Diagnostic and prognostic value of microRNAs in cholangiocarcinoma: a systematic review and meta-analysis

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Background and aim: Several dysregulated microRNAs (miRNAs) have been implicated in the pathogenesis of cholangiocarcinoma (CCA); however, small sample sizes and invariable research designs are limitations, hindering a thorough analysis of miRNAs as diagnostic and prognostic tools for CCA. This study aimed to systematically summarize the clinical value of miRNAs in human CCA both for all available miRNAs and single miRNA with multiple researches.

Methods: Pooled parameters included the area under the curve (AUC), sensitivity, specificity, and hazard ratios (HRs) to separately determine overall diagnostic and prognostic performance. Subgroup and sensitivity analyses were performed only in the event of heterogeneity. Thirty-four studies including 12 diagnostic studies and 22 prognostic studies were eligible for inclusion in this meta-analysis.

Results: We observed that miR-21, miR-26, miR-483, miR-106a, miR-150, miR-192, and miR-194 were employed for distinguishing patients with CCA from healthy controls. Pooled sensitivity, specificity, and AUC were 0.82 (95% confidence interval [CI] 0.77–0.86), 0.83 (95% CI 0.75–0.89), and 0.88 (95% CI 0.85–0.91), respectively. Abnormal expression of miR-21, miR-26a, miR-192, miR-200c, miR-221, miR-29a, miR-191, miR-181c, miR-34a, miR-106a, miR-203, and miR-373 in patients was confirmed to associate with poor survival rate. Pooled HRs and 95% CIs were calculated using STATA, resulting in the pooled HR of 1.47 (95% CI 0.91–2.37) for overall survival (OS), 0.67 (95% CI 0.16–2.81) for disease-free survival (DFS), 2.31 (95% CI 1.59–3.36) for progression-free survival (PFS), and 2.68 (95% CI 0.88–8.15) for relapse-free survival (RFS). Thus, CCA patients with dysregulated miRNA expression were confirmed to have shorter OS, DFS, PFS, and RFS. Data regarding the diagnostic and prognostic roles of miR-21 suggested pooled diagnostic results of miR-21 for sensitivity, specificity, and AUC were 0.85 (95% CI 0.76–0.91), 0.92 (95% CI 0.81–0.97), and 0.93 (95% CI 0.91–0.95), respectively, suggesting better diagnostic performance of miR-21 compared with other miRNAs. Meanwhile, pooled prognostic result of miR-21 for HR was 1.88 (95% CI 1.41–2.51), indicating miR-21 could more appropriately predict shorter OS in patients with CCA.

Conclusion: miRNAs may provide a new approach for clinical application, and miR-21 may be a promising biomarker for diagnosis and prognosis of CCA.

Keywords: microRNAs, miR-21, diagnosis, prognosis, cholangiocarcinoma, meta-analysis

Introduction

Cholangiocarcinoma (CCA) is a rare biliary malignancy prone to lymphatic metastasis. CCA is increasingly common, and currently, it is the most frequent primary hepatic malignancy.¹² In some Asian countries, the elevated incidence of CCA appears to correlate with liver fluke Opisthorchis viverrini (Ov) infections. Liver fluke infections
subsequently result in chronic inflammation of the biliary tree and malignant transformation.\textsuperscript{3,4} Although the accuracy of current diagnostic methods for cancer has greatly improved, most patients with CCA are diagnosed at an unresectable stage of the disease, leading to a 5-year overall survival (OS) of <30\%.\textsuperscript{5} There are currently only few diagnostic techniques for detecting early-stage CCA, and also, biomarker studies available are limited;\textsuperscript{6,7} as such, novel biomarkers for diagnosis and prognosis are needed.\textsuperscript{8}

MicroRNAs (miRNAs) are endogenous small (~22 nt) noncoding RNAs that contribute to cell fate determination, proliferation, and cell death.\textsuperscript{9–11} Several studies indicate that miRNAs can be stably expressed in human plasma and serum, and miRNAs have also been demonstrated to be abnormally expressed in the circulatory system during tumorigenesis and inflammation.\textsuperscript{12,13} Kishimoto et al\textsuperscript{14} found that miR-21 was overexpressed in bile duct cancer (accuracy, 0.89; sensitivity, 84%; and specificity, 98%), indicating that it may be used as a biomarker for distinguishing bile duct cancer patients from normal subjects. Silakit et al\textsuperscript{15} confirmed high expression of miR-192 in sera of CCA patients, which predicted worse OS compared with those with low miR-192 expression. CA19-9 has been frequently used to diagnose and predict CCA patient outcomes.\textsuperscript{16} However, low sensitivity or low specificity prevents CA19-9 from being adopted globally.\textsuperscript{17} Therefore, identifying miRNAs unique to CCA may provide suitable biomarkers for detection and prognosis.

**Methods**

**Search strategy**

We searched PubMed, EMBASE, and the Web of Science for studies of associations between miRNAs and CCA patients. We used these search terms: (microRNA OR miRNA OR miR) and (cholangiocarcinoma OR bile duct cancer) and (diagnosis OR prognosis). We screened titles, abstracts, author information, and results. Studies were included if they focused on miRNAs in CCA patients identified by diagnosis of histopathological confirmation, provided information about the relationship between miRNAs and diagnosis (summary receiver operating characteristic [SROC] curves or sensitivity/specificity) or prognosis (hazard ratio [HR] with 95% confidence interval [CI] or Kaplan–Meier curves), included healthy individuals as controls, and had clearly defined miRNA thresholds. Studies were excluded if they met the following criteria: reviews, letters, commentaries, or meeting records; duplicate publications; used cell lines; combined two or more miRNAs to calculate overall sensitivity/specificity or HR; and lacked sufficient data to evaluate HR and 95% CIs. Three individual investigators screened and evaluated retrieved articles. Disagreement was resolved with detailed discussion.

