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ORIGINAL ARTICLE

MicroRNAs as Biomarkers for Hepatocellular Carcinoma: A Diagnostic Meta-Analysis

QIONG-YING HU [1], HONG JIANG [1], JUN SU [1], YONG-QIAN JIA [2]

[1] *Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, P. R. China*
[2] *Department of Hematology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, P. R. China*

SUMMARY

Background: **Numerous studies reported various microRNAs (miRNAs) could be novel serum biomarkers for hepatocellular carcinoma (HCC). However, the diagnostic ability of different miRNA biomarkers varies among the reports. In this paper, we made a meta-analysis about the diagnostic accuracy of miRNAs for HCC.**
Methods: **We systematically searched The Cochrane Central Register of Controlled Trials, MEDLINE, Pub Med, EMBASE, the Chinese Biomedical Literature Database, the China Academic Journals Full-text Database, and the Chinese Scientific Journals Database for potential studies. Studies were included if they were related to miRNAs and HCC and reported diagnostic outcomes. Diagnostic values analysis was used to summarize the overall test performance of miRNAs.**
Results: **Eight studies were included in this meta-analysis. The ranges of the diagnostic value of miRNAs for HCC were as follows: sensitivity was 0.72 - 0.98, pooled sensitivity was 0.87; specificity was 0.76 - 1.00, pooled specificity was 0.90; positive likelihood ratio was 3.52 - 97.45, pooled positive likelihood ratio was 8.70; negative likelihood ratio was 0.02 - 0.31, pooled negative likelihood ratio was 0.13; and diagnostic odds ratio was 19.06 - 2,646.00, pooled diagnostic odds ratio was 86.69.**
Conclusions: **MiRNAs showed high accuracy in identifying HCC, and could be a useful screening tool for diagnosing HCC patients.**
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KEY WORDS

microRNAs, hepatocellular carcinoma, meta-analysis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, with high morbidity and mortality worldwide. In the United States, in 2012 there were approximately 28,720 new cases and 20,550 deaths of liver cancer [1]. The morbidity and mortality of HCC was even higher in China [2]. It was estimated there were 748,000 new cases of liver cancer worldwide in 2008, causing 696,000 deaths [3]. The 5-year survival rate of 5% - 9% for HCC is very low from the time of clinical diagnosis and many cases of HCC exhibit early intrahepatic and distant metastasis which miss the optimal opportunity for surgical resection [4]. Early detection could raise the 5-year survival rate and improve prognosis. Currently, diagnosis of HCC relies on pathology, liver imaging and measurement of serum alpha-fetoprotein (AFP). Liver biopsy has been considered the 'gold standard' for diagnosing HCC. However, 1iver biopsy can be associated with advanced tumor stages and significant expense, manpower issues, and risk of patient injury and is not available for early cancer screening [2]. Imaging techniques (abdominal ultrasound, MRI and contrast enhanced CT, etc.) show high sensitivity in the diagnosis of cancer, but these procedures are costly and many kinds of imaging methods are not sensitive enough to detect tumors measuring < 1 cm [5]. In the clinical laboratory, AFP is a well-known biomarker for HCC. However, the reported sensitivity (41 - 65%) and specificity (80 - 94%) of AFP is not sufficient for early diagnosis [6]. Thus, discovery of an effective

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tool for early diagnosis of HCC would play a pivotal role in improving the prognosis of patients with HCC. Recently, microRNAs (miRNAs) were considered as novel markers for HCC diagnosis in several clinical reports. MiRNAs are non-coding RNAs which have been identified as post-transcriptional regulators of gene expression [7]. MiRNAs control a wide array of biological processes, such as cell differentiation, proliferation, apoptosis, adhesion, and death [8]. Recently, many studies suggest a link between aberrant expression of miRNAs and various cancers including HCC, which opened a new avenue for the study of molecular mechanisms, diagnosis, and implementation of novel therapeutic targets of HCC [9-12]. However, numerous manuscripts have reported various miRNAs and different diagnostic accuracy in the identification of HCC. Here, we undertook a meta-analysis to assess the overall accuracy of these miRNAs in the diagnosis of HCC.

