Systematic Review and Meta-Analysis

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The diagnostic value of assays for circulating tumor cells in hepatocellular carcinoma

A meta-analysis

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Abstract

Purpose: Circulating tumor cells (CTCs) are considered potential biomarkers for the detection of hepatocellular carcinoma (HCC). Many studies have attempted to explore this role, but the results are variable. We conducted the first comprehensive meta-analysis to evaluate the diagnostic value of CTC assay for HCC patients. Additional prognostic value was also assessed.

Experimental design: All articles included in our study were assessed using QUADAS guidelines after a literature search. Using bivariate generalized linear mixed model and random-effects model, effect measures such as pooled sensitivity/specificity, positive likelihood ratios/negative likelihood ratios (NLRs), diagnostic odds ratios, hazard ratios (HRs), risk ratios, and corresponding 95% confidence intervals (95% CIs) were calculated. We used receiver operating characteristic curves and area under the curve (AUC) to summarize overall test performance. Heterogeneity, publication bias, subgroup, and sensitivity analyses were also performed.

Results: A total of 2256 subjects including 998 HCC patients in 20 studies were recruited in this meta-analysis. Although the overall diagnostic accuracy of the CTC assay was high (AUC 0.93, 95% CI: [0.90–0.95]), there was a high probability of error rate (NLR 0.33, 95% CI: [0.23, 0.48]). The results were more robust when nonmagnetic-activated isolation was used, compared with magnetic-activated isolation subgroup (NLR: 0.18 vs. 0.41; $z=2.118$, $P=.034$). CTCs positivity was significantly associated with relapse-free survival (HR 2.417, 95% CI: [1.421–3.250]; $P<.001$), overall survival (HR 3.59, 95% CI: [1.984–6.495]; $P<.001$), and some clinical characteristics.

Conclusion: CTC assay is not recommended as an independent HCC diagnostic tool, but is associated with poor clinicopathologic characteristics of HCC patients and could indicate poor prognosis.

Abbreviations: AFP = a-fetoprotein, AUC = area under the curve, CI = confidence interval, CTCs = circulating tumor cells, DOR = diagnostic odds ratio, HCC = hepatocellular carcinoma, HR = hazard ratio, NLR = negative likelihood ratio, OS = overall survival, PLR = positive likelihood ratio, RFS = relapse-free survival, RR = relative risk, RT-PCR = reverse transcription-polymerase chain reaction, SROC = summary receiver operating characteristic, TN = true negative, TP = true positive.

Keywords: circulating tumor cells, diagnosis, hepatocellular carcinoma, meta-analysis, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is considered the fifth most common malignancy worldwide. At least 500,000 new cases are diagnosed each year and the mortality rate is still rapidly increasing.[1] Although surgical resection is recommended as the primary treatment,[2] the outcome is not always satisfactory because most patients with HCC have advanced-stage disease, primarily as a result of the lack of an effective means for early diagnosis.[3] Studies exploring effective biomarkers for HCC are continuously ongoing. Some biological factors, such as a-fetoprotein (AFP), golgi protein, and circulating cell-free DNA, have been used to provide diagnostic and prognostic information for HCC, but the results to date have been disappointing.[4,5]

Circulating tumor cells (CTCs) are a type of tumor cells present in peripheral blood, bone marrow, or lymphatic vessels.[6] CTCs can be considered as a new tool for the detection and surveillance of epithelial tumors because they are directly released from primary and metastatic tumors.[6] Assays for CTCs provide an effective approach to bypass the problems of invasive procedures, and can provide diagnostic information and reflect the invasiveness of the tumor. The development of specialized techniques for

Editor: Leyi Wang.

CS and WL contributed equally to this work.

The authors have no funding and conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2017) 96:29(e7513)

Received: 8 April 2017 / Received in final form: 1 June 2017 / Accepted: 17 June 2017

http://dx.doi.org/10.1097/MD.0000000000007513

the isolation and identification of CTCs has boosted enthusiasm for liquid biopsy in cancer patients.[7] At the present time, the application of CTC assays has been reported and well documented in a wide range of malignancies, such as colorectal cancer, breast cancer, and melanoma.[8–10]

However, clinical application of CTC assay in HCC remains in the initial stage, especially in the field of diagnosis. Many studies have attempted to explore whether CTCs can be a potential biomarker for the detection of HCC; however, it is hard to interpret the data because of the varied results. Therefore, we integrated the findings of these published studies and conducted a comprehensive meta-analysis to systematically evaluate the diagnostic value of CTCs for HCC for the first time. Furthermore, we attempted to provide insight into the prognostic value and clinicopathologic correlation of CTCs in patients with HCC.