**Quality assessment**

This meta-analysis had diagnostic and prognostic components, so the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) and the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) were used to evaluate diagnostic and prognostic accuracy of eligible studies.\textsuperscript{18,19} If studies did not meet these criteria, they were excluded. Bias risk and applicability concerns are detailed in Figure 1.

**Data extraction**

Two investigators independently examined each eligible study and its data using a standard protocol. We assessed first author, year of publication, study design, subject number, tumor stage, method of quantifying miRNA expression, and miRNA thresholds to stratify high- and low-subject groups. For diagnostic studies, sensitivity, specificity, true positives (TPs), false positives (FPs), false negatives (FN), and true negatives (TN) were included. For prognostic studies, HRs for OS or disease-free survival (DFS) or progression-free survival (PFS) or relapse-free survival (RFS), and 95% CIs and p values were extracted. If HR and relevant parameters

![Figure 1](https://www.dovepress.com/207.241.231.83) A graph of risk of bias and applicability concerns: a review of authors’ judgments about each domain is presented as percentages across included studies.
were not available, we estimated HRs and 95% CIs using the methods of Parmar et al.20

Statistical analysis
For diagnostic analysis, TPs, FPs, FNs, and TNs were extracted from included studies or recalculated based on the prevalence and sample size of each study. A bivariate model was used to calculate pooled sensitivity, specificity, positive and negative likelihood ratios (LRs), diagnostic odds ratios (DORs), and their 95% CIs.21 SROC curves were plotted by pooling sensitivity and specificity of each study to evaluate diagnostic effects, along with auto-generation of AUCs of SROC curves and the maximum point of intersection between sensitivity and specificity (Q value). Heterogeneity was examined using an F test ($p < 0.1$ or $F > 50\%$ indicated significant heterogeneity).22 Subgroup analysis was performed to identify potential heterogeneity sources, and Deeks’ funnel plot was used to inspect publication bias ($p < 0.1$ indicated statistically significant publication bias).

For prognostic analysis, multivariate analysis was employed to avoid confounding of exposure effects.23 Heterogeneity assessment of combined HRs was performed using Cochran’s Q test and Higgins F statistic ($p < 0.10$ and/or $F > 50\%$ meant significant heterogeneity and the need for a fixed model). Otherwise, a random-effects model was used, and subgroup analysis was conducted to identify the source of heterogeneity. An observed HR $> 1$ indicated elevations of miRNA were detrimental and correlated with worse survival, but HR $< 1$ suggested lows of miRNA were harmful and related to poor survival. Furthermore, Begg’s funnel plots and Egger’s linear regression tests were used to evaluate publication bias.24 All analyses was performed with Stata 12.0 (Stata Corporation, College Station, TX, USA).

Results
As shown in Figure 2, a comprehensive search was performed on 30/04/2017, yielding a total of 373 results from the PubMed, EMBASE, and Web of Science. Among these results, 275 articles were identified as duplicates and irrelevant studies including reviews, textbooks, letters, or animal research after the screening of titles and abstract. The remaining 98 articles meeting the inclusion criteria were further thoroughly assessed. Finally, we found that 64 articles lacked sufficient data to allow for a meta-analysis or were not relevant to diagnoses or prognoses. Therefore, 28 articles containing 34 studies (including 12 for diagnostic analysis and 22 for prognostic analysis) focusing on the relationship between miRNAs and CCA were utilized for the final analysis. The main characteristics of each article are summarized in Tables 1 and 2.

Figure 2 A flowchart of literature search and study selection. Twenty-three case–control studies including 9 studies for diagnoses and 14 studies for prognoses were included in this meta-analysis.
Table 1 Main characteristics of the diagnostic studies included in the meta-analysis

