Digestive and Liver Disease 51 (2019) 621–631

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Meta-Analysis

Circulating microRNAs for the diagnosis of hepatocellular carcinoma

Cheng Peng[a], Yanshuo Ye[a], Zhanpeng Wang[a], Lianyue Guan[a], Suriguga Bao[b], Bo Li[c], Wei Li[a,*]

[a] Department of Hepatobiliary-Pancreatic Surgery, China-Japan Union Hospital of Jilin University, Changchun, China
[b] Department of Hepatobiliary-Pancreatic Surgery, Inner Mongolia people's Hospital, Hohhot, China
[c] Department of Epidemiology, School of Public Health of Jilin University, Changchun, China

ARTICLE INFO

Article history:
Received 10 August 2018
Accepted 17 December 2018
Available online 28 December 2018

Keywords:
Diagnosis
Hepatocellular carcinoma
Meta-analysis
microRNAs

ABSTRACT

Aim: There are no existing biomarkers that demonstrate very reliable performance in the diagnosis of hepatocellular carcinoma (HCC), especially in the early stage. Studies have shown that numerous aberrantly expressed circulating microRNAs (miRNAs) can be used as a diagnostic tool for HCC; however, these studies have produced inconsistent results.
Methods: We performed a meta-analysis to summarize the diagnostic accuracy of circulating miRNAs, alpha-fetoprotein (AFP), and AFP combined with miRNAs in differentiating HCC patients from non-HCC controls, healthy controls and chronic liver disease controls. We also evaluated the diagnostic accuracy of circulating miRNAs for early-stage HCC. Furthermore, we systematically reviewed the diagnostic effectiveness of single miRNAs and individual miRNA panels.
Results: Circulating miRNAs showed good diagnostic performance. Compared with single miRNAs, the diagnostic accuracy of miRNA panels was clearly better. The combination of AFP and miRNAs improved the diagnostic accuracy compared with the use of miRNAs or AFP alone. For early-stage HCC patients, circulating miRNAs exhibited relatively satisfactory diagnostic accuracy.
Conclusions: Circulating miRNAs can be used as an early diagnostic marker of HCC. The combination of miRNAs and AFP has great potential as a novel strategy for the diagnosis of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer, as well as being the third most common cause of mortality and poor-prognosis malignancy due to the recurrence of HCC after surgery and metastasis [1]. HCC accounts for nearly 70%-80% of all cases of primary liver cancer [2].

In clinical practice, the diagnosis of HCC relies mainly on imaging examinations combined with the detection of serum markers. Currently, neither alpha-fetoprotein (AFP) nor Des-gamma-carboxy prothrombin (DCP) have satisfactory diagnostic performance. Although ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) have greatly improved in their accuracy with the progress of science and technology, these modalities are limited in their use due to their costs, availability, reproducibility, and especially their lack of ability in finding small lesions (<1 cm) [3].

Various curative modalities including surgical resection, liver transplantation (LT) and local ablation can be performed if the HCC was early detected. Overall, the 5-year survival rate of HCC patients is 40% after surgical treatment, but for patients who are diagnosed early, the 5-year survival rate is increased to 60%–70% [4–8], even up to 90% for patients with micro-HCC. Therefore, the most urgent need is to find more sensitive and specific biomarkers for early diagnosis of HCC.

MicroRNAs (miRNAs) are a class of small, endogenous, non-coding regulatory RNAs that are nearly 22 nucleotides in length, and which function in RNA silencing and post-transcriptional regulation of gene expression. MicroRNAs regulate approximately 1/3 of total gene expression [9], and hence control a wide array of biological processes, including cellular development, apoptosis, proliferation, differentiation, and tumorigenesis [10]. In the early stage of HCC, abnormal miRNA expression occurs. MiRNAs can remain stable in blood, where RNase is abundant, with the help of their various forms existing in blood, such as microvesicles and so on [11,12]. MiRNAs also have strong resistance to extreme tem-

* Corresponding author at: Department of Hepatobiliary-Pancreatic Surgery, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun 130033, China.
E-mail address: weili888@hotmail.com (W. Li).

https://doi.org/10.1016/j.dld.2018.12.011
1590-8658/

perature and pH levels [13]. These features give circulating miRNAs great potential for becoming a new kind of HCC biomarker.

In recent years, numerous studies have suggested that aberrantly expressed circulating miRNAs can be detected and used as biomarkers for HCC diagnosis. However, the various types of miRNAs and their corresponding diagnostic accuracy have varied among studies. Therefore, we conducted the present meta-analysis of the previous studies to evaluate the clinical applicability of circulating miRNAs.

2. Methods

2.1. Search strategy

Using the following six databases, we conducted a comprehensive literature search to identify studies that evaluated the diagnostic accuracy of circulating miRNAs for HCC: PubMed, Embase, Cochrane Library, Chinese National Knowledge Infrastructure, WanFang Database, and VIP. We did not impose any restrictions on language or publication date. We also performed a manual search of the reference lists of review articles to obtain potential relevant studies.

The medical subject headings (MeSH) and their entry terms employed in the literature search included: (1) "carcinoma hepatocellular" or "hepatocellular carcinoma" or "hepatocellular cancer" or "hepatocellular tumor" or "hepatocellular neoplasm" or "liver cell carcinoma" or "liver cell cancer" or "liver cell neoplasm" or "liver cell tumor" or "HCC"; (2) "microRNAs" or "microRNA" or "miRNAs" or "miRNA" or "miR" or "panel"; (3) "serum" or "plasma" or "blood" or "circulating" or "circulatory"; (4) "diagnosis" or "biomarkers" or "diagnostic" or "screen" or "monitor" or "detect" or "predict" or "predictor" or "prediction" or "specificity" or "sensitivity" or "marker" or "AUC" or "ROC" or "clinical implication".

2.2. Selection criteria

All included references met the following criteria: (1) studies evaluating the diagnostic accuracy of circulating miRNAs for HCC patients; (2) specimen types of plasma, serum, or whole blood; (3) studies with sufficient data.

Studies were excluded if they met any of the following criteria: (1) reviews, meeting abstracts, case reports; (2) studies unrelated to the diagnosis of HCC; (3) animal experiments; (4) studies without complete data; (5) duplications with earlier publications from the same authors or institutions.

2.3. Data extraction

Data extraction was carried out independently by two reviewers according to a standardized table. From each of the included studies, we extracted data on: (1) study features, including the name of the first author, publication year, region, specimen type, and the studied miRNAs and corresponding normalization controls for the polymerase chain reaction (PCR); (2) participant characteristics, including the number of HCC patients and non-HCC controls, age, sex, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection status, liver cirrhosis (LC), and chronic hepatitis B (CHB), chronic hepatitis C (CHC) or other chronic liver disease (CLD) status; (3) data used for meta-analysis, including the number of true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) results. If the article did not provide these four categories of raw data, we performed a data transformation through the number of each group and the diagnostic sensitivity and specificity.

2.4. Study quality assessment

The quality of each included study was assessed using both the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) checklist and the QUADAS-2 checklist [14,15], because each version of the checklist has its own advantages. The QUADAS checklist is comprised of 14 items, with each item scored as "yes", "no", or "unclear". An answer of "yes" indicates that the risk of bias can be judged to be low, while an answer of "no" or "unclear" represents a high risk of bias. Finally, we calculated the total score of each study. The QUADAS-2 tool evaluates the risk of bias for the four domains of patient selection, index test, reference standard, and flow and timing; these are supported by signaling questions to aid judgment on the risk of bias and concerns about applicability, rated as "high", "unclear", and "low".

2.5. Data synthesis and analysis

All statistical analyses were performed using STATA version 14.0 to obtain pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with 95% confidence interval (CI). We also plotted the summary receiver operating characteristic (sROC) curve and obtained the area under the curve (AUC). Meta-DiSc version 1.4 was used to determine whether there was a threshold effect.

A value of $I^2 \geq 50\%$ indicates substantial heterogeneity. A random-effects model was used for further analysis [16,17]; otherwise, the fixed-effects model was applied. Examinations of threshold effect, meta-regression, and subgroup analysis were performed to discover the possible source of heterogeneity. We explored potential publication bias using Deeks' funnel plots [18]. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of the included studies

A total of 928 related published records were retrieved by a primary database search (n = 1349) and a manual search (n = 15) after removing duplicate records (n = 436). After reviewing the titles and abstracts, 778 studies were excluded. We conducted full-text assessment of the remaining 150 studies, and 59 of them were ultimately included for meta-analysis [19–77]. These 59 studies included 5125 HCC patients and 6561 non-HCC controls. The search strategy flow diagram is detailed in Fig. 1(A), and the characteristics of each included study are listed in Table 1. Briefly, the included studies were published from 2010 to 2017. There were 12 Chinese-language reports and 47 English-language reports. Among all the studies, the dominant regions of the studies were China in 43 studies and Japan, India, Italy, Egypt, or the USA in the other 16 studies.