2. Materials and methods

2.1. Literature research strategy and quality assessment

The studies recruited for this meta-analysis were independently retrieved by 2 authors (CS and WL). We used the Medical Subject Heading terms and text words: "circulating tumor cell," "CTC," "circulating tumor cells," "CTCs," "circulating cancer cells," "circulating cancer cell," "liver neoplasms," "hepatocellular carcinoma," "hepatic carcinoma," "liver tumor," "liver cancer," "sensitivity and specificity," and "accuracy" to perform a systematic literature search in the databases of PubMed, Web of Science, Cochrane Library, and Embase. We included articles published up to the beginning of September 2016, with no restriction on the start date. For more comprehensive analysis, the included articles were only in English, although there was no language restriction. We also contacted the authors of these articles to obtain further information when necessary.

All of the articles that met our inclusion criteria were assessed using the guidelines of QUADAS in methodology. This is a tool for quality assessment of diagnostic accuracy studies with a maximum score of 14.[11] In this meta-analysis, the article was awarded 1 point for information reported in or obtained from the studies that was in accordance with the criteria of QUADAS. Conversely, an item that was nonconforming or ambiguous (unclear) would be recorded as scoreless.

2.2. Data extraction

This study had been proved by Ethics Committee of Second Affiliated Hospital of Nanchang University. Data extraction was independently performed by 2 reviewers (ZD and EL), who integrated the results and made the final decision with a third author (QF). The main data extracted from these articles included: author (first), publication year, region, methodological quality score, clinical characteristics of patients, isolation methods, identification methods, cutoff value, outcome, and data on sensitivity and specificity.

2.3. Inclusion criteria

Articles were recruited if they met the following criteria: All of the evaluation indicators were derived from CTC in blood circulation; the sensitivity and specificity for the diagnosis of HCC were reported or obtained in these articles, or could be calculated from the primary data.

2.4. Exclusion criteria

The following conditions were exclusion criteria for recruitment: The indicators and CTC were not related; the evaluation indicators derived from CTC in blood circulation were not used for HCC diagnosis; lack of complete data to describe or calculate the sensitivity and specificity; and reviews, letters, technical reports, case reports, and comments.

2.5. Subgroups

Although various technological advances for isolation and identification have attempted to apply in CTCs analysis, clinical implementation is controversial owing to a lack of applicable standard. In this meta-analysis, we want to find an appropriate technology for CTCs analysis and hope this will provide support for further study. So, subgroups were established according to isolation and identification methods as follows: magnetic-activated isolation (isolation methods included cell search system, magnetic separation, BigEasy magnet or magnetic cell sorting; no limitation on identification methods); nonmagnetic-activated isolation (isolation methods included size of epithelial tumor cells, size-based membrane filters or flow cytometry; no limitation on identification methods); immunostaining (identification methods included immunohistochemistry and immunofluorescence staining; no limitation on isolation methods); and RNA identification (identification methods included nested reverse transcription-polymerase chain reaction [RT-PCR], RT-PCR, and RNA in situ hybridization; no limitation on isolation methods).

2.6. Statistical analysis

The statistical software "stata" (version 12.0; Stata Corporation; College Station, TX) was used to perform meta-analysis. The pooled sensitivity and specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), corresponding 95% confidence interval (CI), and the confidence and prediction contours in summary receiver operating characteristic (SROC) curves were calculated using bivariate mixed-effects binary regression modeling framework. All of these parameters could reflect the test accuracy.[12–14] The SROC curve and the hierarchical SROC curve were used to evaluate the diagnostic performance.[15,16] The area under the curve (AUC) was used to describe the grade of overall accuracy as a potential summary of the SROC curve.[17,18]

We also extracted and pooled hazard ratio (HR) values using a random-effect model. The original HRs and the corresponding CIs were recorded from each study, or calculated as suggested by Tierney if not reported but the relevant data were available.[19] The pooled relative risk (RR) was calculated and used to analyze the association between CTCs and clinical characteristics. Otherwise, a 2-step principal component analysis (PCA) was used to verify these observations. We also used chi-squared tests and Wilcoxon rank-sum test to compare the pooled sensitivity and specificity, PLR and NLR, and HR and RR between different subgroups in this meta-analysis, respectively.

Heterogeneity among these studies was verified by the result of LRT_I^2 (I-square) statistic[20] and LRT_ Q (chi-square) statistic. $I^2 \geq 50\%$ for LRT_I^2 or $P < .10$ for LRT_Q statistics indicates substantial heterogeneity. Meta-regression analysis could be used to explore the source of heterogeneity.[21] A total of 6 variables were estimated in this meta-analysis: publication year, study region, methodological quality score, isolation methods, identification methods, and cutoff value. Sensitivity analysis was used to assess the quality and consistency of results.

To examine the potential publication bias, we performed the Egger test and generated funnel plots according to these studies.[22] The assessment criterion of statistical significance with P values $<.05$ was applied to all analyses in our meta-analysis.