| Study            | Year | miR | Country                | Race     | Specimen | Sample size | Sensitivity | Specificity | AUC | TP  | FP  | FN  | TN  | RNA extraction | Measurements |
|------------------|------|-----|------------------------|----------|----------|-------------|-------------|-------------|-----|-----|-----|-----|-----|----------------|--------------|
| Wang et al<sup>10</sup> | 2015 | 26a | People's Republic of China | Asian    | Serum    | 66          | 84.80%      | 81.80%      | 0.899 | 56  | 12  | 10  | 54  | Qiagen miRNeasy kit | TaqMan       |
| Wang et al<sup>20</sup> | 2015 | 150 | People's Republic of China | Asian    | Plasma   | 15          | 80.60%      | 58.10%      | 0.764 | 13  | 3   | 2   | 12  | Qiagen miRNeasy kit | SYBR         |
| Silakit et al<sup>17</sup> | 2015 | 192 | Thailand               | Asian    | Urine    | 22          | 63.60%      | 66.70%      | 0.682 | 14  | 7   | 8   | 14  | Qiagen miRNeasy kit | TaqMan       |
| Silakit et al<sup>17</sup> | 2015 | 21  | Thailand               | Asian    | Urine    | 22          | 63.60%      | 71.40%      | 0.682 | 14  | 6   | 8   | 15  | Qiagen miRNeasy kit | TaqMan       |
| Wang et al<sup>38</sup>  | 2015 | 21  | People's Republic of China | Asian    | Serum    | 74          | 87.80%      | 90.50%      | 0.908 | 65  | 7   | 9   | 67  | Qiagen miRNeasy kit | TaqMan       |
| Kishimoto et al<sup>14</sup> | 2013 | 21  | Japan                  | Asian    | Plasma   | 94          | 85.10%      | 100.00%     | 0.930 | 80  | 1   | 14  | 49  | miRVana PARIS kit | TaqMan       |
| Cheng et al<sup>17</sup> | 2015 | 106a | People's Republic of China | Asian    | Serum    | 103         | 81.60%      | 85.00%      | 0.890 | 84  | 3   | 19  | 17  | Qiagen miRNeasy kit | TaqMan       |
| Silakit et al<sup>17</sup> | 2014 | 192 | Thailand               | Asian    | Serum    | 51          | 74.00%      | 72.00%      | 0.803 | 38  | 9   | 13  | 23  | Qiagen miRNeasy kit | TaqMan       |
| Selaru et al<sup>20</sup> | 2009 | 21  | USA                    | Caucasian | Tissue  | 20          | 95.00%      | 92.90%      | 0.995 | 19  | 1   | 1   | 13  | TRizol            | TaqMan       |
| Bernuzzi et al<sup>25</sup> | 2016 | 483 | Italy                  | Caucasian | Serum  | 30          | 93.33%      | 66.67%      | 0.770 | 28  | 10  | 2   | 20  | miRVana PARIS kit | TaqMan       |
| Bernuzzi et al<sup>25</sup> | 2016 | 194 | Italy                  | Caucasian | Serum  | 30          | 76.67%      | 73.33%      | 0.740 | 23  | 8   | 7   | 22  | miRVana PARIS kit | TaqMan       |
| Correa-Gallego et al<sup>31</sup> | 2016 | 21  | USA                    | Caucasian | Plasma | 25          | 88.00%      | 100.00%     | 0.940 | 22  | 0   | 3   | 7   | miRVana PARIS kit | TaqMan       |

**Abbreviations:** AUC, area under the curve; Fn, false negative; FP, false positive; TN, true negative; TP, true positive.
## Table 2 Main characteristics of the prognostic studies included in the meta-analysis

| Study          | Year | miR Population | Patient | Method  | Cut-off | Sample | Survival | Follow-up | HR      | 95% CI     | RNA extraction | Measurements | p-value |
|----------------|------|----------------|---------|---------|---------|--------|----------|-----------|---------|------------|---------------|--------------|---------|
| Silakit et al15 | 2014 | Thailand       | 51      | qRT-PCR | 2.0-fold | Serum  | OS       | 78        | 2.520   | 1.306–4.85 | QiaGen miRNEasy kit | TaqMan       | 0.006   |
| Huang et al37   | 2013 | People’s Republic of China | 41      | qRT-PCR | Mean    | Tissue  | OS       | 42        | 1.998   | 1.156–3.456 | DIG tailing kit | TaqMan       | 0.013   |
| Huang et al39   | 2013 | People’s Republic of China | 41      | qRT-PCR | Mean    | Tissue  | RFS      | 38        | 1.825   | 1.041–3.202 | DIG tailing kit | TaqMan       | 0.036   |
| Qiao et al48    | 2015 | People’s Republic of China | 27      | qRT-PCR | Median  | Tissue  | RFS      | 43        | 0.272   | 0.132–0.562 | TRizol        | TaqMan       | 0.000   |
| Wang et al38    | 2015 | People’s Republic of China | 57      | qRT-PCR | Quartile | Tissue  | OS       | 60        | 1.588   | 1.064–2.371 | QiaGen miRNEasy kit | TaqMan       | 0.024   |
| Wang et al38    | 2015 | People’s Republic of China | 57      | qRT-PCR | Quartile | Tissue  | PFs      | 60        | 1.888   | 1.117–3.191 | QiaGen miRNEasy kit | TaqMan       | 0.018   |
| Chu et al42     | 2015 | People’s Republic of China | 254     | qRT-PCR | 7.2-fold | Tissue  | OS       | 107       | 1.926   | 1.156–3.209 | miRVana PARIS kit | TaqMan       | 0.012   |
| Cheng et al47   | 2015 | People’s Republic of China | 103     | qRT-PCR | 1.0-fold | Serum   | OS       | 71        | 0.200   | 0.106–0.38 | TRizol        | TaqMan       | 0.000   |
| Chen et al50    | 2010 | People’s Republic of China | 48      | qRT-PCR | 2.94-fold| Tissue  | OS       | 43        | 0.205   | 0.091–0.463 | miRVana PARIS kit | TaqMan       | 0.000   |
| Chen et al50    | 2010 | People’s Republic of China | 48      | qRT-PCR | 2.94-fold| Tissue  | DFS      | 43        | 0.125   | 0.047–0.337 | miRVana PARIS kit | TaqMan       | 0.000   |
| Li et al60      | 2015 | People’s Republic of China | 25      | qRT-PCR | Median  | Tissue  | DFS      | 42        | 2.540   | 0.960–6.740 | TRizol        | SYBR         | 0.003   |
| Li et al60      | 2015 | People’s Republic of China | 25      | qRT-PCR | Median  | Tissue  | DFS      | 43        | 2.419   | 1.290–4.536 | TRizol        | SYBR         | 0.006   |
| Li et al60      | 2015 | People’s Republic of China | 148     | qRT-PCR | Mean    | Tissue  | OS       | 84        | 0.532   | 0.313–0.905 | MJ Mini PCR   | 0.02        |
| Wang et al69    | 2015 | People’s Republic of China | 66      | qRT-PCR | Mean    | Serum   | OS       | 60        | 3.461   | 1.331–5.364 | QiaGen miRNEasy kit | TaqMan       | 0.013   |
| Wang et al69    | 2015 | People’s Republic of China | 66      | qRT-PCR | Mean    | Serum   | PFs      | 60        | 4.226   | 1.415–10.321 | QiaGen miRNEasy kit | TaqMan       | 0.000   |
| Chusorn et al71 | 2015 | Thailand       | 23      | qRT-PCR | Mean    | Tissue  | OS       | 60        | 2.332   | 1.166–4.664 | TRizol        | TaqMan       | 0.017   |
| Deng and Chen66 | 2017 | People’s Republic of China | 125     | qRT-PCR | Mean    | Tissue  | OS       | 60        | 3.508   | 1.772–6.947 | TRizol        | SYBR         | 0.000   |
| Li et al77      | 2017 | People’s Republic of China | 84      | qRT-PCR | Mean    | Tissue  | OS       | 92        | 2.398   | 1.562–3.683 | TruSeq RNA kit | TruSeq kit   | 0.000   |
| Li et al77      | 2017 | People’s Republic of China | 84      | qRT-PCR | Mean    | Tissue  | DFS      | 92        | 2.267   | 1.124–4.571 | TruSeq RNA kit | TruSeq kit   | 0.022   |
| Wang et al78    | 2016 | People’s Republic of China | 62      | RT-PCR  | Mean    | Tissue  | OS       | 80        | 1.277   | 1.053–3.064 | QiaGen miRNEasy kit | TaqMan       | 0.031   |
| Garajova et al79| 2015 | Italy          | 41      | RT-PCR  | Mean    | Tissue  | OS       | 32        | 4.950   | 1.070–22.980 | QiaGen miRNEasy kit | TaqMan       | 0.041   |
| Garajova et al79| 2015 | Italy          | 41      | RT-PCR  | Mean    | Tissue  | RFS      | 32        | 6.210   | 1.290–29.890 | QiaGen miRNEasy kit | TaqMan       | 0.023   |