MATERIALS AND METHODS

Search strategy and study selection

A comprehensive literature search to identify studies that evaluated the diagnostic accuracy of miRNAs for HCC was conducted. Databases including MEDLINE (1946 to November 2012), the Cochrane Database of Systematic Reviews (2005 to October 2012), the Chinese Biomedical Literature Database (1978 to November 2012), the China Academic Journals Full-text Database (1979 to November 2012), and the Chinese Scientific Journals Database (1989 to November 2012) were searched without language restrictions.

The following search strategy was adopted: 'micro RNA' and ('hepatocellular carcinoma' OR 'HCC' OR 'liver cancer' OR 'liver'). Duplicate articles identified in both Medline and EMBASE were manually deleted using Reference Manager (Thomson Reuters EndNote X5). Results were arbitrated by two investigators (Jun Su and Hong Jiang) on the basis of the title and abstract, and the full paper of each potentially eligible study was then obtained. For further relevant studies, we checked the reference lists of identified trials.

Eligible criteria

All studies that reported data on patients with a confirmed diagnosis of HCC by miRNAs were considered for inclusion. Only studies that reported sufficient data to allow construction of 2 × 2 tables were included. Irrelative studies, such as animal studies, were excluded.

Quality assessment

Two independent reviewers (Jun Su and Hong Jiang) used the Quality Assessment of studies of Diagnostic Accuracy included in Systematic reviews (QUADAS) instrument [13] to assess the quality of selected articles (Table1). Any disagreements were resolved by discussion and consensus, if necessary after contacting the authors for clarification. Study quality was assessed using the QUADAS-list, with each item scored as 'yes', 'no', or 'unclear'. We did not calculate summary scores of quality because the interpretation was problematic and potentially misleading [14].

Data extraction and analysis

One reviewer (Qiong-Ying Hu) extracted data and another reviewer (Hong Jiang) checked them. The data-extraction form was accompanied by a background document that stated how each item on the form should be interpreted. All data collection was performed according to a protocol with the following information being extracted from each study: first author, year of publication, population characteristics, study design, inclusion and exclusion criteria, number of subjects, and the method of HCC determination.

The primary endpoints of miRNAs as biomarkers were sensitivity and specificity (the number of true positive, false negative, true negative and false positive results) for comparison of patients diagnosed with HCC vs. control. We also extracted and calculated sensitivity and specificity from each study and presented the data as diagnostic odds ratio (DOR) and summary receiver operating characteristic (SROC) curves to illustrate the performance of miRNAs in identifying HCC, using the area under the curve (AUC) value. We used Meta-Disc 1.4 software to analyse the plots and curves.

RESULTS

The literature search yielded 574 citations of which 68 were excluded as duplicates. 329 publications were excluded because they failed to meet the eligible criteria on the basis of title and abstract. Of the 177 potentially eligible studies, 169 publications were excluded because they were reviews (n = 6), no diagnostic value studies (n = 155), and unable to extract data (n = 8). Finally, 8 focusing on the target patient spectrum were included (Figure 1). Table 2 describes the general characteristics of the studies included.

Different miRNAs or miRNA panels predicted various diagnostic values. The target diseases and controls were HCC (or related HCC) and healthy groups (or related diseases [15]). Only Liu 2012 [4], Gui 2011 [16], and Tomimaru 2012 [17] reported the time period between biopsy and blood sample tests, which was related to carcinoma development and cancer biomarker variation. But the time periods in these research papers were unclear, which led to heterogeneity. Thus we needed a specific span of time to unify testing time. Usually, at the time point of surgical biopsy, blood miRNA should be tested. Moreover, biomarker detecting should be done before treatment. From raw data, we constructed diagnosis values of the patient population as a 2 × 2 table (Table 3) by methods for diagnostic meta-analysis [18]. The ranges of sensitivity (SEN) and specificity (SPE) of the diagnosis model with miRNAs as identifying of HCC were 0.72 - 0.98 (Figure 2a) and 0.76 -

Table 1. Items of quality assessment chosen from QUADAS checklist [13].