The QUADAS scores are listed in Table 1. There were 42 studies with a score ≥ 7, while 17 studies had a score <7. The QUADAS-2 tool suggested that the major biases focused on "patient selection" and "index test". In the domain of "patient selection", 49 of the included studies were case-control designed, which resulted in a risk of bias in patient selection. In addition, the cut-off values of circulating miRNAs were set by the ROC curve instead of pre-specified in the studies, which led to a risk of bias in the domain of "index test". The review authors' judgments about each domain for each included study and the percentages across the included studies are shown in Fig. 1(B–C).

Table 1
Characteristics of studies included in the meta-analysis.

Author	Year	Region	Specimen	Conference test	Index test	Study design	Markers studied	Normalization controls	HCC patients		Non-HCC controls		QUADAS score
									No.	Population	No.	Population	
Li-Min Li	2010	China	Serum	Histology	RT-PCR	Cohort	10a, 125b, 23b, 423, 375, 23a, 342-3p, 25, 7f	U6	120	HBV-HCC(120)	345	HBV(55)CHB(80)HC(210)	8
Jian Zhou	2011	China	Plasma	Histology	RT-PCR	Cohort	122, 223, 26a, 27a, 192, 21, 801	miR-1228	196	HBV-HCC(196)	194	HC(66)CHB(72)HBV-LC(56)	10
Tomimaru, Y	2011	Japan	Plasma	Histology	RT-PCR	Case-control	21, AFP	miR-16	126	HBV-HCC(25)HCV-HCC(84) HBV + HCV-HCC(3)other(14)	80	CH(30)HC(50)	11
Jian Xu	2011	China	Serum	n.a.	RT-PCR	Case-control	21, 122, 223	miR-181a miR-181c	101	HBV-HCC(76)other(25)	89	HC(89)	4
Kevin Z, Qu	2011	USA	Serum	Histology	RT-PCR	Case-control	16, 199a, AFP	U6	105	HBV-HCC(20)HCV-HCC(66) HBV + HCV-HCC(1)other(18)	107	CHB(8)CHC(59)other(40)	9
Peng Qi	2011	China	Serum	Histology	RT-PCR	Cohort	122	miR-16	70	HBV-HCC(70)	82	HC(34)HBV(48)	11
Lihua Li	2012	China	Serum	Histology	RT-PCR	Case-control	18a	U6	101	HBV-HCC(101)	90	HC(60) CHB or HBV-LC(30)	9
Angela M Liu	2012	China	Serum	Histology	RT-PCR	Cohort	15b, 130b, 21, 183	n.a.	57	HBV-HCC(57)	59	HC(30)CHB(29)	11
Zhoujing Zhang	2013	China	Serum	Histology	RT-PCR	Case-control	483-5p, AFP	Ath-miR-156a	112	HBV-HCC(79)other(33)	141	HC(56)CHB(85)	7
Jie Luo	2013	China	Plasma	Histology	RT-PCR	Case-control	122a, AFP	U6	85	HBV-HCC(75)other(10)	85	HC(16)HBV(69)	7
Jing Shen	2013	USA	Plasma	Histology	RT-PCR	Case-control	483-5p	cel-miR-39	49	HBV-HCC(6)HCV-HCC(32)other(11)	49	HC(39)HBV(7)HCV(1)HBV + HCV(2)	10
Jianjian Zheng	2013	China	Serum	Histology	RT-PCR	Case-control	29b, AFP	U6	87	HCC(87)	96	HC(96)	6
Yun Xie	2014	China	Serum	Histology	RT-PCR	Case-control	101, AFP	cel-miR-39	67	HBV-HCC(67)	170	HC(30)CHB(79)HBV-LC(61)	9
Youwen Tan	2014	China	Serum	Histology	RT-PCR	Case-control	206, 141-3p, 433-3p, 1228-5p, 199a-5p, 122-5p, 192-5p, 26a-5p, AFP	miR-24	238	HBV-HCC(238)	360	HC(150)HBV-LC(210)	8
Tao Li	2014	China	Plasma	Histology	RT-PCR	Case-control	139, AFP	miR-16	31	HCC(31)	31	CHB(31)	8
Zhuqing Zhang	2014	China	Plasma	Histology or imaging	RT-PCR	Case-control	143, 215	U6	95	HCC(95)	127	HC(127)	8
Hassan El-Garem	2014	Egypt	Serum	n.a.	RT-PCR	Case-control	221	SNORD68	30	HCV-HCC(30)	60	CHC(30)HCV-LC(30)	6
Liwei Qiu	2014	China	Serum	Histology	RT-PCR	Case-control	96, 182, AFP	miR-16	60	HBV(49)other(11)	180	HC(60)LC(60)CHB(60)	10
Fanlong Meng	2014	China	Serum	Histology	RT-PCR	Case-control	106b	cel-miR-39	47	HBV(47)	53	HC(31)CLDs(22)	8
Zhifeng Yang	2014	China	Serum	Histology	RT-PCR	Case-control	221, 338	U6	80	HCC(80)	140	CLDs(60)HC(80)	5
Yang Wen	2015	China	Plasma	Histology or imaging	RT-PCR	Cohort	20a-5p, 25-3p, 30a-5p, 92a-3p, 132-3p, 185-5p, 320a, 324-3p	cel-miR-39	67	HBV-HCC(67)	82	HBV(82)	6
Li Jiang	2015	China	Plasma	n.a.	RT-PCR	Case-control	106b	U6	47	HBV-HCC(37)other(10)	36	HC(36)	5
Tarek K. Motawi	2015	Egypt	Serum	Imaging	RT-PCR	Case-control	19a, 195, 192, 146a, 296, 130a, 34a, AFP	SNORD68	112	HCV-HCC(112)	167	HC(42)HCV-LC early(75)HCV-LC late(50)	10
L.Yang	2015	China	Serum	Histology	RT-PCR	Case-control	218, AFP	RNU6B	156	HBV-HCC(113)other(43)	64	HC(64)	9
Fujun Yu	2015	China	Serum	Histology	RT-PCR	Case-control	150	miR-16	120	HBV-HCC(120)	230	HC(120)CHB(110)	10
Nevine E. EL-Abd	2015	Egypt	Serum	Imaging	RT-PCR	Case-control	16, AFP	RNU48 RNU6B	40	HCV-HCC(40)	40	HCV-LC(40)	6
Y.Wang	2015	China	Serum Plasma	n.a.	RT-PCR	Case-control	183	U6, cel-miR-39	38	HCC(38)	52	CH(19)LC(12)HC(21)	4
Kun He	2015	China	Serum	Histology	RT-PCR	Case-control	301	GAPDH	42	HBV-HCC(29)other(13)	38	HC(20)LC(9)CHB(9)	8
Yi Chen	2015	China	Plasma	Histology	RT-PCR	Cohort	15b-5p, 338-5p, 764	miR-16	47	HCC(47)	60	HC(31)LC(29)	8
Jian Yin	2015	China	Serum	Histology	RT-PCR	Case-control	375, 199a-3p	U6	78	HBV-HCC(49)other(29)	156	HC(156)	10
Feng Wang	2015	China	Serum	Histology	RT-PCR	Case-control	148a, 148b, 152	cel-miR-39	76	HBV-HCC(55)other(21)	117	HC(55)CH(35)LC(21)FLD(4)ALD(2)	8
Duo Zuo	2015	China	Serum	Histology	RT-PCR	Case-control	125b, 223, 27a, 26a, AFP	U6	145	HBV-HCC(90)early HCC HBV(55)	60	HC(30)CHB(30)	11
Lin Chen	2015	China	Serum	Histology	RT-PCR	Case-control	182, 331-3p, AFP	U6	103	HBV-HCC(72)other(31)	95	LC(39)CH(47)FLD(9)	9

Table 1 (*Continued*)