**Abbreviations:** CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; RT-PCR, reverse transcription PCR; qRT-PCR, quantitative reverse transcription PCR.
Diagnostic and prognostic value of miRNAs for CCA

Twelve studies containing 552 patients and 380 healthy controls evaluated the diagnostic value of miRNAs for CCA. Various miRNAs were detected and shown to be associated with the diagnosis of CCA, including miR-21 (n = 5), miR-192 (n = 2), miR-26 (n = 1), miR-194 (n = 1), miR-106a (n = 1), miR-150 (n = 1), and miR-482 (n = 1). Out of all the investigations, eight studies were conducted in Asian populations, and the remaining four studies were performed in Caucasian populations. Different samples including serum (n = 6), plasma (n = 3), urine (n = 2), and tissue (n = 1) were used in these different studies. All studies used quantitative reverse transcription PCR (qRT-PCR) to measure the expression of miRNAs. Sensitivity, specificity, and DOR data are shown in Figure 3A, indicating that miRNAs have relatively high diagnostic value for CCA. F data are shown in Figure 4A. Meta-regression analysis revealed that plasma may be a source of heterogeneity, while other covariates, such as RNA extraction, measurements, specimen, and different races, may not contribute to heterogeneity (all p > 0.05) (Table 3). Pooled positive and negative LR data are shown in Figure 5A. Studies that included 1,155 patients from the People’s Republic of China, Italy, and Thailand were collected, and 12 miRNAs were identified in CCA patients. Tissue and sera were sample sources, and 13 reports assessed the correlations between abnormal miRNA expression and OS, while four studies assessed the relationship between miRNAs expression and DFS. Limited studies were included for PFS (n = 3) and RFS (n = 2); thus, pooled HRs were respectively analyzed in the systemic review but without further heterogeneity analysis.

Increased expression of miR-21, miR-26a, miR-192, miR-200c, miR-191, miR-181c, miR-29a, and miR-221 was associated with a poor prognosis, as was decreased expression of miR-34a, miR-106a, miR-203, and miR-373 (Table 2). Heterogeneity data are shown in Figure 6A. A random-effects model was applied for the OS, DFS, and RFS subgroups, and the data are given in Table 4. Through subgroup analysis of OS determined by different elements, we found that the up–downregulation of miRNAs might be the source of heterogeneity (p = 0.001), while other factors (such as sample type, sample size, RNA extraction, and different measurements) may not lead to significant heterogeneity (all p > 0.05) (Table 4). The pooled results indicated that miRNAs were significant prognostic biomarkers for CCA patients (OS: HR = 1.47, 95% CI 0.91–2.37, n = 13; DFS: HR = 0.67, 95% CI 0.16–2.81, n = 4; PFS: HR = 2.31, 95% CI 1.59–3.36, n = 3; RFS: HR = 1.46, 95% CI 1.00–2.14, n = 2). Sensitivity analysis data are shown in Figure 7. No obvious publication bias was found in the quantitative synthesis when Begg’s funnel plot and Egger’s test were applied to assess publication bias (p = 0.326) (Figure 8A).

Diagnostic and prognostic values of miR-21 for CCA

Notably, we found that abnormal expression of miR-21 was identified by five studies for diagnostic analysis and four studies for prognostic analysis of all included studies. Thus, we had to conduct a meta-analysis for evaluating the potential diagnostic and prognostic value of miR-21 for CCA.