3. Results

3.1. Characteristics of these studies

A total of 20 studies,[23–42] 2256 subjects included 998 HCC patients were recruited in this meta-analysis. The remaining 1258 individuals belonged to the control group that contained healthy volunteers in 15 studies[24–26,29,30,32,33,35–42] and patients with various hepatic and tumorous diseases in 15 studies.[3,24,27–30,32,34–36,38–42] The flowchart for inclusion and exclusion of these studies is shown in Fig. 1. Five of these studies came from Europe,[23,24,33–35] 13 from Asia,[25,26,28–32,36–39,41,42] and the rest from the United States and Egypt.[27,40] Four studies were published before 2010.[23,24,28,36] Magnetic-activated isolation methods were used in 15 studies[23,26–36,38,39,41] and nonmagnetic-activated isolation methods were used in the other 5.[24,25,37,40,42] Immunohistochemistry and immunofluorescence staining were used in 13 studies[23,24,26,30–35,37,38,41,42] and RNA identification methods were used in 7 studies.[25,28–30,36,39,40] Only 1 study used Next Generation Sequencing as an identification method.[27] In addition, among these 20 studies, 6 trials evaluated the association between CTCs and overall survival (OS), relapse-free survival (RFS) or time to recurrence.[27,29,32,34,35,39] Seven studies assessed the association between CTCs and various clinical characteristic parameters.[27,29–32,34,37] The detailed data are shown in Table 1. All of these trials were prospective studies.

Some of the articles needed more explanation. Guo et al[29] recruited a total of 299 patients, but only 122 HCC patients were enrolled in the diagnostic trial therefore the data in the experimental group were adjusted from 299 to 122. Similarly, data in the study by Mu et al were adjusted from 62 to 30.[41] In the study of Kelley et al, the data in the control group were adjusted from 10 to 9 because 1 volunteer was lost to follow-up.[27] The study of Yao et al was rejected for evaluation of the association between CTC and serum AFP level (AFP ≥ 400 ng/mL) because the cutoff in this study was set at 20 ng/mL.[28]

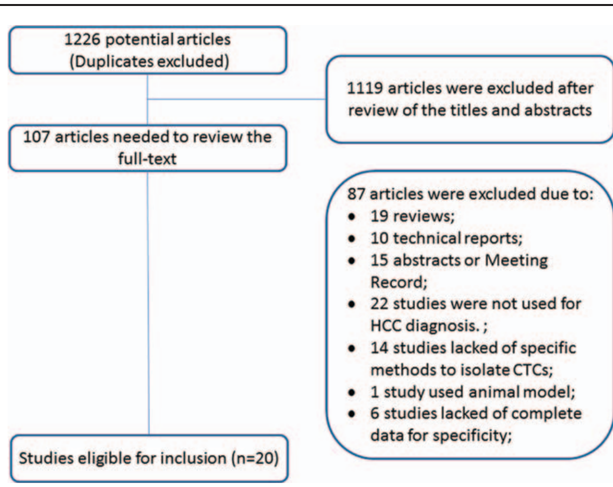

Figure 1. Flowchart for inclusion and exclusion of studies in the meta-analysis. CTCs = circulating tumor cells, HCC = hepatocellular carcinoma.

3.2. Diagnostic accuracy

The pooled sensitivity of CTCs as a diagnostic tool for HCC in all of these studies was 0.67 (95% CI: 0.55, 0.78). The pooled specificity was 0.98 (95% CI: 0.93, 0.99) and 1.0 (95% CI: 0.80, 1.00) when various hepatic and tumorous diseases and healthy volunteers was used as control group only. From our calculations, the overall PLR was 43.5 (95% CI: 11.5, 164.6), NLR was 0.33 (95% CI: 0.23, 0.48), and DOR was 131 (95% CI: 33, 528). These results indicated that an approximately 40-fold greater chance of true positive (TP) would be indicated by a positive test results and an error rate of approximately 33% would be presented when true negative (TN) was determined in a negative test. LRT_ I2 (I-square) statistic was 99 (95% CI: 98, 99), indicating that an evident heterogeneity existed in these 20 studies. LRT_ Q (chi-square) statistic was 183.701 ($P<.001$), implying that the heterogeneity was likely to come from nonthreshold effects.

The overall results for subgroup analyses were also calculated and are described in Supplementary Table S1, http://links.lww.com/MD/B799. We found that HCC diagnostic sensitivity in the subgroup of nonmagnetic-activated isolation was superior to that in the subgroup of magnetic-activated isolation ($\chi^2=47.02$, $P<.001$), but this superiority did not apply to the specificity ($\chi^2=0.95$, $P=.33$). Comparison between the subgroups of immunostaining and RNA identification indicated no statistical significance for sensitivity and specificity in these 2 subgroups ($\chi^2=0.81$, $P=.37$ and $\chi^2=0.52$, $P=.47$).