Author	Year	Region	Specimen	Conference test	Index test	Study design	Markers studied	Normalization controls	HCC patients		Non-HCC controls		QUADAS score
									No.	Population	No.	Population	
Xuejia Lin	2015	China	Serum	Histology or imaging	RT-PCR	Cohort	29a, 29c, 133a, 143, 145, 192, 505, AFP	cel-miR-67	310	HBV-HCC(27)other(283)	438	HC(159)CHB(119)HBV-LC(118) HBV(42)	8
Xuguang Mi	2015	China	Serum	Histology or imaging	RT-PCR	Case-control	199a/b-3p, AFP	U6	30	HCC(30)	60	CLDs(30)HC(30)	6
Francesca Fornari	2015	Italy	Serum	n.a.	RT-PCR	Case-control	939, 595, 519d	cel-miR-39	85	HCC(85)	30	LC(30)	8
Chunbo Zhuan	2015	China	Serum	Histology	RT-PCR	Case-control	21, 26a, 101, AFP	cel-miR-39, U6	52	HBV-HCC(33)HCV-HCC(2)other(17)	85	HC(43)CHB(28)CHC(5)other CH(9)	9
Chao-Hung Hung	2016	China	Serum	Histology	RT-PCR	Case-control	122, 7b, AFP	U6	120	HBV-HCC(120)	30	HBV-DN(30)	8
Amit Ghosh	2016	India	Plasma	Histology or imaging	RT-PCR	Case-control	126, 142-3p, AFP	cel-miR-39	49	HBV-HCC(49)	38	HBV-LC(20)CHB(18)	6
Sourav Bhattacharya	2016	USA	Serum	n.a.	RT-PCR	Case-control	30e, 223	miR-39	39	HBV-HCC(14)HCV-HCC(14)other(11)	31	CLD(17)HC(14)	5
Shanshan Chen	2016	China	Plasma	Histology or imaging	RT-PCR	Case-control	125b	U6	64	HBV-HCC(64)	178	HC(56)CHB(63)HBV-LC(59)	8
Khalda Said Amr	2016	Egypt	Serum	Histology	RT-PCR	Case-control	21, 199a, AFP	18S RNA	23	HBV-HCC(3)HCV-HCC(19)HBV + HCV-HCC(1)	17	CHB(5)CHC(10)PBC(1)PSC(1)	10
Ling Lin	2016	China	Serum	Histology	RT-PCR	Cohort	224, AFP	cel-miR-39	96	HBV-HCC(96)	146	HBV-LC(39)CHB(51)HC(56)	8
Xiaohong Chen	2016	China	Serum	Histology	RT-PCR	Case-control	21	miR-16	62	HCC(62)	62	HC(62)	9
Maofeng Wu	2016	China	Serum	Histology	RT-PCR	Case-control	205-5p	U6	34	HBV-HCC(34)	60	HBV-LC(21)CHB(20)HC(19)	10
Pan Wang	2016	China	Serum	n.a.	RT-PCR	Case-control	21, 4429, AFP	n.a.	69	HBV-HCC(21)other(48)	87	CHB(47)HC(40)	4
Tarek M. K. Motawi	2016	Egypt	Serum	Histology or imaging	RT-PCR	Case-control	222	SNORD68	60	HCV-HCC(60)	40	HCV(40)	7
Haotu Zhu	2016	China	Serum	Histology	RT-PCR	Cohort	27b-3p, 192-5p	cel-miR-39	212	HBV-HCC(174)other(38)	216	HBV-LC(106)HC(110)	9
Shanshan Chen	2016	China	Plasma	Histology	RT-PCR	Case-control	205, AFP	U6	64	HBV-HCC(64)	115	HBV-LC(59)HC(56)	8
Yu Zhang	2016	China	Serum	Histology	RT-PCR	Case-control	335, AFP	U6	50	HCC(50)	40	HC(40)	6
Xin Guo	2017	China	Serum	Histology	RT-PCR	Cohort	21, AFP	U6	175	HCC(175)	278	CHB(64)LC(78)HC(136)	10
Hamdy E. Abouzeid Ali	2017	Egypt	Serum	n.a.	RT-PCR	Case-control	126, 93, 21, 130a, 30c, 193b, 122, 222, 125b	miR-16	34	HCV-HCC(25)other(9)	77	HCV(52)HC(25)	6
Amal Ahmed Mohamed	2017	Egypt	Serum	Imaging	RT-PCR	Case-control	23a, 34, 203, 338, 16, AFP	U6	57	HCC(57)	114	LC(57)HC(57)	8
Moustafa Nouh Elemeery	2017	Egypt	Serum	Imaging	RT-PCR	Case-control	1269, 494, 125b, 138b, 214-5p, 375, 145	SNORD68	224	HCV-HCC(224)	334	CHC(250)HC(84)	9
Marwa Tarek	2017	Egypt	Serum	n.a.	RT-PCR	Case-control	7	SNORD68	30	HBV-HCC(3)HCV-HCC(26)other(1)	60	HC(30)CLDs(30)	6
Olfat Shaker	2017	Egypt	Serum	n.a.	RT-PCR	Case-control	221, 101-1	SNORD68	37	HCV-HCC(37)	78	HCV(46)HC(32)	6
Jian Zhang	2017	China	Serum	Histology	RT-PCR	Case-control	143	GAPDH	131	HBV-HCC(56)other(75)	122	HC(122)	8
Chuhong Zhang	2017	China	Serum	n.a.	RT-PCR	Case-control	122a, 6086, AFP	n.a.	45	HBV-HCC(45)	45	HC(45)	8
Tian Hu	2017	China	Serum	Histology or imaging	RT-PCR	Case-control	4281	miR-39	45	HBV-HCC(19)other(26)	45	HC(45)	5

CHB:chronic hepatitis B;CHC:chronic hepatitis C;HC:healthy control;LC:liver cirrhosis;CH:chronic hepatitis;n.a.:not available;HBV:hepatitis B virus infection;HCV:hepatitis C virus infection;DN:dysplastic nodule;CLDs:chronic liver diseases;PBC:primary biliary cirrhosis;PSC:primary sclerosing cholangitis; ALD:alcoholic liver disease; FLD:fatty liver disease.

Table 2
The results of meta-analysis.

	Sensitivity(95%CI)	Specificity(95%CI)	PLR(95%CI)	NLR(95%CI)	DOR(95%CI)	AUC(95%CI)	Number of data sets	Number of HCC	Number of non-HCC
Single miRNAs									
HCC vs non-HCC	0.80(0.78–0.82)	0.73(0.71–0.76)	3.03(2.79–3.28)	0.27(0.24–0.30)	11.27(9.67–13.13)	0.84(0.80–0.87)	212	16651	14349
HCC vs CLDs	0.77(0.74–0.80)	0.71(0.68–0.74)	2.67(2.39–2.97)	0.32(0.28–0.37)	8.25(6.78–10.05)	0.80(0.77–0.84)	102	7954	6828
HCC vs HC	0.85(0.81–0.88)	0.79(0.76–0.81)	4.03(3.57–4.54)	0.19(0.16–0.24)	20.71(15.51–27.66)	0.87(0.84–0.89)	65	5783	3556
miRNAs panel									
HCC vs non-HCC	0.83(0.79–0.87)	0.87(0.83–0.91)	6.64(4.89–9.00)	0.19(0.15–0.24)	34.91(21.88–55.69)	0.92(0.89–0.94)	40	3709	3252
HCC vs CLDs	0.81(0.73–0.87)	0.83(0.77–0.88)	4.84(3.34–7.02)	0.23(0.15–0.33)	21.46(11.07–41.58)	0.89(0.86–0.92)	19	1749	1289
HCC vs HC	0.85(0.75–0.91)	0.94(0.82–0.98)	13.63(4.12–45.10)	0.16(0.09–0.29)	84.64(15.51–461.90)	0.94(0.92–0.96)	9	849	543
miRNAs(single + panel)									
HCC vs non-HCC	0.81(0.79–0.83)	0.76(0.74–0.78)	3.40(3.11–3.71)	0.25(0.23–0.28)	13.49(11.55–15.74)	0.85(0.82–0.88)	252	20360	17601
HCC vs CLDs	0.78(0.75–0.81)	0.73(0.70–0.76)	2.92(2.61–3.27)	0.30(0.27–0.35)	9.62(7.85–11.78)	0.82(0.79–0.85)	121	9703	8117
HCC vs HC	0.85(0.81–0.88)	0.81(0.78–0.84)	4.53(3.85–5.34)	0.19(0.15–0.23)	24.15(17.52–33.30)	0.89(0.86–0.92)	74	6632	4099
AFP									
HCC vs non-HCC	0.64(0.59–0.68)	0.92(0.87–0.95)	7.66(5.09–11.52)	0.40(0.36–0.44)	19.29(12.51–29.72)	0.80(0.76–0.83)	52	4722	4320
HCC vs CLDs	0.61(0.55–0.67)	0.89(0.82–0.94)	5.79(3.58–9.35)	0.43(0.38–0.50)	13.35(8.05–22.13)	0.78(0.74–0.81)	27	2495	1704
HCC vs HC	0.71(0.60–0.80)	0.96(0.92–0.98)	17.00(8.33–34.72)	0.31(0.22–0.44)	55.33(21.27–143.90)	0.95(0.93–0.97)	8	675	391
Single miRNAs combine AFP									
HCC vs non-HCC	0.86(0.83–0.89)	0.87(0.83–0.91)	6.88(5.08–9.32)	0.16(0.13–0.20)	43.11(28.22–65.85)	0.93(0.90–0.95)	28	2303	1760
HCC vs CLDs	0.86(0.81–0.90)	0.85(0.79–0.90)	5.73(4.02–8.16)	0.17(0.12–0.23)	33.84(19.28–59.38)	0.92(0.89–0.94)	17	1363	874
HCC vs HC	0.87(0.81–0.92)	0.87(0.79–0.92)	6.75(4.20–10.85)	0.15(0.10–0.22)	46.39(23.66–90.97)	0.94(0.91–0.95)	6	509	340
miRNAs panel combine AFP									
HCC vs non-HCC	0.83(0.80–0.86)	0.89(0.86–0.91)	7.65(6.08–9.62)	0.19(0.15–0.23)	41.27(28.56–59.65)	0.93(0.90–0.95)	27	2214	2119
HCC vs CLDs	0.80(0.73–0.85)	0.89(0.86–0.91)	7.18(5.36–9.61)	0.23(0.17–0.31)	31.17(18.41–52.77)	0.92(0.89–0.94)	13	1073	843
HCC vs HC	0.90(0.85–0.93)	0.90(0.81–0.95)	8.95(4.44–18.04)	0.11(0.07–0.17)	80.99(31.59–207.61)	0.94(0.91–0.96)	4	252	133
miRNAs(single + panel) combine AFP									
HCC vs non-HCC	0.85(0.82–0.87)	0.88(0.86–0.90)	7.17(5.92–8.68)	0.17(0.15–0.20)	41.17(31.15–54.42)	0.93(0.90–0.95)	55	4517	3879
HCC vs CLDs	0.83(0.79–0.86)	0.87(0.84–0.90)	6.39(5.00–8.17)	0.19(0.16–0.24)	32.88(22.24–48.63)	0.92(0.89–0.94)	30	2436	1717
HCC vs HC	0.88(0.85–0.91)	0.89(0.83–0.93)	7.74(5.05–11.88)	0.13(0.10–0.17)	59.18(33.01–106.09)	0.94(0.92–0.96)	10	761	473