Item	Yes	No	Unclear
1. Was the spectrum of patients representative of those who will receive the test in practice?	( )	( )	( )
2. Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
3. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
4. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
5. Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
6. Was the reference standard independent of the index test (i.e., the index test did not form part of the reference standard)?	( )	( )	( )
7. Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
8. Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
9. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
10. Were uninterruptible/intermediate test results reported?	( )	( )	( )
11. Were withdrawals from the study explained?	( )	( )	( )

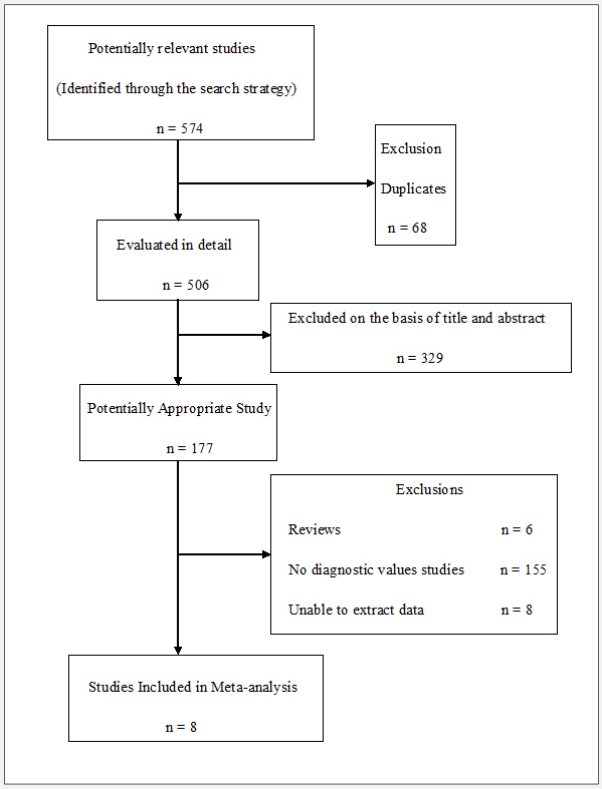

Figure 1. Flow diagram of study selection process for the Meta-analysis.

Table 2. Characteristics and quality assessment of the included studies with miRNAs.

	Li 2010 [34]	Gui 2011 [16]	Peng 2011 [36]	Qu 2011 [15]	Zhou 2011 [19]	Li 2012 [20]	Liu 2012 [4]	Tomimaru 2012 [17]
MiRNA types	microRNA panel†	miR-885-5p	miR-122	miR-16	microRNA panel‡	miR-18a	miR-15b & miR-130b	miR-21
Patient spectrum	HBV related HCC	liver pathologies	HCC	HCC	HBV related HCC	HBV related HCC	HCC	HCC
Sample	Serum	Serum	Serum	Serum	Plasma	Serum	Serum	Plasma
Detec-tion method	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR
Age group	53.43 ± 9.10◆	54.2 (mean)	24-55	55 (mean)	53 ± 12◆	54 (25-82)	< 60 and ≥ 60	63 ± 10◆
Study design	CSS	CSS	CSS	CSS	CSS	CSS	CSS	CSS
*Item 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
*Item 2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
*Item 3	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes
*Item 4	yes	yes	yes	yes	yes	yes	yes	yes
*Item 5	yes	yes	yes	yes	yes	yes	yes	yes
*Item 6	yes	yes	yes	yes	yes	yes	yes	yes
*Item 7	yes	yes	yes	yes	yes	yes	yes	yes
*Item 8	yes	yes	yes	yes	yes	yes	yes	yes
*Item 9	yes	yes	yes	yes	yes	yes	yes	yes
*Item 10	no	no	no	no	no	no	no	no
*Item 11	unclear	unclear	unclear	unclear	unclear	unclear	unclear	unclear

CSS: Cross sectional study
*: Items chosen to rate from QUADAS checklist in Table 1
miRNA panel†: miR-23b, miR-423, miR-375, miR-23a, miR-342-3p, miR-25 and let-7f
miRNA panel‡: miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801
qRT-PCR: real-time quantitative reverse transcription PCR
◆: Mean ± SD.