For PLR, there was no significant statistical difference between the subgroups of nonmagnetic-activated isolation and magnetic-activated isolation ($z=-0.707$, $P=.480$). This result indicated that a similar fold greater chance of TP would be presented by positive test results. However, the NLR value in the subgroup of nonmagnetic-activated isolation was superior to that in the magnetic-activated isolation subgroup (NLR: 0.18 vs. 0.41; $z=2.118$, $P=.034$). In other words, the error rate was lower and the result was more robust when the TN was determined in the nonmagnetic-activated negative test. Moreover, we found that the values of PLR and NLR had no significant statistical significance in the 2 subgroups of immunostaining and RNA identification ($z=-0.926$, $P=.355$ and $z=-1.149$, $P=.251$). Concordant with these findings, no significant statistical significance for DOR was found in these subgroups ($z=-1.65$, $P=.099$ and $z=0.463$, $P=.643$). The detailed data are shown in Supplementary Table S1, http://links.lww.com/MD/B799 and the forest plots are presented in Fig. 2 and Supplementary Fig. S5, http://links.lww.com/MD/B799.

The SROC curve is 1 kind of statistical analysis method to estimate the overall diagnostic performance in meta-analysis. It can demonstrate the trade-off between sensitivity and specificity in multistudies.[15] In Fig. 3, the observed data, together with the confidence and predictive ellipses are shown in the graph of SROC curve for all of these studies. The AUC was 0.93 (95% CI: 0.90, 0.95), signifying a high level of overall accuracy.

3.3. Survival analysis and clinical correlation

We also executed pooled analysis of the association of CTCs with survival of HCC patients from these included studies. The HR was taken to imply a poor prognosis in the CTC-positive groups

Table 1

Characteristics of studies included in the meta-analysis.

Authors	Year	Region	Score	Isolation methods	Identification methods	Cutoff	No. of P/C	TP	FP	FN	TN	Sensitivity, %	Specificity, %	Outcome	HR extraction
Sabile et al	1999	France	7	Magnetic beads	Immunohistochemistry	1 cells/10^7 PBLs	19/13	0	0	19	13	0	100	NA	NA
Vona et al	2000	France	8	ISET	Immunostaining	1 cells/mL‡	7/16	5	0	2	16	71.4	100	NA	NA
Liu et al	2016	China	6	Size-based membrane filters	RNA-ISH	NA	33/10	33	0	0	10	100	100	NA	NA
Choi et al	2014	Korea	6	Ficoll-paque + Dynabeads FlowComp Flexi Kit	Immunocytochemistry	12 cells/8.5 mL	7/1	3	0	4	1	42.9	100	NA	NA
Kelley et al	2015	USA	10	CellSearch System	NGS	2 cells/7.5 mL	20/9	8	1	12	8	40	88.9	OS	Date extrapolated
Yao et al	2005	China	7	Immunomagnetic beads	Nested RT-PCR	18.1 ng/3 mL	43/36	31	12	12	24	72.1	66.7	NA	NA
Guo et al	2014	China	11	CellSearch system	qRT-PCR	2.0§	122/120	52	4	70	116	42.6	96.7	TTR	Reported in text
Xu et al	2011	China	12	DGS + AutoMACS Pro Separator	Immunofluorescence	1 cell/5 mL	85/71	69	0	16	71	81	100	NA	NA
					RT-PCR	NA	85/71	26	1	59	70	31	98.6	NA	NA
Li et al	2013	China	9	DGS + magnetic separation	Immunofluorescence	NA	60/30	46	0	14	30	76.7	100	NA	NA
Sun et al	2012	China	12	CellSearch system	Immunofluorescence	2 cells/7.5 mL	123/15	51	0	41	15	41.5	100	TTR	Date extrapolated
Dent et al	2015	UK	7	Magnetic nanoparticles	Immunofluorescence	1 cell/4 mL	6/53	4	0	2	53	66.7	100	NA	NA
Schulze et al	2013	Germany	11	CellSearch system	Immunofluorescence	1 cell/7.5 mL	59/19	18	1	41	18	30.5	94.7	OS	Date extrapolated
Ogle et al	2016	UK	12	BigEasy magnet	Immunofluorescence	1 cell/4 mL	69/13	45	0	24	31	65.2	100	OS	Reported in text
Waguri et al	2003	Japan	9	Immunomagnetic beads	RT-PCR	NA	50/44	29	0	26	44	52.7	100	NA	NA
Wu et al	2015	China	6	Size-based membrane filters	Immunofluorescence	1 cell/5 mL	40/20	24	0	16	20	60	100	NA	NA
Li et al	2014	China	8	Ficoll-paque + magnetic separation	Immunofluorescence	1 cell/5 mL	27/49	24	0	3	49	88.9	100	NA	NA
Cheng et al	2013	Taiwan	10	Ficoll-paque + magnetic separation	RT-PCR	NA	96/60	32	3	64	57	33.3	95	RFS	Reported in text
Bahnassy et al	2014	Egypt	8	DGS + + flow cytometry	Anti-CK19*	3 cells/7.5 mL	70/63	61	11	9	52	87.1	82.5	NA	NA
					Anti-CD90*	3 cells/7.5 mL	70/63	57	6	13	56	81	89.6	NA	NA
					RT-PCR	NA	70/63	39	0	31	63	55.71	100	NA	NA
Mu et al	2014	China	7	DGS + magnetic separation	Anti-GPC3†	1 cell/7.5 mL	30/22	27	6	3	16	90	71.4	NA	NA
					Anti-ASGPR†	1 cell/7.5 mL	30/22	28	5	2	17	93.1	75	NA	NA
					Anti-CK†	1 cell/7.5 mL	30/22	25	16	5	6	82.6	28.6	NA	NA
Liu et al	2014	China	7	Fluorescent-labeled antibodies + flow cytometry	Immunofluorescence	1 cell/5 mL	32/77	29	0	3	77	90.6	100	NA	NA