Five studies containing 235 patients assessed the diagnostic value of miR-21 for CCA. The pooled sensitivity, specificity, positive LR (PLR), negative LR (NLR), and DOR were 0.85 (95% CI 0.76–0.91), 0.92 (95% CI 0.81–0.97), 10.9 (95% CI 4.0–29.5), 0.17 (95% CI 0.10–0.28), and 66 (95% CI 16–272), respectively, indicating that miR-21 has a higher diagnostic accuracy than the pooled miRNAs for CCA patients (Figure 4B). Additionally, the AUC was 0.93 (95% CI 0.91–0.95), indicating miR-21 has a stronger diagnostic value for CCA when compared with the pooled miRNAs (Figure 3B). Furthermore, there was no significant publication bias in this meta-analysis (Figure 5B).

Meanwhile, we performed the prognostic analysis on the relationship of miR-21 and the OS of CCA patients (n = 4) (Figure 6B). As heterogeneity among all studies was not observed (OS: F = 0.0%; p = 0.452), a fixed-effects model was used. We found significant association between abnormal miR-21 expression and poor OS (HR = 1.88, 95% CI 1.41–2.51). Publication bias was evaluated with funnel plots and Begg’s tests. No significant publication biases were found (Figure 8B).

Discussion

CCA is the second most common primary hepatic malignancy arising from the bile duct epithelium,25 but it has few distinguishable symptoms, and patients are mostly diagnosed at the advanced tumor phase, resulting in the stubbornly high mortality in the recent years.26–28 Although the OS of patients with CCA is dismal, there is a large discrepancy between patients diagnosed at early and late stages, indicating an urgent need for novel diagnostic and prognostic biomarkers.29

Current biomarkers for CCA have low sensitivity and specificity. Ince et al10 constructed a receiver operating characteristic (ROC) curve and collected optimal thresholds for serum and biliary CA19-9 (serum: sensitivity, 49%; specifici-
Figure 3 SROC curves describing the diagnostic performance of miRNAs in discriminating cancer patients from healthy subjects: (A) miRNAs and (B) miR-21.

Abbreviations: AUC, area under the curve; miRNA, microRNA; SENS, sensitivity; SPEC, specificity; SROC, summary receiver operating characteristic.
Figure 4 Forest plots of sensitivity and specificity of each included publication: (A) miRNAs and (B) miR-21.

Abbreviations: CI, confidence interval; miRNA, microRNA.
Diagnostic and prognostic value of miRNAs in cholangiocarcinoma

By 84.5%; accuracy, 64%; biliary: sensitivity, 74%; specificity, 34%; accuracy, 69%) and noted the low sensitivity and specificity of these markers. They also reported sensitivity and specificity of biliary carcinoembryonic antigen (CEA) of 57% and 68%, respectively. Thus, CEA may be not ideal for distinguishing CCA from normal people. Huang et al31 identified elevated soluble fragments of cytokeratin 19 (CYFRA 21-1) from CCA patients sera and reported an AUC of 0.879 and a high specificity (96.2%) but a low sensitivity (75.6%). Therefore, traditional biomarkers are not suitable for improving the accuracy of diagnosis and prognosis.

miRNAs contribute to tumorigenesis by affecting cellular processes, including the cell cycle, angiogenesis, invasion, and metastasis.32–34 Because miRNAs are protected from RNases and remain stable in plasma and serum, they may have a potential role as biomarkers for early cancer detection.35 However, studies focusing on diagnostic or prognostic values of miRNAs in CCA are inconsistent. For example, Selaru et al36 performed qRT-PCR on 18 primary CCAs and 12 normal liver specimens and found that miR-21 was 95% sensitive and 100% specific in distinguishing between CCA and normal tissues (AUC 0.995). Silakit’s report37 revealed that the sensitivity and specificity of urine-derived miR-21 were 63.6% and 71.4%, respectively, for differentiating CCA patients from healthy controls (AUC 0.682). Wang et al38 suggested that high tissue-derived miR-21 expression was associated with a higher risk of intrahepatic cholangiocarcinoma (ICC) death compared to low tissue miR-21 expression and was an independent predictor of poor OS of ICC patients (HR = 3.519, 95% CI 1.411–5.702, p = 0.021). Huang et al39 reported that CCA patients with high miR-21 expression had a mean 3-year OS of 15%, whereas patients with low miR-21 expression had a 33% 3-year OS (HR = 1.620, 95% CI 0.440–5.960, p = 0.046). Differences in specimen samples (ie, tissue or urine), different inclusion criteria, and different microarray techniques may explain discrepancies among these studies, but a lack of systematic evaluation complicates this conclusion. Knowledge on the diverse value of miRNAs is vague. Thus, we carried out this comprehensive and up-to-date research in a clinical context to precisely evaluate the diagnostic and prognostic accuracy of miRNAs.