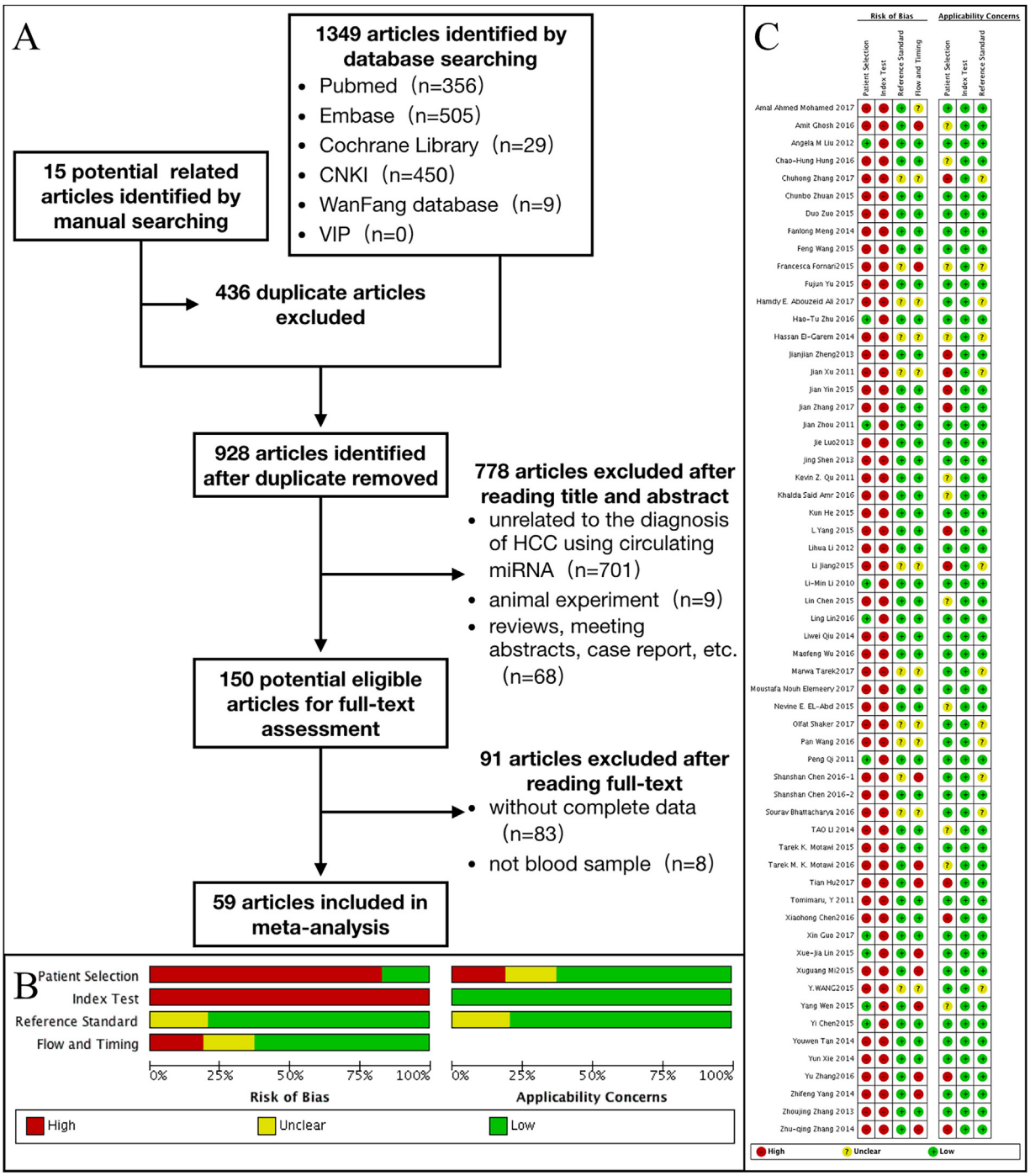

Fig 1. Reference search strategy and quality assessment of studies selected for analysis. (A) Flow diagram of study identification and selection for meta-analysis. (B) Risk of bias and applicability concerns graph: review authors' judgments about each domain presented as percentages across included studies. (C) Risk of bias and applicability concerns summary: review authors' judgments about each domain for each included study.

3.2. Diagnostic accuracy of circulating miRNAs

The sensitivity and specificity of circulating miRNAs (including single miRNAs and miRNA panels) in discriminating cases of HCC from cases of non-HCC were 0.81 (95% CI: 0.79–0.83) and 0.76 (95% CI: 0.74–0.78), respectively. The PLR, NLR, DOR, and AUC with their corresponding 95% CIs were 3.40 (95% CI: 3.11–3.71), 0.25 (95% CI: 0.23–0.28), 13.49 (95% CI: 11.55–15.74), and 0.85 (95% CI: 0.82–0.88), respectively. For single miRNAs, the pooled sensitivity, specificity, PLR, NLR, DOR, and AUC were 0.80 (95% CI: 0.78–0.82), 0.73 (95% CI: 0.71–0.76), 3.03 (95% CI: 2.79–3.28), 0.27 (95% CI: 0.24–0.30), 11.27 (95% CI: 9.67–13.13), and 0.84 (95% CI: 0.80–0.87), respectively. The combination of multiple miRNAs (miRNA panels) improved the diagnostic power with an increase in sensitivity of 0.83 (95% CI: 0.79–0.87), specificity of 0.87 (95% CI: 0.83–0.91), PLR of 6.64 (95% CI: 4.89–9.00), NLR of 0.19 (95% CI: 0.15–0.24), DOR of 34.91 (95% CI: 21.88–55.69), and AUC of 0.92 (95% CI 0.89–0.94). The results are detailed in Table 2 and Fig. 2(A–C).

We also conducted pooled analysis of circulating miRNAs in discriminating patients with HCC from healthy controls (HC) or CLD controls. Briefly, the results showed that the diagnostic effectiveness of an miRNA panel is clearly better than that of single miRNAs.