Table 3. Diagnosis values of the patients who participated in the studies which were included in the Meta-analysis.

Study (year)	n	TP(a)	FP(b)	FN(c)	TN(d)	Sensitivity (%)	Specificity (%)
Li 2010*	513	53	1	2	49	97	99
Li 2010**	513	54	1	1	49	98	99
Li 2010***	513	53	0	2	50	96	100
Gui 2011	151	86	12	9	44	91	79
Peng 2011	152	57	6	13	28	82	83
Qu 2011	283	76	12	29	95	72	89
Zhou 2011	934	163	4	33	62	83	94
Li 2012	191	74	34	12	11	86	75
Liu 2012	212	56	5	1	54	98	92
Tomimaru 2012	216	110	4	16	46	87	92

*: miRNA panel: miR-23b, miR-423, miR-375, miR-23a, miR-342-3p
**: miRNA panel: miR-375, miR-25, let-7f
***: miR-375 alone.

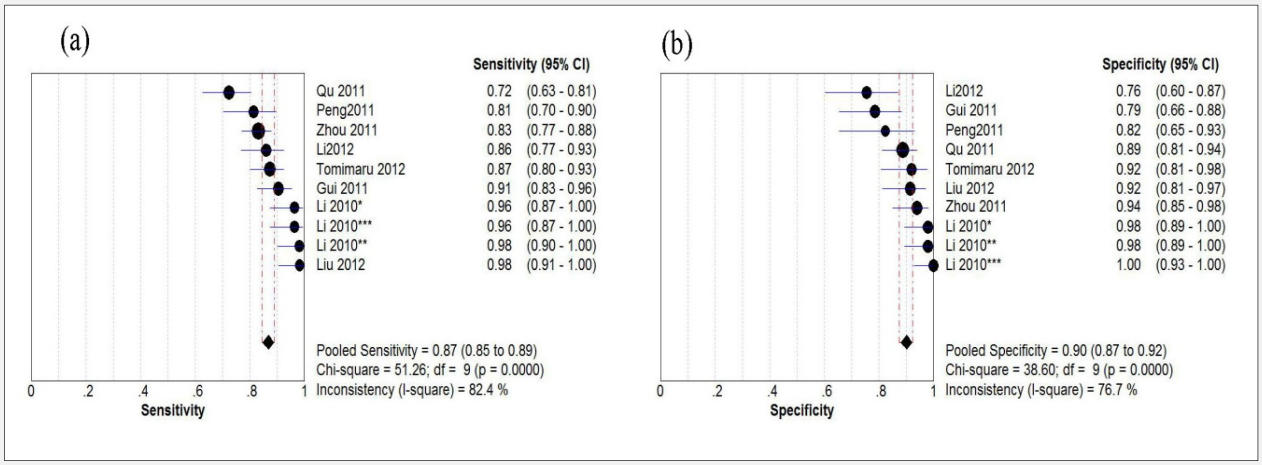

Figure 2. Sensitivity (SEN) (a) and Specificity (SPE) (b) of diagnostic model with miRNAs as identifying HCC.