In this systematic review, 12 diagnostic and 22 prognostic studies were included to study whether miRNAs are useful biomarkers for CCA. We noted that miRNAs had a relatively high diagnostic accuracy, and yielded a combined AUC of 0.88 (95% CI 0.85–0.91), with a pooled sensitivity of 0.82 and a pooled specificity of 0.81 for discriminating CCA cases. DOR, which combines the strength of sensitivity and specificity, is a useful indicator of diagnostic accuracy. In this meta-analysis, the DOR value for miRNAs was 22 (95% CI 11–43), indicating moderate diagnostic accuracy. However, the PLR and NLR were 4.8 (95% CI 3.1–7.3) and 0.22 (95% CI 0.16–0.29), respectively, suggesting that miRNAs may be insufficient in distinguishing patients with CCA because

| Year       | Sensitivity | Specificity | AUC   |
|------------|-------------|-------------|-------|
| Before 2010| 0.95        | 0.93        | 0.99  |
| After 2010 | 0.81        | 0.82        | 0.87  |

### Table 3

Multivariate meta-regression analysis for the associations of miRNAs with susceptibility to various cancers

| Results of subgroup and meta-regression analysis in the diagnosis meta-analysis |
|---------------------------------|-----------------|-----------------|---------------|
| Sensitivity 95% CI Specificity 95% CI AUC 95% CI Regression |
| Year               | 0.95 (0.77–0.98) | 0.82 (0.74–0.90) | 0.87 (0.84–0.9) | 0.2 |
| Race              |Asian (0.8)       | 0.74 (0.85)     | 0.83 (0.9)     | 0.87 (0.84–0.9) | 0.13 |
|Race              |Caucasian (0.88)  | 0.83 (0.94)     | 0.8 (0.58–0.92) | 0.92 (0.89–0.94) |
|Sample size       |<100 (0.85)       | 0.72 (0.89)     | 0.75 (0.67–0.81) | 0.81 (0.78–0.85) | 0.07 |
|Sample size       |≥100 (0.85)       | 0.8 (0.88–0.9)  | 0.8 (0.81–0.9)  | 0.87 (0.83–0.89) |
|Specimen          |Serum (0.83)      | 0.78 (0.87)     | 0.8 (0.72–0.86) | 0.87 (0.84–0.9)  |
|Specimen          |Plasma (0.86)     | 0.78 (0.91)     | 0.95 (0.76–0.99) | 0.87 (0.84–0.90) |
|Specimen          |Tissue (0.95)     | -              | 0.93 (0.81–0.95) | 0.87 (0.83–0.89) |
|RNA extraction    |QIAGEN miRNeasy kit (0.81) | 0.76 (0.86)     | 0.81 (0.73–0.90) | 0.88 (0.85–0.91) |
|RNA extraction    |Others (0.85)     | 0.78 (0.91)     | 0.85 (0.75–0.96) | 0.86 (0.83–0.89) |
|Measurements      |TaqMan (0.82)     | 0.78 (0.86)     | 0.83 (0.76–0.90) | 0.88 (0.85–0.91) |
|Measurements      |SYBR (0.87)       | 0.69 (1.00)     | 0.81 (0.53–1.00) | 1.00 |

Note: The en-dashes indicate the 95% CI could not be calculated due to limited studies.

Abbreviations: AUC, area under the curve; CI, confidence interval; miRNA, microRNA.
Figure 5 Graph of Deeks’ funnel plot asymmetry test: (A) miRNAs ($p = 0.30$) and (B) miR-21 ($p = 0.64$).

Abbreviations: ESS, effective sample size; miRNA, microRNA.
PLR > 10 and NLR < 0.1 are thresholds representing high accuracy. Subgroup and meta-regression analyses were applied if heterogeneity occurred in the diagnostic meta-analysis. We compared factors that may influence the heterogeneity and suggest that plasma specimens may be the chief sources of heterogeneity ($p = 0.05$). Tumor-derived circulating miRNA may have utility as a noninvasive blood biomarker, and both the sensitivity and specificity of blood-based assays (serum and plasma) exceeded 0.8, so they may have a high diagnostic value.

Elevated expression of miRNAs (miR-21, miR-26a, miR-29a, miR-181c, miR-191, miR-192, miR-200c, and miR-221) was associated with poor survival of CCA patients, and decreased expression of miRNAs (miR-34a, miR-106a, miR-203, and miR-373) was associated with a worse prognosis. Pooled HR values of OS correlated with miRNA expression for CCA patients, and this suggested that specific miRNAs are independent risk factors for prognosis and may have use for clinical decision-making. The forest plot revealed heterogeneity in this meta-analysis ($I^2 = 88.1\%$, $p < 0.001$), so we performed meta-regression analysis to explore the source. Results showed that the up–downregulation of miRNAs was significantly related to heterogeneity ($p = 0.001$) and may partly explain the heterogeneity of the OS analysis. We also evaluated the relationship between miRNA expression and RFS, DFS, and PFS. The pooled HR of PFS from the