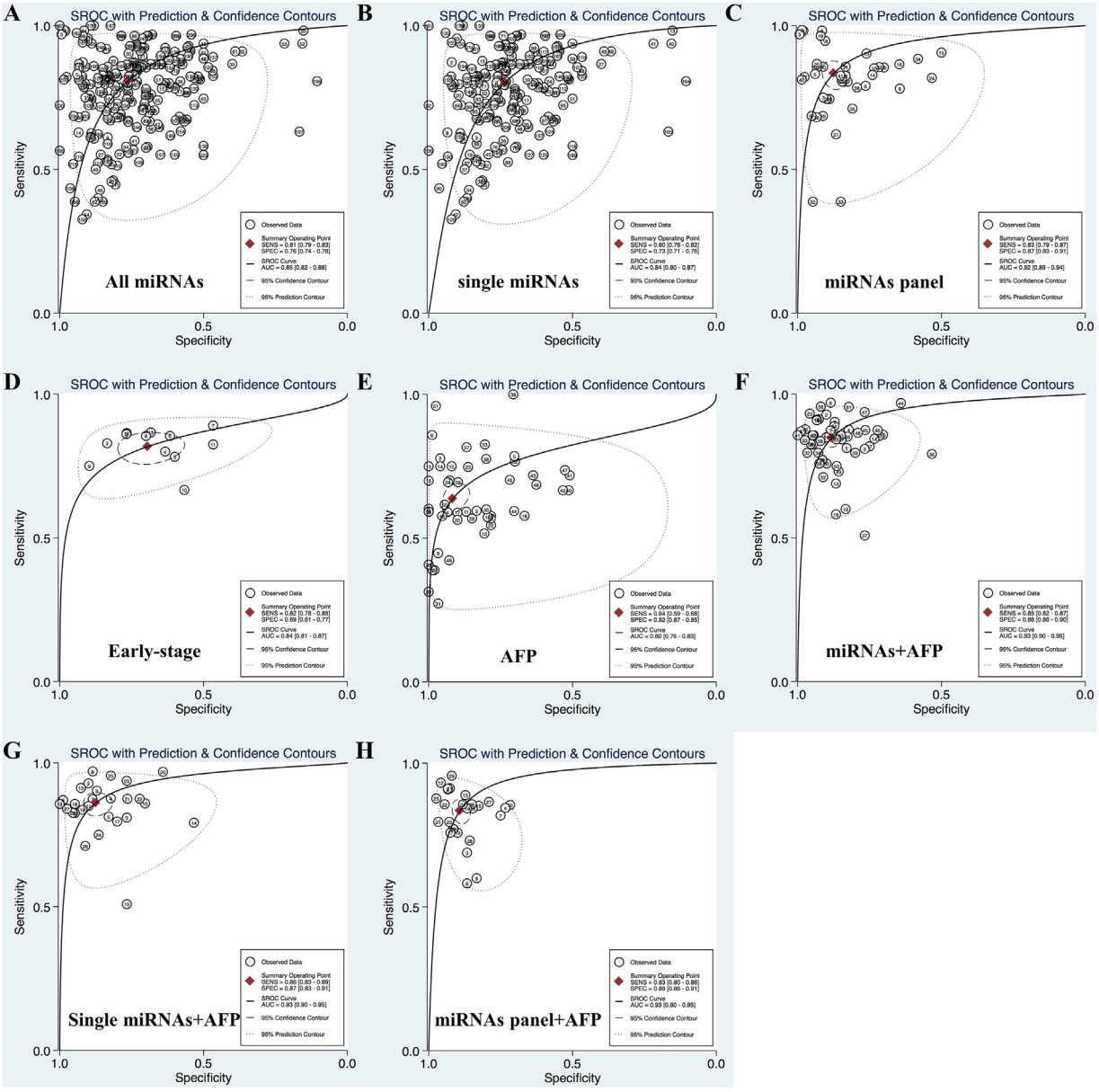

Fig. 2. Summary receiver operating characteristic (sROC) curve describes the diagnostic performance of circulating microRNAs (miRNAs), alpha-fetoprotein (AFP), and miRNAs combined with AFP in discriminating hepatocellular carcinoma (HCC) from non-HCC controls. (A) sROC of all miRNAs; (B) sROC of single miRNAs; (C) sROC of miRNA panels; (D) sROC of circulating miRNAs for the diagnosis of early-stage HCC patients; (E) sROC of AFP; (F) sROC of miRNAs combined with AFP; (G) sROC of single miRNAs combined with AFP; (H) sROC of miRNA panels combined with AFP. Every solid circle represents a study.

In addition, the diagnostic accuracy of circulating miRNAs, single miRNAs, and miRNA panels in distinguishing HCC from HC is higher than that of distinguishing HCC from CLD. These results are detailed in Table 2.

In reviewing the 59 articles included in the present study, we also analyzed the diagnostic accuracy of circulating miRNAs for early-stage HCC patients, which refers to HCC patients with Barcelona Clinic Liver Cancer (BCLC) stage 0 or stage A. We extracted 12 data sets, including 940 HCC patients and 1527 non-HCC controls; the results showed that the pooled sensitivity, specificity, and AUC were 0.82 (95% CI: 0.78–0.85), 0.69 (95% CI: 0.61–0.77) and 0.84 (95% CI: 0.81–0.87), respectively. The results are detailed in Fig. 2(D).

A total of 80 different single miRNAs and 17 miRNA panels were mentioned in our included studies, of which 33 were studied in a single data set. From this data set, we extracted the sensitivity, specificity, PLR, NLR and DOR from the studies (listed in Supplemental Table 1). For the other 64 single miRNAs and miRNA panels, we conducted pooled analysis to summarize their diagnostic accuracy. Among these 64 single miRNAs or miRNA panels, miR-132-3p, 145, 146a, 185-5p, 193b, 205, 30e, and 375 showed high sensitivity (>90%), while miR-199a, 29b, 338-5p, and 595 exhibited high specificity (>90%). The miRNAs miR-130b, 150, 182, 215, and 96 yielded satisfactory sensitivity (>80%) as well as specificity (>80%). Additionally, 5 miRNA panels showed high sensitivity (>90%) and specificity (>90%), such as miR-10a combined with 125b. The results are given in Supplemental Table 2.

3.3. *Diagnostic accuracy of AFP and miRNAs combined with AFP*

The pooled diagnostic accuracy of AFP in discriminating HCC from non-HCC was: sensitivity 0.64 (95% CI: 0.59–0.68), specificity 0.92 (95% CI: 0.87–0.95), PLR 7.66 (95% CI: 5.09–11.52), NLR 0.40 (95% CI: 0.36–0.44), DOR 19.29, (95% CI: 12.51–29.72), and AUC 0.80

Table 3
The results of subgroup analysis and meta-regression.

	Sensitivity(95%CI)	Specificity (95%CI)	PLR (95%CI)	NLR (95%CI)	DOR (95%CI)	AUC (95%CI)	Number of data sets	Number of HCC	Number of non-HCC	Heterogeneity ($OR\text{-}I^2$)	tau^2	$Adj\text{-}R^2$
Region											0.99	−0.57%
China	0.79(0.77–0.81)	0.77(0.74–0.79)	3.46(3.12–3.84)	0.27(0.24–0.29)	12.99(11.03–15.30)	0.85(0.82–0.88)	166	12262	11119	74.3%		
Egypt	0.85(0.82–0.88)	0.72(0.67–0.76)	3.00(2.54–3.55)	0.21(0.16–0.26)	14.63(10.06–21.29)	0.85(0.82–0.88)	67	6777	5706	88.9%		
USA, Italy, Japan, India	0.74(0.63–0.83)	0.84(0.78–0.89)	4.68(3.16–6.93)	0.31(0.20–0.46)	15.34(7.50–31.35)	0.87(0.84–0.90)	19	1321	776	77.1%		
Specimen											0.97	1.46%
Serum	0.82(0.80–0.84)	0.77(0.74–0.79)	3.53(3.19–3.91)	0.23(0.21–0.26)	15.14(12.57–18.23)	0.86(0.83–0.89)	193	16837	14517	83.3%		
Plasma	0.76(0.72–0.80)	0.74(0.69–0.79)	2.98(2.53–3.51)	0.32(0.27–0.38)	9.34(7.32–11.91)	0.82(0.78–0.85)	59	3523	3084	66.6%		
Conference test											0.99	−0.19%
Histology	0.79(0.77–0.81)	0.80(0.77–0.82)	3.87(3.40–4.41)	0.26(0.23–0.29)	14.79(12.08–18.10)	0.86(0.83–0.89)	123	9918	8115	76.0%		
Histology or imaging	0.75(0.69–0.80)	0.71(0.65–0.77)	2.62(2.18–3.14)	0.35(0.30–0.42)	7.40(5.66–9.67)	0.80(0.76–0.83)	44	2445	2766	72.5%		
Imaging	0.88(0.83–0.91)	0.70(0.64–0.76)	2.96(2.39–3.66)	0.17(0.12–0.25)	17.27(10.21–29.22)	0.86(0.83–0.89)	42	5925	4509	91.9%		
Unclear	0.82(0.79–0.85)	0.77(0.72–0.80)	3.49(2.92–4.17)	0.24(0.20–0.28)	14.82(10.58–20.75)	0.86(0.83–0.89)	43	2072	2211	68.8%		
Study design											0.99	−0.55%
Case-control	0.81(0.79–0.83)	0.76(0.73–0.78)	3.31(3.01–3.64)	0.25(0.22–0.28)	13.20(11.02–15.82)	0.85(0.82–0.88)	184	15368	12863	82.9%		
Cohort	0.80(0.77–0.83)	0.78(0.73–0.82)	3.60(2.93–4.43)	0.26(0.22–0.30)	14.06(10.41–18.99)	0.86(0.83–0.89)	68	4992	4738	73.5%		
miRNA											0.86	12.32%
Single	0.80(0.78–0.82)	0.73(0.71–0.76)	3.03(2.79–3.28)	0.27(0.24–0.30)	11.27(9.67–13.13)	0.84(0.80–0.87)	212	16651	14349	79.7%		
Panel	0.83(0.79–0.87)	0.87(0.83–0.91)	6.64(4.89–9.00)	0.19(0.15–0.24)	34.91(21.88–55.69)	0.92(0.89–0.94)	40	3709	3252	77.2%		
Control population											0.95	11.61%
CLDs	0.78(0.75–0.81)	0.73(0.70–0.76)	2.92(2.61–3.27)	0.30(0.27–0.34)	9.63(7.87–11.77)	0.82(0.79–0.85)	122	9878	8195	79.6%		
HC	0.85(0.81–0.88)	0.81(0.78–0.84)	4.53(3.85–5.34)	0.19(0.15–0.23)	24.15(17.52–33.30)	0.89(0.86–0.92)	74	6632	4099	80.1%		
CLDs + HC	0.81(0.78–0.84)	0.74(0.69–0.79)	3.16(2.60–3.84)	0.26(0.22–0.29)	12.37(9.26–16.52)	0.85(0.82–0.88)	56	3850	5307	82.7%		
QUADAS score											0.96	2.5%
≥7	0.82(0.79–0.84)	0.78(0.75–0.80)	3.64(3.26–4.06)	0.24(0.21–0.27)	15.36(12.65–18.64)	0.87(0.83–0.89)	171	16551	13129	83.9%		
<7	0.79(0.75–0.82)	0.73(0.69–0.77)	2.92(2.55–3.35)	0.29(0.25–0.34)	10.06(7.94–12.76)	0.83(0.79–0.86)	81	3809	4472	69.3%		