DGS = density gradient separation, Ficoll-paque = Ficoll-paque + gradient centrifugation, FN = false negative, FP = false positive, immunofluorescence = immunofluorescence staining, HR = hazard ratio, ISET = size of epithelial tumor cells, NA = not applicable, NGS = Next Generation Sequencing, OS = overall survival, PBL = peripheral blood leukocyte, qRT-PCR = quantitative real-time polymerase chain reaction, RFS = relapse-free survival, RNA-ISH = RNA in situ hybridization, RT-PCR = reverse transcription-polymerase chain reaction, TN = true negative, TP = true positive, TTR = time to recurrence.

* Fluorescent-labeled antibodies.

† Immunofluorescence antibodies.

‡ Cells/mL blood.

§ $2^{-\Delta\Delta C_q}$ algorithm transformation.

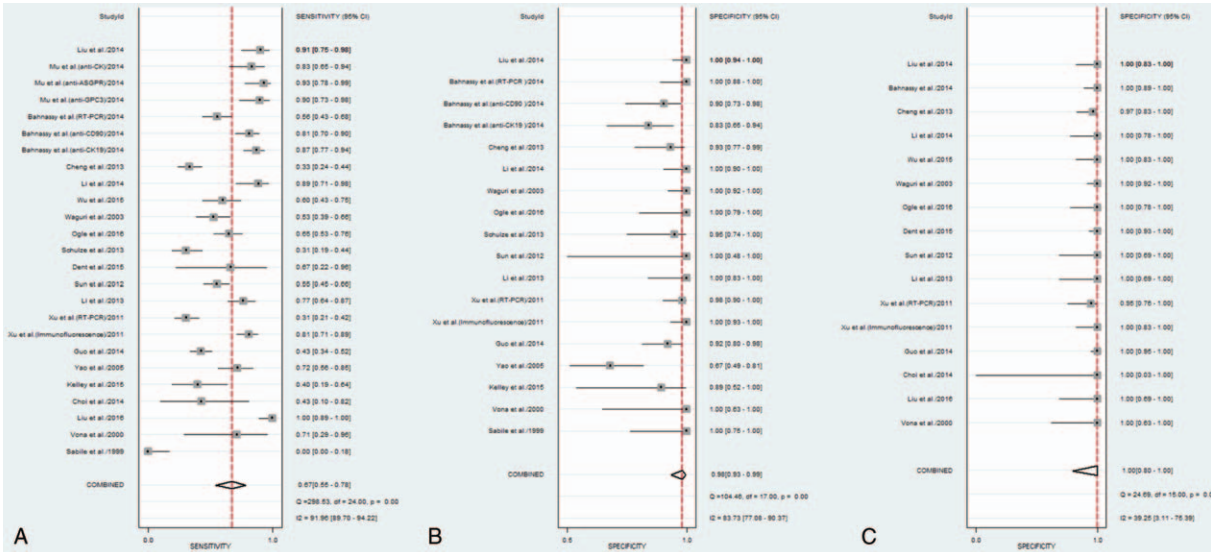

Figure 2. Forest plots of estimates of sensitivity and specificity. The point estimates are shown as brown squares and 95% CIs are shown as error bars. (A) The forest plots of sensitivity for all studies; (B) the forest plots of specificity for studies with diseases control only; (C) the forest plots of specificity for studies with healthy control only. CI = confidence interval.

when its numerical value was >1. Data on OS were available in 3 of the studies,[27,34,35] and the pooled HRs showed that poor OS was associated with CTC positivity status (HR, 2.417; 95% CI: 1.421–3.250; $P<.001$). Similar results were presented for the data for RFS in 3 studies[29,32,39]; the pooled HRs showed that CTC positivity was significantly associated with a higher risk of disease recurrence in HCC patients (HR, 3.59; 95% CI: 1.984–6.495; $P<.001$). The forest plots are shown in Fig. 4.