| Study ID (year/miR-expression) | HR (95% CI) | % Weight |
|-------------------------------|------------|----------|
| OS                            |            |          |
| Silak et al$^{15}$ (2014/192/↑) | 2.52 (1.31–4.85) | 4.74     |
| Huang et al$^{39}$ (2013/21/↑) | 2.00 (1.16–3.46) | 4.95     |
| Wang et al$^{38}$ (2015/21/↑) | 1.59 (1.06–2.37) | 5.19     |
| Chu et al$^{36}$ (2015/200/↑) | 1.93 (1.16–3.21) | 5.01     |
| Wang et al$^{39}$ (2015/26a/↑) | 3.46 (1.33–5.36) | 4.66     |
| Chusorn et al$^{35}$ (2013/21/↑) | 2.33 (1.17–4.66) | 4.66     |
| Li et al$^{39}$ (2017/191/↑) | 2.40 (1.56–3.68) | 5.15     |
| Wang et al$^{38}$ (2016/181c/↑) | 1.80 (1.05–3.06) | 4.97     |
| Deng and Chen$^{26}$ (2017/29a/↑) | 3.51 (1.77–6.95) | 4.68     |
| Garajova et al$^{39}$ (2015/21/↑) | 4.96 (1.07–22.98) | 2.92     |
| Chen et al$^{33}$ (2010/373/↓) | 0.20 (0.09–0.46) | 4.41     |
| Cheng et al$^{17}$ (2015/106a/↓) | 0.53 (0.31–0.90) | 4.98     |
| Li et al$^{47}$ (2015/203/↓) | 0.20 (0.11–0.38) | 4.77     |
| Subtotal ($I^2 = 88.1\%$, $p = 0.000$) | 1.47 (0.91–2.37) | 61.10    |
| DFS                            |            |          |
| Chen et al$^{33}$ (2010/373/↓) | 0.13 (0.05–0.34) | 4.03     |
| Li et al$^{39}$ (2015/221/↑) | 2.54 (0.96–6.74) | 4.06     |
| Qiao et al$^{36}$ (2015/34a/↓) | 0.27 (0.13–0.56) | 4.60     |
| Li et al$^{37}$ (2017/191/↑) | 2.27 (1.12–4.57) | 4.65     |
| Subtotal ($I^2 = 91.6\%$, $p = 0.000$) | 0.67 (0.16–2.81) | 17.33    |
| PFS                            |            |          |
| Li et al$^{39}$ (2015/221/↑) | 2.42 (1.29–4.54) | 4.79     |
| Wang et al$^{38}$ (2015/21/↑) | 1.89 (1.12–3.19) | 4.99     |
| Wang et al$^{39}$ (2015/26a/↑) | 4.23 (1.41–10.32) | 4.01     |
| Subtotal ($I^2 = 0.4\%$, $p = 0.366$) | 2.31 (1.59–3.36) | 13.80    |
| RFS                            |            |          |
| Garajova et al$^{39}$ (2015/21/↑) | 6.21 (1.29–29.89) | 2.85     |
| Huang et al$^{39}$ (2013/21/↑) | 1.83 (1.04–3.20) | 4.92     |
| Subtotal ($I^2 = 51.7\%$, $p = 0.150$) | 2.68 (0.88–8.15) | 7.77     |
| Overall ($I^2 = 86.9\%$, $p = 0.000$) | 1.46 (1.00–2.14) | 100.00   |

Note: Weights are from random-effects analysis

Figure 6 (Continued)
B

| Study ID (year/miR-/expression) | HR (95% CI) | % Weight |
|---------------------------------|-------------|----------|
| OS                              |             |          |
| Huang et al39 (2013/21/↑)       | 2.00 (1.16–3.46) | 27.63    |
| Wang et al38 (2015/21/↑)        | 1.59 (1.06–2.37) | 51.61    |
| Chusorn et al55 (2013/21/↑)     | 2.33 (1.17–4.66) | 17.24    |
| Garajova et al59 (2015/21/↑)    | 4.95 (1.07–22.98) | 3.52     |
| Subtotal (I² = 0.0%, p = 0.452) | 1.88 (1.41–2.51) | 100.00   |
| Overall (I² = 0.0%, p = 0.452)  | 1.88 (1.41–2.51) | 100.00   |

Note: Weights are from random-effects analysis

Table 4 Subgroup analysis for association of miRNAs with OS in CCA

| Variables | Coefficient | Standard error | t      | p value | 95% CI     |
|-----------|-------------|----------------|--------|---------|------------|
| Up–down   | −2.055098   | 0.34408        | −5.97  | 0.001   | −2.869494  |
|           |             |                |        |         | −1.240703  |
| Sample type | −0.0456237 | 0.3268338     | −0.14  | 0.893   | −0.818462  |
|           |             |                |        |         | 0.7272155  |
| Year      | 0.115651    | 0.380489       | 0.3    | 0.772   | −0.7924804 |
|           |             |                |        |         | 1.023782   |
| Sample size | 0.0126298  | 0.3844345      | 0.03   | 0.975   | −0.896413  |
|           |             |                |        |         | 0.921673   |
| RNA extraction | 0.798387   | 0.4680503     | 1.71   | 0.12    | −0.2445424 |
|           |             |                |        |         | 1.84122    |
| Measurements | −0.4936747 | 0.5209893      | −0.95  | 0.37    | −1.654511  |
|           |             |                |        |         | 0.6671618  |

Abbreviations: CCA, cholangiocarcinoma; CI, confidence interval; miRNA, microRNA; OS, overall survival.

Figure 6 Forest plots of studies evaluating miRNAs expression level and cancer prognosis: (A) miRNAs and (B) miR-21.

Note: “↑” and “↓” indicate that elevated and decreased expression of microRNAs correlate with poor survival rate.

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; miRNA, microRNA; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival.

Table 4 Subgroup analysis for association of miRNAs with OS in CCA

| Reference (year/miR-/expression) | Meta-analysis estimates, given named study is omitted |
|---------------------------------|------------------------------------------------------|
|                                 | Lower CI limit | Estimate | Upper CI limit |
| Silakit et al15 (2014/192/↑)    |                 |          |               |
| Huang et al39 (2013/21/↑)       |                 |          |               |
| Wang et al38 (2015/21/↑)        |                 |          |               |
| Chu et al52 (2015/200c/↑)       |                 |          |               |
| Wang et al40 (2015/26a/↑)       |                 |          |               |
| Chusorn et al55 (2013/21/↑)     |                 |          |               |
| Li et al57 (2017/191/↑)         |                 |          |               |
| Wang et al58 (2016/181c/↑)      |                 |          |               |
| Deng and Chen56 (2017/29a/↑)    |                 |          |               |
| Garajova et al59 (2015/21/↑)    |                 |          |               |
| Chen et al53 (2010/373/↓)       |                 |          |               |
| Li et al54 (2015/203/↓)         |                 |          |               |
| Cheng et al17 (2015/106a/↓)     |                 |          |               |

Figure 7 Sensitivity analyses assessing the influence of individual studies on the pooled abnormal miRNAs expression in OS subgroup.