(95% CI: 0.76–0.83). Similar to circulating miRNAs, the diagnostic accuracy of AFP in distinguishing HCC from HC was significantly higher than that of HCC from CLD. The combination of miRNAs and AFP improved the diagnostic accuracy compared with miRNAs alone. The results are shown in Table 2 and Fig. 2(E–H). A total of 29 different combinations of miRNAs and AFP were mentioned in our included studies, the combination of miR-331-3p and AFP, miR-182, 331-3p and AFP, miR-21, 4429 and AFP showed high sensitivity (>90%) and specificity (>90%), the results are given in Supplemental Table 3.

3.4. Results of subgroup analysis and meta-regression

As considerable heterogeneity was revealed in our meta-analysis, for the sake of exploring potential sources of heterogeneity, subgroup analysis and meta-regression were performed based on region, specimen type, conference test, study design, control population, QUADAS score, and miRNA type. However, after being divided into different subgroups, the I^2 of each subgroup was still >50%, which showed that the above factors did not significantly affect heterogeneity. The values of τ^2 and adjusted R^2 in meta-regression also supported that the above factors were not sources of heterogeneity. The pooled sensitivity, specificity, PLR, NLR, DOR, AUC and the values of I^2, τ^2, and adjusted R^2 of each group are shown in Table 3.

3.5. Results of sensitivity analysis and publication bias

In the sensitivity analysis, the removal of any individual study did not affect the overall outcome significantly. Most results had overlapping confidence intervals, ensuring that our findings were not significantly influenced by any individual study. To further evaluate potential publication bias, Deeks' funnel plot asymmetry test was conducted. The P value of every group in our meta-analysis was >0.05 (0.06–0.96), indicating that no significant publication bias was detected. These tests all confirmed the validity of our meta-analysis results.

4. Discussion

AFP is the most commonly used biomarker for HCC, but it has only modest sensitivity and accuracy and fails to detect HCC in half of patients. On the other hand, AFP is also elevated in patients with hepatitis and cirrhosis [78]. The progression of chronic hepatitis to cirrhosis or HCC may occur slowly over several decades, but by the time HCC is detected, prospects for successful treatment are often poor. Therefore, more sensitive biomarkers may help to achieve earlier detection. The dysregulation of miRNAs is involved in all stages of hepatocarcinogenesis, miRNAs have the potential to discriminate HCC patients from healthy subjects as well as those with other liver diseases [79]. Circulating miRNAs are stable in body fluids and are detectable, representing a promising approach to monitoring the progression of HCC.

In our meta-analysis, we mainly studied the diagnostic value of circulating miRNAs, single miRNAs, and miRNA panels in differentiating HCC patients from non-HCC controls, healthy controls, and patients with CLD (including HBV/HCV infection, chronic hepatitis, cirrhosis, etc). The results showed that the overall sensitivity and specificity of circulating miRNAs in distinguishing HCC patients from non-HCC controls were 0.81 (95% CI: 0.79–0.83) and 0.76 (95% CI: 0.74–0.78), respectively. The pooled PLR and NLR values were 3.40 (95% CI: 3.11–3.71) and 0.25 (95% CI: 0.23–0.28), respectively. The pooled DOR value was 13.49 (95% CI: 11.55–15.74). The corresponding AUC value was 0.85 (95% CI: 0.82–0.88) in the overall sROC curve. A PLR value of 3.40 suggests that patients with HCC have a 3.4-fold higher chance of having a positive miRNA assay than those without HCC. The pooled NLR was found to be 0.25, suggesting that if the miRNA assay result was negative, the probability of the patient suffering from HCC is approximately 25%. The closer the ROC curve is to the upper left corner, the closer the AUC value is to 1, and the better the diagnostic effectiveness [80]. A value of AUC between 0.80 and 0.90 is considered satisfactory, and a value >0.90 indicates a very high diagnostic accuracy. As for DOR, the greater, the better [81]. Therefore, miRNAs demonstrate good diagnostic potential. The sensitivity and specificity of using single miRNAs to distinguish between HCC and non-HCC controls were 0.80 (95% CI: 0.78–0.82) and 0.73 (95% CI: 0.71–0.76), respectively. The pooled PLR, NLR and DOR values were 3.03 (95% CI: 2.79–3.28), 0.27 (95% CI: 0.24–0.30), and 11.27 (95% CI: 9.67–3.13) respectively. The AUC was 0.84 (95% CI: 0.80–0.87). When multiple miRNAs were combined, the sensitivity and specificity rose to 0.83 (95% CI: 0.79–0.87) and 0.87 (95% CI: 0.83–0.91), respectively. The PLR, NLR and DOR values were 6.64 (95% CI: 4.89–9.00), 0.19 (95% CI: 0.15–0.24), and 34.91 (95% CI: 21.88–55.69) respectively. The AUC was 0.92 (95% CI: 0.89–0.94). This suggests that the use of miRNA panels is an ideal choice for the diagnosis of HCC, with high sensitivity and specificity, which reduces the rate of missed diagnosis and the rate of misdiagnosis. In addition, we also found that the use of multiple miRNAs, single miRNAs, or miRNA panels was more effective when differentiating HCC from HC than HCC from CLD.

The expression level of circulating miRNAs changes at the early stage of HCC, and is expected to be a new marker for early diagnosis. Our meta-analysis also suggested that for patients with BCLC stage 0 or stage A, circulating miRNAs yield a sensitivity of 0.82 (95% CI: 0.78–0.85), a specificity of 0.69 (95% CI: 0.61–0.77) and an AUC of 0.84 (95% CI: 0.81–0.87). The results of our analysis were relatively satisfactory, indicating that circulating miRNAs can be used as an early diagnostic marker. However, the data were not quite sufficient, and need further verification.

In order to verify the diagnostic effectiveness of the traditional index of AFP, we also extracted data from the literature about the diagnostic accuracy of AFP, and concluded that the sensitivity and specificity of AFP in distinguishing HCC from non-HCC controls were 0.64 (95% CI: 0.59–0.68) and 0.92 (95% CI: 0.87–0.95), respectively. The pooled PLR, NLR and DOR values were 7.66 (95% CI: 5.09–11.52), 0.40 (95% CI: 0.36–0.44), and 19.29 (95% CI: 12.51–29.72) respectively. The AUC was 0.80 (95% CI: 0.76–0.83). Hence, one can see that AFP has high specificity, although it reduces the misdiagnosis rate, but its low sensitivity is not conducive to early screening for HCC. In using the combination of AFP and single miRNAs, the diagnostic accuracy was significantly improved; the pooled sensitivity, specificity, PLR, NLR, DOR, and AUC were 0.86 (95% CI: 0.83–0.89), 0.87 (95% CI: 0.83–0.91), 6.88 (95% CI: 5.08–9.32), 0.16 (95% CI: 0.13–0.20), 43.11 (95% CI: 28.22–65.85), and 0.93 (95% CI: 0.90–0.95), respectively. The combination of AFP and miRNAs panels improved the diagnostic accuracy slightly compared with using miRNA panels alone, but not significantly. These results indicate that the combination of AFP and miRNAs can provide a new strategy for the diagnosis of HCC.

The present study also systematically reviewed the diagnostic effectiveness of 80 kinds of single miRNAs,17 miRNAs panels and 29 combinations of miRNAs and AFP in discriminating HCC from non-HCC controls. The results showed that miR-132-3p, 145, 146a, 185-5p, 193b, 205, 30e, 375, 199a, 29b, 338-5p, 595, 130b, 150, 182, 215, and 96 yielded a satisfactory sensitivity and/or specificity. Five miRNAs panels and Three combinations of miRNAs and AFP, such as miR-10a combined with 125b, miR-331-3p combined AFP, showed excellent diagnostic performance with both sensitivity and specificity over 90%. All of the above miRNAs are expected to be novel markers for HCC.

Heterogeneity comes mainly from two aspects; one is the threshold effect and the other is the non-threshold effect. Spearman

correlation analysis showed that the Spearman correlation coefficient was 0.162 and the P value was 0.10 (>0.05), indicating that there was no threshold effect. Deeks' funnel plot asymmetry test found no evidence of publication bias in this meta-analysis, indicating that publication bias could not be identified as a source of heterogeneity. Meta-regression and subgroup analysis also failed to identify heterogeneous sources, suggesting that the influencing factors are complex. We conjecture that heterogeneity comes mainly from the following aspects: (1) cut-off values varied among studies, because the studies determined the cut-off value through an ROC curve instead of predefining; (2) there were too many kinds of microRNAs and normalization control miRNAs in the 59 included studies; (3) forty-five of the studies included in this meta-analysis were in Asian populations, which may have resulted in population selection bias.

