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

A meta-analysis

Chi Sun, MMa, Weniun Liao, MDb, Zefu Deng, MMb, Enliang Li, MMb, Qian Feng, MMb, Jun Lei, MDb, Rongfa Yuan, MDb, Shubing Zou, MDb, Yilei Mao, PhD, Jianghua Shao, MDb, Linquan Wu, MD³,*, Chao Zhang, MMa, Wenjun Liao, MD, Zefu Deng, MMb, Enliang Li, MD, Qian Feng, MMb, Jun Lei, MD, Rongfa Yuan, MD, Shubing Zou, MD, Yilei Mao, PhD, Jianghua Shao, MD, and Chi Sun, MMa, Wenjun Liao, MD, Zefu Deng, MMb, Enliang Li, MD, Qian Feng, MMb, Jun Lei, MD, Rongfa Yuan, MD, Shubing Zou, MD, Yilei Mao, PhD, Jianghua Shao, MD.

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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 = α-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 α-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
the isolation and identification of CTCs has boosted enthusiasm for liquid biopsy in cancer patients.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[12-14]} The SROC curve and the hierarchical SROC curve were used to evaluate the diagnostic performance.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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_F (I-square) statistic\textsuperscript{[20]} and LRT_Q (chi-square) statistic. $F \geq 50\%$ for LRT_F or $P < 0.10$ for LRT_Q statistics indicates substantial heterogeneity. Meta-regression analysis could be used to explore the source of heterogeneity.\textsuperscript{[21]} A total of 6 variables were estimated in this meta-analysis: publication year, study region, methodological quality score, isolation methods, identifi-
cation 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. 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, 2256 subjects included 998 HCC patients were recruited in this meta-analysis. The remaining 1238 individuals belonged to the control group that contained healthy volunteers in 15 studies and patients with various hepatic and tumorous diseases in 15 studies. The flowchart for inclusion and exclusion of these studies is shown in Fig. 1. Five of these studies came from Europe, 13 from Asia, and the rest from the United States and Egypt. Four studies were published before 2010. Magnetic-activated isolation methods were used in 15 studies and nonmagnetic-activated isolation methods were used in the other 5. Immunohistochemistry and immunofluorescence staining were used in 13 studies and RNA identification methods were used in 7 studies. Only 1 study used Next Generation Sequencing as an identification method. 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. Seven studies assessed the association between CTCs and various clinical characteristic parameters.

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. LRT1 (I-square) statistic was 99 (95% CI: 98, 99), indicating that an evident heterogeneity existed in these 20 studies. LRT2 (chi-square) statistic was 183.701 (99), indicating that an evident heterogeneity existed in these 20 studies. LRT3 (chi-square) statistic was 183.701 (99), indicating that an evident heterogeneity existed in these 20 studies.

The overall results for subgroup analyses were also calculated and are described in Supplementary Table S1. For PLR, there was no significant statistical difference between the subgroups of nonmagnetic-activated isolation and magnetic-activated isolation (z = –0.99, P = .33). Comparison between the subgroups of immunostaining and RNA identification indicated no statistical significance for sensitivity and specificity in these 2 subgroups (z = 0.81, P = .37 and x² = 0.52, P = .47).

For PLR, there was no significant statistical difference between the subgroups of nonmagnetic-activated isolation and magnetic-activated isolation (z = –0.70, 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 subgroup of magnetic-activated isolation (z = 4.70, P < .001), but this superiority did not apply to the specificity (z = 0.95, P = .33). 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. 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. 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.