Abbreviations: CI, confidence interval; miRNA, microRNA; OS, overall survival.
Diagnostic and prognostic value of miRNAs in cholangiocarcinoma

In the included studies was 2.31 (95% CI 1.59–3.36), indicating high expression of miRNAs might predict poor PFS for cancer patients. The included studies had low heterogeneity ($I^2 = 0.4\%$; $p = 0.366$), and the pooled HRs of DFS and RFS were 0.67 (0.16–2.81) and 2.68 (0.88–8.15), respectively, which showed that miRNAs can be used to monitor the therapeutic effects of radical resection or chemotherapy. However, due to limited studies, meta-regression and subgroup analyses for DFS and RFS subgroups were not performed, although there was significant heterogeneity.

Several miRNAs were confirmed to be associated with CCA patient variables, but most were assessed in one study, and only miR-21 was reported in at least three studies, suggesting it requires more investigation. For the diagnostic meta-analysis, four studies involving 210 patients and 159 healthy controls were included, and the pooled sensitivity and specificity of miR-21 were 0.85 (95% CI 0.76–0.91) and 0.92 (95% CI 0.81–0.97), respectively, with an ROC of 0.93 (95% CI 0.91–0.95). Compared with the pooled parameters of miRNAs, miR-21 had a higher diagnostic accuracy than other miRNAs and a better DOR (53, 95% CI 12–239), NLR (0.18, 95% CI 0.10–0.32), and PLR (9.4, 95% CI 3.5–25.4), indicating miR-21 was adequate to distinguish CCA patients from normal subjects. Data showed that patients with increased miR-21 had a higher risk of poor survival compared to those with low miR-21 (HR 1.88, 95% CI 1.41–2.51). There was no statistical heterogeneity among the included studies, so miR-21 may be an ideal prognostic marker for clinical decision-making. In conclusion, these findings proved that miR-21 could be a more suitable clinical biomarker with a higher capacity for discriminating CCA patients and healthy people. Exploring the sources of heterogeneity is necessary in a meta-analysis.

In the present study, different measures such as sample size, ethnicity, RNA extraction, and measurement methods were used to extract miRNAs in different studies. However, there is no evidence that these variables may influence the heterogeneity. In addition, high expression of miR-21 has been found to relate with hepatocellular carcinoma (HCC). Our study further concluded that miR-21 correlate with diagnosis and prognosis of CCA with numerous miRNAs. It may not be possible for miR-21 to distinguish HCC and CCA. Thus, clinical features and techniques such as physical examination and CT/MRI scanning may need to be combined to differentiate CCA from HCC.

miRNAs have been implicated in the pathogenesis of CCA because upregulated miRNAs suggest an oncogenic role and downregulation of miRNAs may be suppressive, so dysregulated miRNAs are correlated with the adverse clinical features, diminished survival, and poor prognosis of CCA patients. Selaru et al proved that miR-21 was oncogenic by inhibiting PDCD4 and TIMP3 suppressor genes in the biliary tree. Wang et al confirmed the functional and mechanistic links between miR-21 and tumor suppressor genes (such as PTPN14 and PTEN) in the pathogenesis of ICC, which influenced the proliferation and tumor progression in ICC. Li et al reported that miR-221 promoted extrahepatic CCA (EHCC) invasion and metastasis by targeting PTEN and formed a positive feedback loop with the $\beta$-catenin/c-Jun signaling pathways. Moreover, results from Qiao’s study suggested that miR-34a inhibited invasion and migration by targeting Smad4 to suppress the epithelial–mesenchymal transition via the TGF-$\beta$/Smad signaling pathway in human EHCC. In conclusion, altered miRNA expression is a key to tumorigenesis and can be used to identify clinicopathologic features of the disease.
Our study was limited by finding miR-21 in four studies, but up to 17 miRNAs were identified to be valuable for diagnosis or prognosis, and study heterogeneity occurred. Subgroup analysis was performed to find the source of heterogeneity, but we could not fully explain it. Likely differences in patients’ baseline characteristics (ethnicity, gender, age, and tumor stage and grade), different thresholds for miRNA expression, and the way samples were prepared and preserved (ie, paraffin-fixed, formalin-fixed, freshly frozen tumors, or blood) made a difference. In addition, some HRs were calculated based on data extracted from survival curves, which might be less powerful than data obtained from articles directly.

Despite these limitations, our review had several important strengths. First, a relatively thorough systematic search was performed, and diagnostic and prognostic values of miRNAs in CCA were independently evaluated and verified. Our methods were rigorous and followed guidelines for conducting and reporting systematic reviews. Besides, further analysis and research was carried out to attest the diagnostic and prognostic roles of miR-21 expression in CCA patients.

**Conclusion**

miRNAs, especially miR-21, were identified to be highly correlated with CCA and could be potential and promising biomarkers in distinguishing CCA patients from healthy people; they could also contribute to predicting the progression of CCA. Furthermore, to better understand and use miRNAs as biomarkers in clinical detection, more large-scale and high-quality investigations are needed to confirm our results.

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**Disclosure**

The authors report no conflicts of interest in this work.

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