostic and prognostic studies, (A) diagnostic studies and (B) prognostic studies.

Overall, the findings remain strong and provide relevant, cross-platform information regarding the let-7 family's diagnostic and prognostic significance in BC. Future research should focus on conducting larger, multicenter studies to validate the diagnostic and prognostic value of let-7 miRNAs, particularly in diverse populations. To enhance reliability, studies should adopt a unified analytical method, focusing on each member separately or performing subgroup analyses to strengthen the clinical applicability of the let-7 as a biomarker.

## Conclusions

This meta-analysis highlights the diagnostic and prognostic potential of the let-7 miRNA family in breast cancer, with miR-202 showing exceptional promise as a non-invasive biomarker. let-7a and let-7c are also significant for identifying aggressive subtypes. Despite limitations such as high heterogeneity and publication bias in prognostic studies, these findings underscore the value of let-7 miRNAs in breast cancer management. Future research should focus on validating these findings in diverse populations and s standardizing detection methods to enhance clinical applicability.

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## Supplementary material

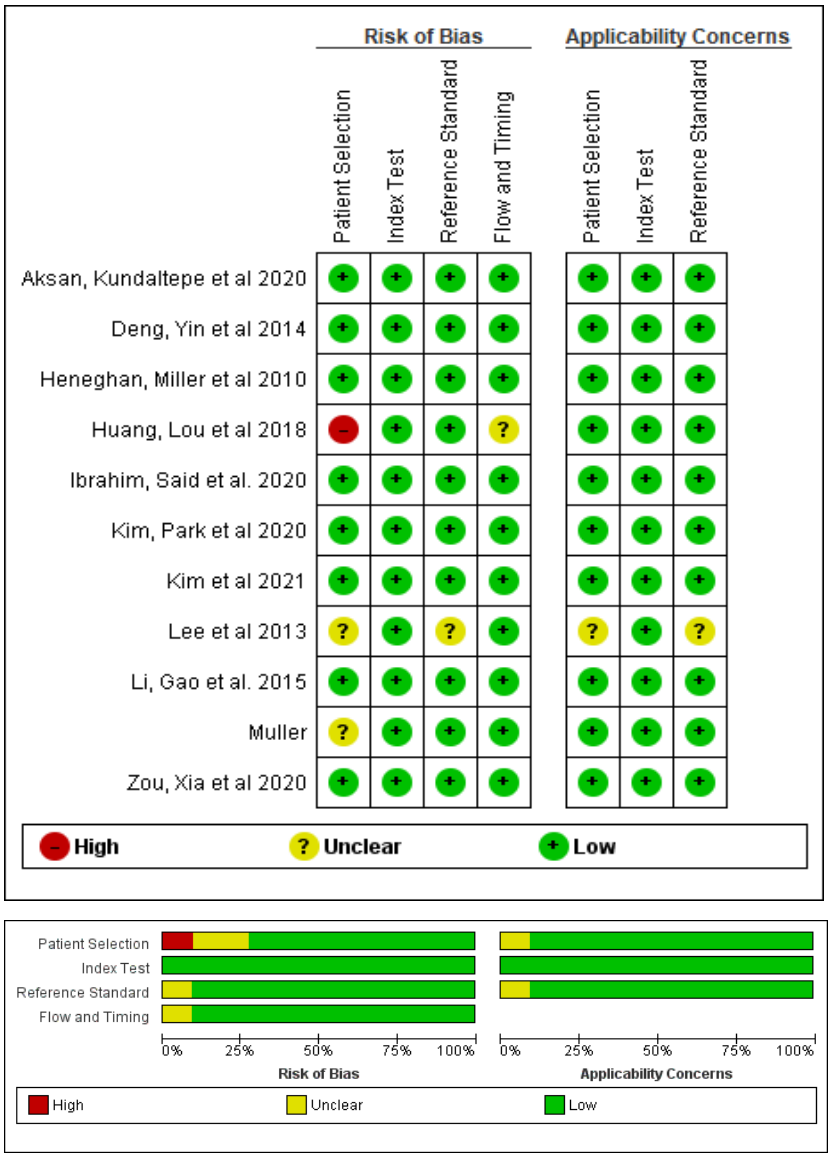


**Figure S1** Quality assessment for diagnosis studies using QUADAS-2.

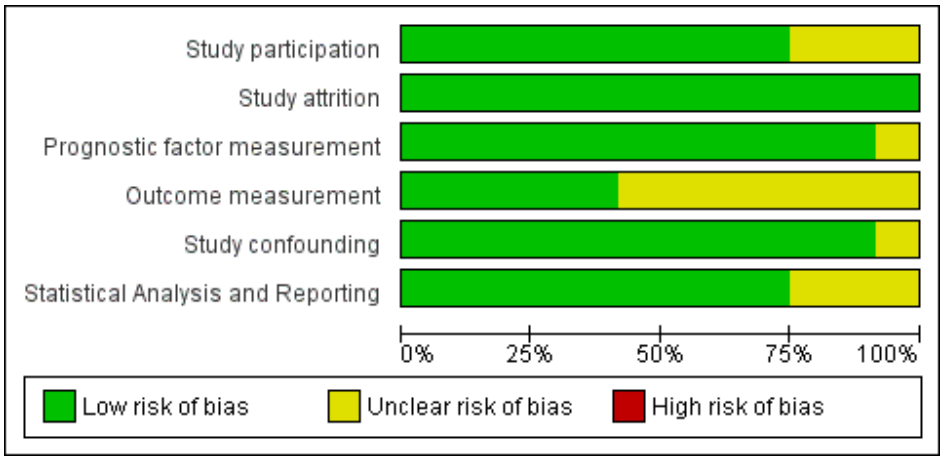


**Figure S2** Quality assessment of prognostic studies using QUIPS.