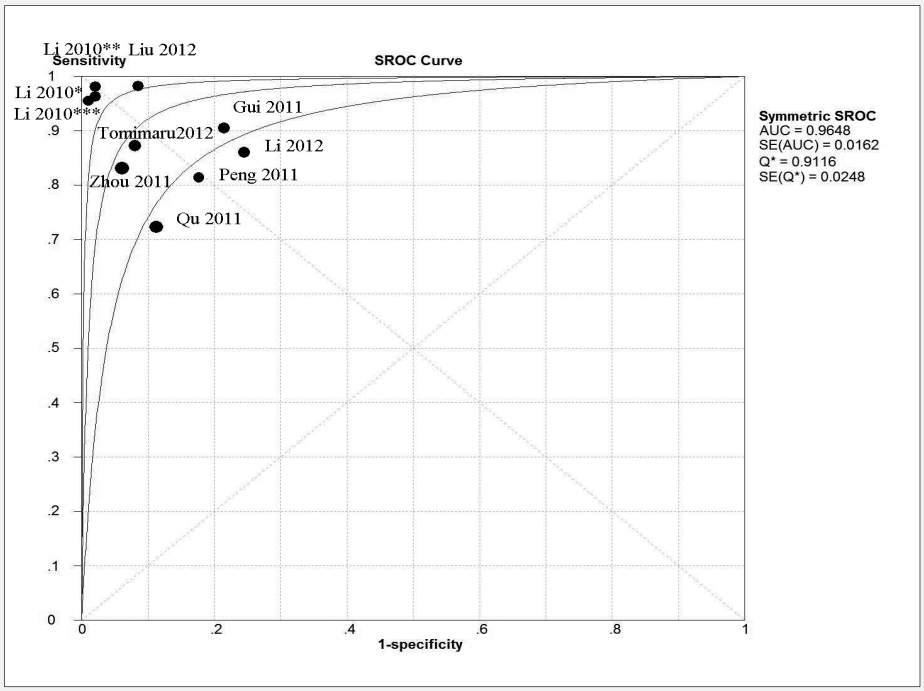

Figure 3. Summary receiver operator characteristic curve of diagnostic model with miRNAs as identifying HCC.

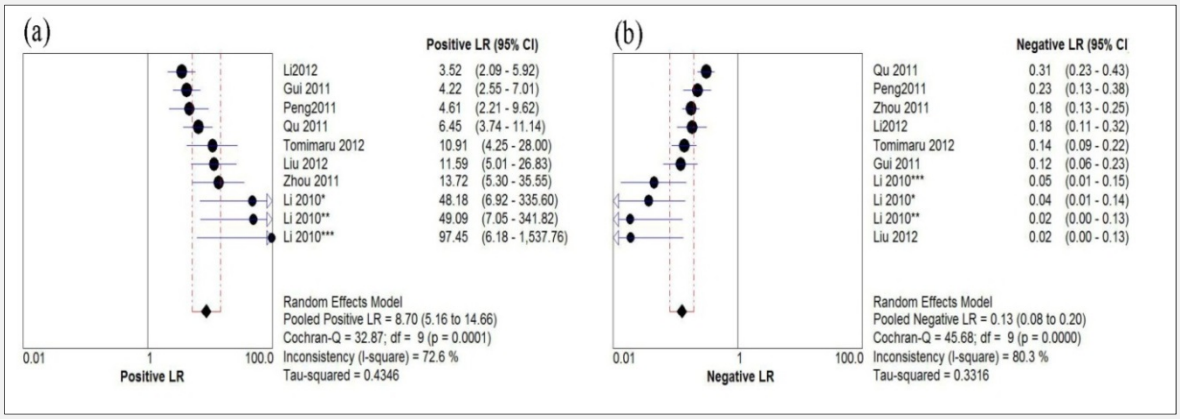

Figure 4. Positive LR (PLR) (a) and Negative LR (NLR) (b) of diagnostic model with miRNAs as identifying HCC.