The present study had some limitations. First, a high-quality diagnostic study should enroll a consecutive or random sample of eligible patients with suspected disease to prevent the potential for bias. The patients enrolled in the included studies of our meta-analysis were clearly diagnosed by the conference method which might overestimate the diagnostic effectiveness of miRNAs [15]. Second, heterogeneity was present in our meta-analysis, a situation common in meta-analyses of diagnostic accuracy [82,83]. Third, the majority of HCC patients (3447 out of 5125) we included were associated with HBV or HCV. Regrettably, for the rest of the patients, there was no detailed information being described in the papers. However, the proportion of patients with HCC developing as a consequence of metabolic disorders or metabolic syndrome, which is also referred to as metabolic HCC, has increased in recent years, especially in the western countries [84]. The value of circulating miRNAs in the diagnosis of metabolic HCC remains unknown and is interested in being further evaluated in the future.

But the present study also had advantages. First, we established a well-designed search strategy and inclusion criteria with no restrictions on language or publication year, and 59 articles were included for analysis. Compared with the studies by He [85] and Hu [86], the results of the present study may be more representative thanks to the larger sample size. Second, we not only summarized the diagnostic accuracy of circulating miRNAs, AFP, and miRNAs combined with AFP, but also systematically reviewed the diagnostic effectiveness of every individual single miRNAs, miRNAs panel and combination of miRNAs and AFP, providing valuable reference data for further research. Third, we evaluated the diagnostic effectiveness of circulating miRNAs for early-stage HCC patients, which had not previously been investigated.

In conclusion, the present study demonstrated that circulating miRNAs provide satisfactory diagnostic accuracy for HCC and can serve as markers comparable to conventional AFP, and could complement the use of AFP. The combination of miRNAs and AFP provides a more satisfactory diagnostic performance, showing the potential of being a novel non-invasive tool for the early diagnosis of HCC.

Conflict of interest

None declared.

Acknowledgements

The study was sponsored by the National Natural Science Foundation of China (NSFC) (81273264); International Cooperation Fund of Jilin Province Science & Technology Bureau (20160414022GH).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.12.011.

References

[1] Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet 2012;379(9822):1245–55.
[2] Nordenstedt H, White DL, Elserag HB. The changing pattern of epidemiology in hepatocellular carcinoma. Dig Liver Dis 2010;42(Suppl 3 (2)):S206.
[3] Colli A, Fraquelli M, Casazza G, et al. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. Am J Gastroenterol 2006;101(3):513–23.
[4] Zhang W, Zhao G, Wei K, et al. Adjuvant sorafenib reduced mortality and prolonged overall survival and post-recurrence survival in hepatocellular carcinoma patients after curative resection: a single-center experience. Biosci Trends 2014;8(6):333–8.
[5] Sasaki K, Matsuda M, Ohkura Y, et al. The influence of histological differentiation grade on the outcome of liver resection for hepatocellular carcinomas 2 cm or smaller in size. World J Surg 2015;39(5):1134–41.
[6] Zhou Z, Lei J, Li B, et al. Liver resection and radiofrequency ablation of very early hepatocellular carcinoma cases (single nodule <2 cm): a single-center study. Eur J Gastroenterol Hepatol 2014;26(3):339–44.
[7] Guo R, Feng X, Xiao S, et al. Short- and long-term outcomes of hepatectomy with or without radiofrequency-assist for the treatment of hepatocellular carcinomas: a retrospective comparative cohort study. Biosci Trends 2015;9(1):65–72.
[8] Ge S, Huang D. Systemic therapies for hepatocellular carcinoma. Drug Discov Ther 2015;9(5):352–62.
[9] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10(10):704–14.
[10] Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 2010;11(4):252–63.
[11] Arrese M, Eguchi A, Feldstein AE. Circulating microRNAs: emerging biomarkers of liver disease. Semin Liver Dis 2015;35(1):43–54.
[12] Giordano S, Columbano A. MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? Hepatology 2013;57(2):840–7.
[13] Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18(10):997–1006.
[14] Whiting P, Rutjes AW, Reitsma JB, et al. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. Chin J Evid Based Med 2003;3(3):25.
[15] Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155(8):529–36.
[16] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Br Med J 2003;327(7414):557–60.
[17] Jackson D, White IR, Thompson SG. Extending DerSimonian and Laird's methodology to perform multivariate random effects meta-analyses. Stat Med 2010;29(12):1282–97.
[18] Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58(9):882–93.
[19] Li LM, Hu ZB, Zhou ZX, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. Cancer Res 2010;70(23):9798–807.
[20] Qi P, Cheng SQ, Wang H, et al. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. PLoS One 2011;6(12):e28486.
[21] Qu KZ, Zhang K, Li H, et al. Circulating microRNAs as biomarkers for hepatocellular carcinoma. J Clin Gastroenterol 2011;45(4):355–60.
[22] Xu J, Wu C, Che X, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog 2011;50(2):136–42.
[23] Zhou J, Yu L, Gao X, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol 2011;29(36):4781–8.
[24] Li L, Guo Z, Wang J, et al. Serum miR-18a: a potential marker for hepatitis B virus-related hepatocellular carcinoma screening. Dig Dis Sci 2012;57(11):2910–6.
[25] Liu AM, Yao TJ, Wang W, et al. Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. BMJ Open 2012;2(2):e000825.
[26] Tomimaru Y, Eguchi H, Nagano H, et al. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. J Hepatol 2012;56(1):167–75.
[27] Luo J, Chen M, Huang H, et al. Circulating microRNA-122a as a diagnostic marker for hepatocellular carcinoma. Onco Targets Ther 2013;6:577–83.
[28] Shen J, Wang A, Wang Q, et al. Exploration of genome-wide circulating microRNA in hepatocellular carcinoma: MiR-483-5p as a potential biomarker. Cancer Epidemiol Biomarkers Prev 2013;22(12):2364–73.
[29] Zhang Z, Ge S, Wang X, et al. Serum miR-483-5p as a potential biomarker to detect hepatocellular carcinoma. Hepatol Int 2013;7(1):199–207.