RR was used to analyze the association between CTC and clinical characteristics in our meta-analysis. We found that CTC positivity was associated with AFP ≥ 400 ng/mL (overall positive rate, 56.61% vs. 34.17%; RR, 1.664; 95% CI: 1.117–2.478; $P=.012$). When portal vein tumor thrombus (PVT) was present, the CTC positivity was greater (overall positive rate, 93.83% vs. 44.32%; RR, 2.059; 95% CI: 1.625–2.609; $P<.001$). In addition, pooled RRs showed that CTC positivity in tumor lymph nodes metastasis (TNM) stage I and II was less than that in stage III and IV (overall positive rate, 61.18% vs. 89%; RR, 0.614; 95% CI: 0.403–0.936; $P=.023$), and was also associated with tumor size (overall positive rate, 49.82% vs. 54.74%; RR, 0.871; 95% CI: 0.765–0.992; $P=.038$). None of the other clinical characteristics had an obvious correlation with CTC positivity. We also used principal component analysis to verify these results. The detailed data are shown in Table 2 and the forest plots are shown in Supplementary Fig. S1, http://links.lww.com/MD/B799.

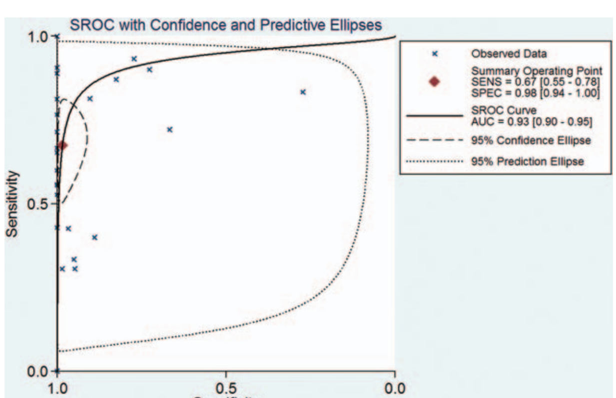

Figure 3. Summary receiver operating characteristic (SROC) curves for circulating tumor cells assay. The confidence ellipse indicates that the mean values for sensitivity and specificity were more likely to be in this region. The prediction ellipse indicates that individual values for sensitivity and specificity were more likely to be in this region. AUC = area under the curve.

3.4. Heterogeneity, meta-regression analysis, and sensitivity analysis

Our results indicated that obvious heterogeneity from non-threshold effects was present in these 20 studies. We used meta-regression analysis to evaluate some of the covariates used in these studies and tried to find the source of the heterogeneity. The study characteristics included "publication year (year 2010)," "region (Europe)," "methodological quality score (score 10)," "isolation methods (isolation)," "identification methods (identification)," and "cutoff value (cutoff4cellml)" (Table 1). The results of meta-regression suggested that the covariate of "methodological quality score" and "cutoff value" might be potential sources of heterogeneity in sensitivity, and the covariates of "region" and "cutoff value" might produce major heterogeneity in specificity in our diagnostic meta-analysis. The detailed data for meta-regression analysis are presented in Supplementary Fig. S2, http://links.lww.com/MD/B799.

Sensitivity analysis was used to investigate the influence of each individual study from the overall pooled analysis. The result indicated that no individual study dominated this meta-analysis because neither the direction nor the magnitude of the estimated pooled results was obviously affected (Supplementary Fig. S3, http://links.lww.com/MD/B799).

3.5. Publication bias

The Egger test, 1 kind of linear regression of log odds ratios on inverse root of effective sample sizes as a test for funnel plot