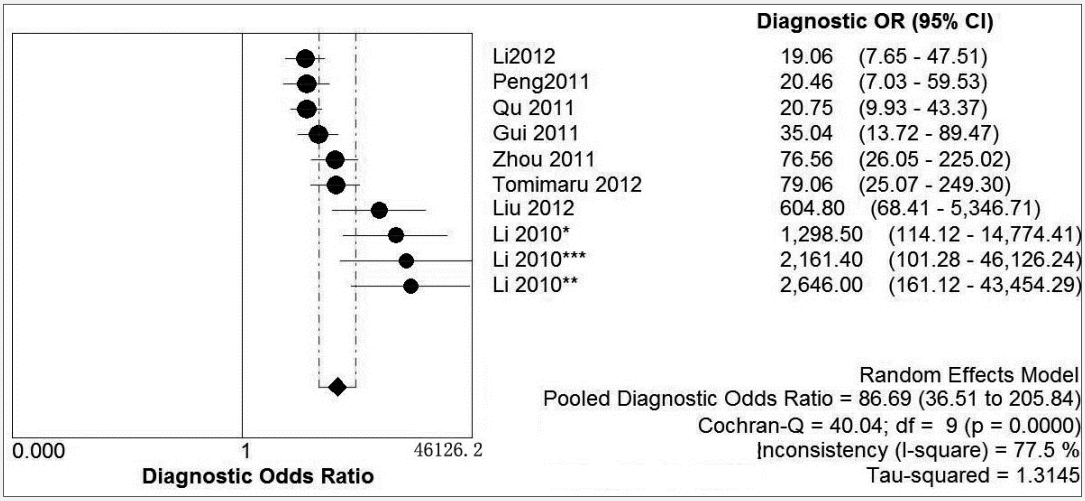

Figure 5. Diagnostic odds ratio (DOR) of diagnostic model with miRNAs as identifying HCC.

1.00 (Figure 2b), respectively, with an overall AUC value of SROC curve of 0.9648 (Figure 3). The ranges of positive likelihood ratio (PLR) (Figure 4a) and negative likelihood ratio (NLR) (Figure 4b) were 3.52 - 97.45 and 0.02 - 0.31, respectively. The range of diagnostic odds ratio (DOR) was 19.06 - 2,646.00 (Figure 5). 8 studies were included in the meta-analysis. The pooled diagnostic values of miRNAs for HCC were as follows: SEN 0.87, SPE 0.90, PLR 8.70, NLR 0.13, DOR 86.69. All of the studies were free of commercial funding and provided a clear definition of what was considered to be a 'positive' result and the technology of the index test was unchanged since the study was carried out. All studies were pre-specified objectively. Data on instrument variation and data on observer variation were not available. All papers failed to describe whether test operators had appropriate training. Five studies clearly pointed out that treatment was withheld until both the index test and reference standard were performed. However, experiment details of Qu 2011 [15], Zhou 2011 [19], and Li 2012 [20] were not available.

DISCUSSION

Currently, imaging and biochemical biomarkers are the two main methods for the diagnosis of HCC. Although remarkable advances of other methods have been made, it is difficult to detect HCC, especially early-stage HCC [19], because of their unsatisfactory diagnostic performance. The present studies demonstrated that circulating miRNA levels were significantly increased in cancer plasma or serum samples compared to the healthy controls [21-25], including liver cancer [12,26-28]. However, different expression of miRNAs has been reported in human HCC, and few studies to date have examined whether circulating miRNA levels could be useful in differentiating between HCC and healthy groups. The reliability of these miRNAs is still debatable.

Moreover, the same miRNA could be biomarkers in diverse diseases [29-32]. Some references and additional relevant information of miRNA's reported to relate to HCC were found in supplemental table. This study therefore aimed at the identification and evaluation of circulating miRNAs to predict HCC.