[30] Zheng JJ, Yu FJ, Dong PH, et al. Expression of miRNA-29b and its clinical significances in primary hepatic carcinoma. Zhonghua Yi Xue Za Zhi 2013;93(12):888–91.
[31] El-Garem H, Ammer A, Shehab H, et al. Circulating microRNA, miR-122 and miR-221 signature in Egyptian patients with chronic hepatitis C related hepatocellular carcinoma. World J Hepatol 2014;6(11):818–24.
[32] Li T, Yin J, Yuan L, et al. Downregulation of microRNA-139 is associated with hepatocellular carcinoma risk and short-term survival. Oncol Rep 2014;31(4):1699–706.
[33] Meng FL, J W, Xu GL, Li JS, Wang W, Sun QK. Serum miR-106b expression and its clinical significance in hepatocellular carcinoma. Acta Univ Med Anhui 2014;(3):334–8.
[34] Qiu LW, W W, Sai WL, Yang JL, Zhang HJ, Zheng WJ, et al. Diagnostic application and comparative analysis of serum miR-96 and miR-182 in patients with hepatocellular carcinoma. J Nantong Univ (Med Sci) 2014;(5):358–61.
[35] Tan Y, Ge G, Pan T, et al. A serum microRNA panel as potential biomarkers for hepatocellular carcinoma related with hepatitis B virus. PLoS One 2014;9(9):e107986.
[36] Xie Y, Yao Q, Butt AM, et al. Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Cancer Biol Ther 2014;15(9):1248–55.
[37] Yang ZF, C H, Jiang XM. Serum miR-221 and miR-338 expression and its clinical significance in hepatocellular carcinoma. Guangdong Med J 2014;(6):841–3.
[38] Zhang ZQ, Meng H, Wang N, et al. Serum microRNA 143 and microRNA 215 as potential biomarkers for the diagnosis of chronic hepatitis and hepatocellular carcinoma. Diagn Pathol 2014;9:135.
[39] Chen L, Chu F, Cao Y, et al. Serum miR-182 and miR-331-3p as diagnostic and prognostic markers in patients with hepatocellular carcinoma. Tumour Biol 2015;36(10):7439–47.
[40] Chen Y, Chen J, Liu Y, et al. Plasma miR-15b-5p, miR-338-5p, and miR-764 as biomarkers for hepatocellular carcinoma. Med Sci Monit 2015;21:1864–71.
[41] El-Abd NE, Fawzy NA, El-Sheikh SM, et al. Circulating miRNA-122, miRNA-199a, and miRNA-16 as biomarkers for early detection of hepatocellular carcinoma in Egyptian patients with chronic hepatitis C virus infection. Mol Diagn Ther 2015;19(4):213–20.
[42] Fornari F, Ferracin M, Trere D, et al. Circulating microRNAs, miR-939, miR-595, miR-519d and miR-494, identify cirrhotic patients with HCC. PLoS One 2015;10(10):e0141448.
[43] He K, Hu Z, Ruan J, et al. MicroRNA301 is a potential diagnostic biomarker for hepatocellular cancer. Int J Clin Exp Pathol 2015;8(5):5603–8.
[44] Jiang L, Li X, Cheng Q, et al. Plasma microRNA might as a potential biomarker for hepatocellular carcinoma and chronic liver disease screening. Tumour Biol 2015;36(9):7167–74.
[45] Lin XJ, Chong Y, Guo ZW, et al. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. Lancet Oncol 2015;16(7):804–15.
[46] Mi XG, L L, Li SQ, Fang YQ. Value of serum microRNA-199a/b-3p on early diagnosis of hepatocellular carcinoma. Chin J Immunol 2015;(5):683–5.
[47] Motawi TK, Shaker OG, El-Maraghy SA, et al. Serum microRNAs as potential biomarkers for early diagnosis of hepatitis C virus-related hepatocellular carcinoma in Egyptian patients. PLoS One 2015;10(9):e0137706.
[48] Wang Y, Liang Z, Gao Y, et al. Factors influencing circulating microRNA level in the studies of hepatocellular carcinoma biomarker. Neoplasma 2015;62(5):798–804.
[49] Wen Y, Han J, Chen J, et al. Plasma miRNAs as early biomarkers for detecting hepatocellular carcinoma. Int J Cancer 2015;137(7):1679–90.
[50] Yin J, Hou P, Wu Z, et al. Circulating miR-375 and miR-199a-3p as potential biomarkers for the diagnosis of hepatocellular carcinoma. Tumour Biol 2015;36(6):4501–7.
[51] Yu F, Lu Z, Chen B, et al. microRNA-150: a promising novel biomarker for hepatitis B virus-related hepatocellular carcinoma. Diagn Pathol 2015;10:129.
[52] Amr KS, Ezzat WM, Elhosary YA, et al. The potential role of miRNAs 21 and 199-a in early diagnosis of hepatocellular carcinoma. Gene 2016;575(1):66–70.
[53] Bhattacharya S, Steele R, Shrivastava S, et al. Serum miR-30e and miR-223 as novel noninvasive biomarkers for hepatocellular carcinoma. Am J Pathol 2016;186(2):242–7.
[54] Chen SS, C H, Gao SS, Zhou H, Qiu SL, Yu MX, et al. Differential expression of plasma miR-205 in HBV-related liver diseases and diagnostic potential for hbv-induced hepatocellular carcinoma. Med J Wuhan Univ 2016;37(3):445–50.
[55] Chen XH, W Y, Zhang L, Yin D, Gao Y, Zhang ZX. Diagnostic value of serum miR-21 combined with ultrasound in hepatocellular carcinoma. Med J Commun 2016;30(5):465–7.
[56] Ghosh A, Ghosh A, Datta S, et al. Hepatic miR-126 is a potential plasma biomarker for detection of hepatitis B virus infected hepatocellular carcinoma. Int J Cancer 2016;138(11):2732–44.
[57] Hung CH, Hu TH, Lu SN, et al. Circulating microRNAs as biomarkers for diagnosis of early hepatocellular carcinoma associated with hepatitis B virus. Int J Cancer 2016;138(3):714–20.
[58] Lin L, Lu B, Yu J, et al. Serum miR-224 as a biomarker for detection of hepatocellular carcinoma at early stage. Clin Res Hepatol Gastroenterol 2016;40(4):397–404.
[59] Motawi TM, Sadik NA, Shaker OG, et al. Elevated serum microRNA-122/222 levels are potential diagnostic biomarkers in Egyptian patients with chronic hepatitis C but not hepatic cancer. Tumour Biol 2016;37(7):9865–74.
[60] Wang F, Ying H, He B, et al. Circulating miR-148/152 family as potential biomarkers in hepatocellular carcinoma. Tumour Biol 2016;37(4):4945–53.
[61] Wu MF, Z J, Wu XM. Expression and clinical value of serum miR-205-5p in HBV-related hepatocellular carcinoma. J Trop Med 2016;(1):31–4.
[62] Yang L, Xu Q, Xie H, et al. Expression of serum miR-218 in hepatocellular carcinoma and its prognostic significance. Clin Transl Oncol 2016;18(8):841–7.
[63] Zhang YSJ, Chen L, Bian ZL, Guan HT. Evaluate the significance of serum miR-335 in the diagnosis of hepatocellular carcinoma. J Nantong Univ (Med Sci) 2016;36(3):174–8.
[64] Zhuang C, Jiang W, Huang D, et al. Serum miR-21, miR-26a and miR-101 as potential biomarkers of hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2016;40(4):386–96.
[65] Zuo D, Chen L, Liu X, et al. Combination of miR-125b and miR-27a enhances sensitivity and specificity of AFP-based diagnosis of hepatocellular carcinoma. Tumour Biol 2016;37(5):6539–49.
[66] Ali HEA, Abdel Hameed R, Effat H, et al. Circulating microRNAs panel as a diagnostic tool for discrimination of HCV-associated hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2017;41(4):e51–62.
[67] Chen S, Chen H, Gao S, et al. Differential expression of plasma microRNA-125b in hepatitis B virus-related liver diseases and diagnostic potential for hepatitis B virus-induced hepatocellular carcinoma. Hepatol Res 2017;47(4):312–20.
[68] Elemeery MN, Badr AN, Mohamed MA, et al. Validation of a serum microRNA panel as biomarkers for early diagnosis of hepatocellular carcinoma post-hepatitis C infection in Egyptian patients. World J Gastroenterol 2017;23(21):3864–75.
[69] Guo X, Lv X, Lv X, et al. Circulating miR-21 serves as a serum biomarker for hepatocellular carcinoma and correlated with distant metastasis. Oncotarget 2017;8(27):44050–8.
[70] Hu TLJ, Yang XF, Zhang CH, Li S, Lv X, He S, et al. Study of circulating microRNA-4281 for diagnosis and prognostic valuation in hepatocellular carcinoma. J Univ South China (Med Ed) 2017;45(1):57–60.
[71] Mohamed AA, Ali-Eldin ZA, Elbedewy TA, et al. MicroRNAs and clinical implications in hepatocellular carcinoma. World J Hepatol 2017;9(23):1001–7.
[72] Shaker O, Alhelf M, Morcos G, et al. miRNA-101-1 and miRNA-221 expressions and their polymorphisms as biomarkers for early diagnosis of hepatocellular carcinoma. Infect Genet Evol 2017;51:173–81.
[73] Tarek M, Louka ML, Khairy E, et al. Role of microRNA-7 and selenoprotein P in hepatocellular carcinoma. Tumour Biol 2017;39(5), 1010428317698372.
[74] Wang PMJ, Zhu J, Li ZY. Serum miR-21 and miR-4429 levels detected by droplet digital PCR for diagnosis of hepatocellular carcinoma. Zhejiang Med J 2017;39(3):170–2.
[75] Zhang CH, Hu T, Yang XF, Li S, Lv X, He S, et al. Role of circulating miRNA-122a and miRNA-6086 in diagnosis and prognosis of hepatocellular carcinoma. J Hunan Normal Univ (Med Sci) 2017;14(2):12–6.
[76] Zhang J, Lin H, Wang XY, et al. Predictive value of microRNA-143 in evaluating the prognosis of patients with hepatocellular carcinoma. Cancer Biomark 2017;19(3):257–62.
[77] Zhu HT, Liu RB, Liang YY, et al. Serum microRNA profiles as diagnostic biomarkers for HBV-positive hepatocellular carcinoma. Liver Int 2017;37(6):888–96.
[78] Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology 2011;53(3):1020–2.
[79] Borel F, Konstantinova P, Jansen PLM. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol 2012;56(6):1371–83.
[80] Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med 2002;21(9):1237–56.
[81] Glas AS, Lijmer JG, Prins MH, et al. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol 2003;56(11):1129–35.
[82] Gatsonis C, Paliwal P. Meta-analysis of diagnostic and screening test accuracy evaluations: methodologic primer. AJR Am J Roentgenol 2006;187(2):271–81.
[83] Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med 2002;21(11):1525–37.
[84] Agosti P, Sabba C, Mazzocca A. Emerging metabolic risk factors in hepatocellular carcinoma and their influence on the liver microenvironment. Biochim Biophys Acta Mol Basis Dis 2018;1864(2):607–17.
[85] He S, Hu XW, Wang D, et al. Accuracy of microRNAs for the diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. Clin Res Hepatol Gastroenterol 2016;40(4):405–17.
[86] Hu QY, Jiang H, Su J, et al. MicroRNAs as biomarkers for hepatocellular carcinoma: a diagnostic meta-analysis. Clin Lab 2013;59(9–10):1113–20.