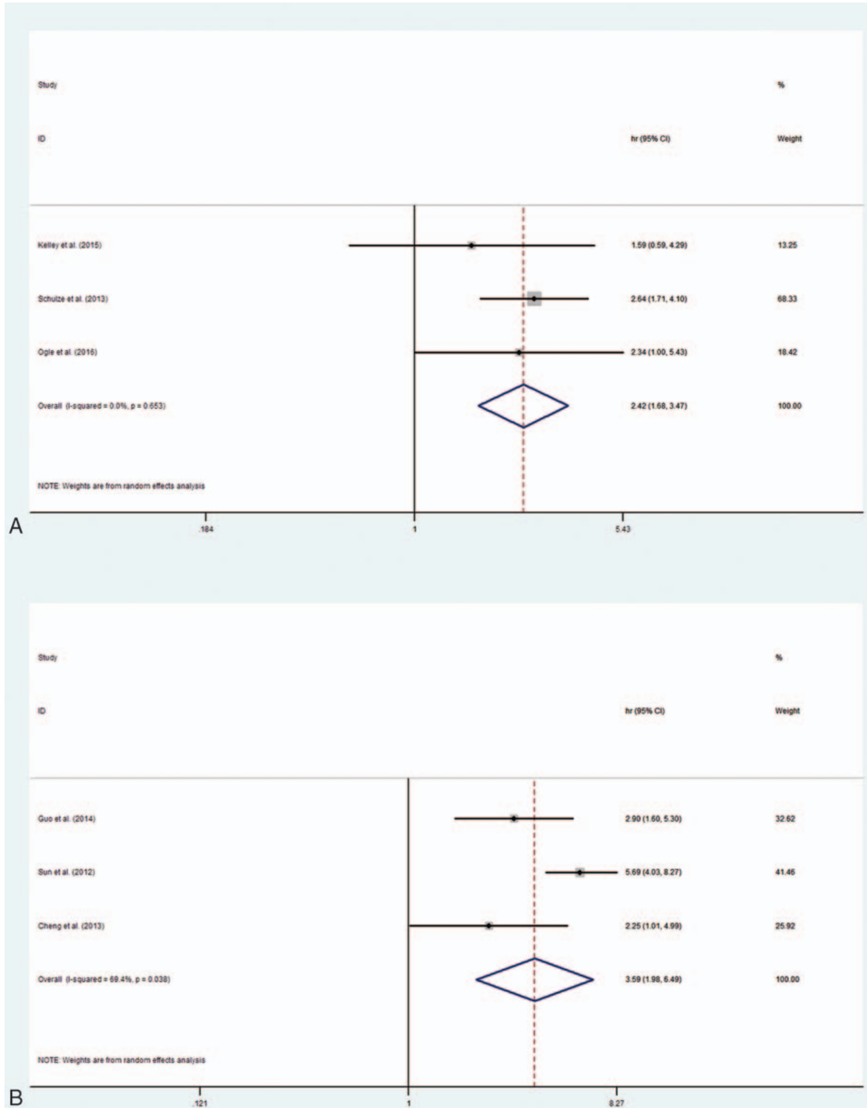

Figure 4. Summary estimates of hazard ratio for OS (A) and RFS (B) between CTC-positive and CTC-negative patients for HCC. CTC = circulating tumor cells, OS = overall survival, RFS = relapse-free survival.

asymmetry in diagnostic meta-analyses, can be used to evaluate the publication bias.[22] The funnel plot had a coefficient of −4.998 (95% CI: −25.017, 15.019) and *P* value of .61, which indicated that the funnel plot was symmetric and publication bias was not present (Supplementary Fig. S4, http://links.lww.com/MD/B799).

4. Discussion

Assays for CTCs have attracted increasing attention because this kind of noninvasive biomarker can be used to provide diagnostic and prognostic information for personalized medicine. However, the results from dozens of studies are disparate and lack statistical power, and the clinical significance of CTCs in HCC patients is still controversial. We therefore performed this meta-analysis to integrate these published results and systematically evaluate the clinical application of CTC assays.

The results of our meta-analysis indicated that CTC assay presented satisfactory pooled sensitivity and specificity. The numerical values of 0.67 (sensitivity) and 0.98/1.00 (specificity) were superior to those of the AFP assay alone (pooled sensitivity and specificity was 0.54 and 0.909 in our previous study).[43] We also used the SROC curve and the corresponding AUC to estimate the overall diagnostic performance in meta-analysis. The evaluation criteria can be divided into 3 levels: low (AUC: 0.5–0.7), moderate (AUC: 0.7–0.9), or high (AUC: 0.9–1) accuracy.[17] In this meta-analysis, the AUC value for CTC assay was 0.93, indicating a high level of overall accuracy category.

To further evaluate diagnostic accuracy, we analyzed DOR, PLR, and NLR. The DOR value ranges from 0 to infinity and facilitates formal meta-analysis of studies on diagnostic test performance.[12] In our meta-analysis, the DOR value was 131 and indicated that a high level of accuracy was present. The LRs indicate the amount by which the odds of disease would increase or decrease for a positive and negative test. That is to say, the probability of a true-positive and the value of PLR exhibit a direct ratio when the test is positive. Analogously, a higher value of NLR indicates a higher probability of a false-negative when the test is negative. In our results, the PLR for CTC assay was 43.5, indicating a high chance of a true-positive would be present in a

Table 2

Correlation of circulating tumor cells with clinical characteristics.

Clinical characteristics	Over positive rate, %	RR	95% CI	P
Gender				
Male	40.77	0.845	0.636–1.123	.246
Female	50.62			
Age, y				
≤50	45.05	0.983	0.797–1.211	.869
>50	46.67			
HBsAg				
Negative	50.00	0.984	0.438–2.212	.969
Positive	45.26			
Liver cirrhosis				
Positive	46.28	1.061	0.768–1.467	.720
Negative	42.37			
Serum AFP, ng/mL				
≥400	56.61	1.664	1.117–2.478	**.012***
<400	34.17			
Vascular invasion				
Positive	53.21	2.034	0.936–4.421	.073
Negative	32.46			
Tumor number				
Single	43.63	0.853	0.678–1.075	.178
Multiple	51.85			
Tumor size, cm				
≤5	49.82	0.871	0.765–0.992	**.038***
>5	54.74			
Tumor encapsulation				
Complete	47.52	1.250	0.913–1.710	.163
Incomplete	38.13			
Satellite lesion				
Positive	52.94	1.214	0.310–4.748	.781
Negative	41.46			
PVT				
Positive	93.83	2.059	1.625–2.609	<**.001***
Negative	44.32			
Child-Pugh				
Stage A	46.70	1.296	0.869–1.933	.203
Stage B	37.21			
Edmondson stage				
Stage I–II	41.07	0.768	0.443–1.330	.345
Stage III–IV	51.65			
BCLC stage				
Stage 0–A	41.92	0.940	0.591–1.497	.795
Stage B–C	38.18			
TNM				
Stage I–II	61.18	0.614	0.403–0.936	**.023***
Stage III–IV	89.00			

AFP = a-fetoprotein, BCLC = barcelona clinic liver cancer, CI = confidence interval, PVT = portal vein tumor thrombus, RR = relative risk, TNM = tumor lymph nodes metastasis.