We studied several possible sources of heterogeneity: groups of patients, study design features, and effect of cut-off value. In the studies included, miRNA types included single miRNA and miRNA panels. Only one research article calculated diagnostic values for a single miRNA (miR-375 alone) and miRNA panels (panel of miR-23b, miR-423, miR-375, miR-23a, miR-342-3p or panel of miR-375, miR-25, let-7f). However, comparing diagnostic efficiency of single miRNA with miRNA panels, we could hardly conclude which one was better. In order to validate and improve these miRNAs' diagnostic values, more studies should be done. The methodological quality of the eight studies is relatively good, and all of them reported the age and gender of the patients included. The patient spectrum had slight variations, though the target disease was HCC. Interestingly, one report involving miR-21 made an opposite conclusion in different population groups when we selected the studies: the serum miR-21 level was a marker for necroinflammatory activity, but did not differ between patients with HCV and HCV-induced HCC [33]. So if the spectrum was different, the representation of patients may be poor, which lead to population bias. In addition, different samples could influence circulating miRNAs' types and amount. And serum or plasma samples may provide different miRNA spectra. The shorter the time period between pathology examination and miRNA qRT-PCR, the less the influence of target condition changed between the two tests. Only Li 2010 [34], Qu 2011 [15], and Zhou 2011 [19] indicated the interval of two tests definitely. A main source of heterogeneity in diagnostic test accuracy is the difference of the cut-off value applied among the studies [35]. Different miRNAs and statistical analyses would influence the cut-off value. Gui 2011 [16], Qu 2011 [15], Zhou 2011 [19], and Liu 2012 [4] used a logistic regression model to analyze, meanwhile, Li 2010 [34] and Tomimaru 2012 [17] applied paired *t*-tests and Wilcoxon's signed-rank test, respectively. Peng 2011 [36] and Li 2012 [20] established Receiver Operating Characteristic (ROC) curves to discriminate patients with or without HCC. Accordingly, the cut-off values of Gui 2011 [16], Qu 2011 [15], Peng 2011[36], Li 2012 [20], Liu 2012 [4], and Tomimaru 2012 [17] were 1.06 (normalized), 6, 0.475, 1.765, -0.611, and -0.108, respectively, but the remaining rests were not available.

We analyzed all included data to draw plots, and they showed the pooled sensitivity and specificity of miRNAs as identifying HCC to be 0.87 (95% confidence interval 0.85 to 0.89) and 0.90 (95% confidence interval 0.87 to 0.92), respectively. With an overall AUC, the value of the SROC curve was 0.9648. In Figure 3, the top and low curves represented the 95% confidence interval of the SROC curve; moreover, the curve between the top and low curves was the ultimate SROC curve. The AUC summarized the diagnostic performance as a single number: a perfect test will have an AUC close to 1. Accordingly, our meta-analysis calculated the AUC value (0.9648) which indicates an excellent ability. Another useful statistic is the Q^* (0.9116) index, defined by the point where sensitivity and specificity are equal, which is the point closest to the ideal top-left corner of the ROC space. The pooled PLR, NLR, and DOR were 8.7 (95% confidence interval 5.16 to 14.66), 0.13 (95% confidence interval 0.08 to 0.20), and 86.69 (95% confidence interval 36.51 to 205.84), respectively. In conclusion, this Meta-analysis suggests that detecting circulating miRNAs is a valuable method for diagnosing HCC, but it needs more supporting data.

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Declaration of Interest:
None.

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Correspondence:
Dr. Yong-Qian Jia
Department of Hematology
West China Hospital, Sichuan University
Chengdu610041, Sichuan Province, P. R. China
Email: jia_yq@scu.edu.cn

Supplemental table: Some references and additional relevant information of miRNA's reported to relate to HCC.

Year	Authors	miRNAs	Target diseases
2006 [1]	Murakami Y, Yasuda T, Saigo K, et al.	miR-16, miR-22 and miR-29	HCC
2008 [2]	Li W, Xie L, He X, et al.	mir-18, mir-224, mir-199a, mir-199a*and mir-195	HCC
2011 [3]	Bihrer V, Waidmann O, Friedrich-Rust M, et al.	miR-21	CHC and CHC-associated HCC
2011 [4]	Braconi C, Henry JC, Kogure T, et al.	review	HCC
2011 [5]	Murakami Y, Toyoda H, Tanaka M, et al.	miR-199a, 199a*, 200a and 200b	Chronic hepatitis C, liver cirrhosis and hepatocellular carcinoma
2011 [6]	Xu J, Wu C, Che X, et al.	miR-21, miR-122 and miR-223	HCC
2011 [7]	Zhang ZZ, Liu X, Wang DQ, et al.	miR-18a/miR-18b, miR-106a, miR-221 and miR-101	HBV and HCC
2012 [8]	Giordano S, Columbano A.	review	HCC
2012 [9]	Romilda C, Marika P, Alessandro S, et al.	miR-92	HCC

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