Bold values signify significant correlations were observed between CTC positivity status and serum AFP level, PVT and TNM stage in our meta-analysis.

positive test result. However, the NLR was 0.33, indicating an approximately 33% error rate for a true-negative was determined in the negative test. This might represent a limitation when the CTC assay is used independently for the detection of HCC, despite a high level of diagnostic efficiency.

We also performed subgroups analysis and found that the diagnostic sensitivity for HCC in the subgroup of magnetic-activated isolation was worse than that in the subgroup of nonmagnetic-activated isolation. However, simple pooled sensitivity and specificity was inappropriate because this approach ignored threshold differences.[44,45] Therefore, we analyzed PLR and DOR further and found no significant statistical difference in these 2 subgroups. As a result, we still have reservations regarding whether the diagnostic ability with nonmagnetic-activated isolation is really superior to that with magnetic-activated isolation in CTC assays. In addition, although the diagnostic specificity of HCC in these subgroups was similar, the result was more robust when nonmagnetic-activated isolation was used in CTC assays because the error rate was lower. The reason for the lower robustness of diagnosis for magnetic-activated isolation might be that the strategies employed in most of these studies solely targeted epithelial cell adhesion molecule,[27,29,32,34,39] leukocyte common antigen,[33] or other monoclonal antigen.[23] A narrow detection range might miss the target CTCs and cause a higher probability of a false-negative when the negative test is executed. For the other 2 subgroups, no significant statistical difference for these indicators was found.

We also investigated the impact of CTCs on survival of HCC patients. The results showed that a poor OS and RFS were associated with CTC positivity status, similar to the result of Fan et al.[46] Thus, the CTC assay could indeed be considered as a prognostic marker for HCC. In addition, we analyzed the association between CTC assay data and various clinicopathologic parameters. Significant correlations were observed between CTC positivity status and serum AFP level, PVT and TNM stage in our meta-analysis. It is easy to understand this result because VPT status or high-grade TNM stage always accompany more aggressive disease for HCC, while AFP mRNA has been confirmed as a pivotal predictive marker for HCC metastasis as well.[47] This result could be considered sound evidence for the application of CTC assay as a predictive marker for poor clinicopathologic factors in the progression of HCC.

We found that obvious heterogeneity from nonthreshold effects was present in these studies. Results of a meta-regression analysis revealed the covariates of “methodological quality score,” “cutoff value,” and “region” as potential sources of heterogeneity in our meta-analysis. In addition, we were also concerned about the effect of publication bias because positive results were more likely to be published; however, the results of the Egger test did not indicate a publication bias in our meta-analysis.

Our meta-analysis had some limitations that should be noted. First, the pooled data might be argued because these included studies had obvious heterogeneity. However, as a new biomarker, the exploration of CTC assay is still in the primary stage and many standards are not established yet. Therefore, the integration of CTC assay data from nonhomogeneous covariates was inevitable. Second, the sources of heterogeneity might be extensive and it was impossible for us to determine all of them. We could not collect some covariates that were absent in these included articles. Finally, the inclusion of only English-language studies in this analysis might have introduced some bias.

The ongoing development of CTC assays provides a powerful tool for identifying biomarkers and bio-signatures of HCC. We systematically synthesized diverse study results in our meta-analysis and provide powerful evidence for the potential clinical value of CTC assay, as a diagnostic marker, prognostic marker, and indicator of clinicopathologic predictive factors. However, the clinical application of CTC assay is currently limited because this assay may produce a high probability of error rate. Whereas, nonmagnetic-activated isolation may be a promising technology that shows more robust results than other approaches. Furthermore, the heterogeneity that is revealed in our meta-analysis indicates that standardization of methodologies is still lacking for this emerging technology. Nonetheless, the results of our meta-analysis provide convincing evidence for future applications of CTC assay in the management of HCC, although further studies are needed before its adoption in clinical practice.

5. Conclusion

The results of our meta-analysis suggest that the CTC assay could be used in HCC detection because it has a high level of overall accuracy. However, the current diagnostic value is limited because independent application of this assay may produce a high probability error rate. Although the sensitivity of nonmagnetic-activated isolation is superior to that of magnetic-activated isolation in CTC assays, we still have reservations regarding whether the ability for HCC diagnosis with the former isolation method is actually greater. Nonetheless, it is clear that the results will have a lower probability of error rate and will be more robust for a negative HCC diagnostic test when nonmagnetic-activated isolation is used. Furthermore, use of CTC assay may allow evaluation of the poor clinicopathologic characteristics, and indicate a poor clinical prognosis for HCC patients.

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