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. 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 0.59 (95% CI: 0.40, 0.59), signifies 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    | FN    | TN    | Sensitivity, % | Specificity, % | Outcome | HR extraction |
|--------------|------|-------------|-------|------------------------------------|-------------------------------------------------|-----------------|------------|-------|-------|-------|----------------|-----------------|---------|---------------|
| Sabile et al | 1999 | France      | 7     | Magnetic beads                     | Immunochemistry                                  | 1 cells/10^7 PBLs | 19/13     | 0     | 0     | 19    | 0               | 100             | NA      | NA            |
| Vona et al   | 2000 | France      | 8     | ISET                              | Immunoabsorbing                                   | 1 cells/mL       | 7/16      | 5     | 2     | 16    | 71.4           | 100             | NA      | NA            |
| Liu et al    | 2016 | China       | 6     | Size-based membrane filters        | RNA-ISH                                          | NA              | 33/10     | 33    | 0     | 10    | 100            | 100             | NA      | NA            |
| Choi et al   | 2014 | Korea       | 6     | Ficoll-paque + Dynabeads           | Immunochemistry                                   | 12 cells/8.5 mL  | 7/1       | 3     | 0     | 4     | 42.9           | 100             | NA      | NA            |
| Kelley et al | 2015 | USA         | 10    | CellSearch System                 | FlowComp Flexi Kit                                | RT-PCR           | NA        | 85/71 | 31    | 16    | 100            | 100             | NA      | NA            |
| Yao et al    | 2005 | China       | 7     | Immunomagnetic beads              | Nested RT-PCR                                    | 1 cells/mL       | 7/16      | 8     | 1     | 12    | 40             | 88.9           | OS      | Date extrapolated|
| Liu et al    | 2014 | China       | 11    | CellSearch system                 | qRT-PCR                                          | 2.0             | 122/120  | 52    | 4     | 70    | 42.6           | 96.7           | NA      | TTR            |
| Choi et al   | 2014 | Korea       | 6     | Ficoll-paque + magnetic separation | Immunochemistry                                   | 12 cells/8.5 mL  | 7/1       | 3     | 0     | 4     | 42.9           | 100             | NA      | NA            |
| Kelley et al | 2015 | USA         | 10    | CellSearch System                 | FlowComp Flexi Kit                                | RT-PCR           | NA        | 85/71 | 31    | 16    | 100            | 100             | NA      | NA            |
| Guo et al    | 2014 | China       | 11    | CellSearch system                 | FlowComp Flexi Kit                                | RT-PCR           | NA        | 85/71 | 31    | 16    | 100            | 100             | NA      | NA            |
| Li et al     | 2013 | China       | 9     | DGS + magnetic separation         | Immunochemistry                                   | 1 cells/5 mL     | 85/71     | 69    | 0     | 16    | 81             | 100             | NA      | NA            |
| Sun et al    | 2012 | China       | 12    | CellSearch system                 | Immunochemistry                                   | 1 cells/5 mL     | 85/71     | 69    | 0     | 16    | 81             | 100             | NA      | NA            |
| Dent et al   | 2015 | UK          | 7     | Magnetic nanoparticles            | Immunochemistry                                   | 1 cells/4 mL     | 6/53      | 4     | 0     | 2     | 66.7           | 100             | NA      | NA            |
| Schulze et al| 2013 | Germany     | 11    | CellSearch system                 | Immunochemistry                                   | 1 cells/7.5 mL   | 59/19     | 18    | 1     | 41    | 30.5           | 94.7           | OS      | Date extrapolated|
| Ogle et al   | 2015 | UK          | 12    | BigEasy magnet                    | Immunochemistry                                   | 1 cell/4 mL      | 6/53      | 4     | 0     | 2     | 66.7           | 100             | NA      | NA            |
| Waquri et al | 2013 | Japan       | 9     | Immunomagnetic beads              | RT-PCR                                           | NA              | 50/44     | 29    | 0     | 26    | 52.7           | 100             | NA      | NA            |
| Wu et al     | 2015 | China       | 6     | Size-based membrane filters        | Immunochemistry                                   | 1 cells/5 mL     | 40/20     | 16    | 0     | 20    | 60             | 100             | NA      | NA            |
| Li et al     | 2014 | China       | 8     | Ficoll-paque + magnetic separation | Immunochemistry                                   | 1 cells/5 mL     | 27/49     | 24    | 0     | 3     | 88.9           | 100             | NA      | NA            |
| Cheng et al  | 2013 | Taiwan      | 10    | Ficoll-paque + magnetic separation | Immunochemistry                                   | 1 cells/5 mL     | 27/49     | 24    | 0     | 3     | 88.9           | 100             | NA      | NA            |
| Bahnassy et al| 2014 | Egypt       | 8     | DGS + flow cytometry              | Anti-CK19 | 3 cells/7.5 mL | 70/63     | 61    | 11    | 52    | 87.1           | 82.5           | NA      | NA            |
| Mu et al     | 2014 | China       | 7     | DGS + magnetic separation         | Anti-ASGPR†                                      | 1 cell/7.5 mL    | 30/22     | 28    | 5     | 2    | 93.1           | 75             | NA      | NA            |
| Liu et al    | 2014 | China       | 7     | Fluorescent-labeled antibodies     | Immunochemistry                                   | 1 cell/5 mL      | 32/77     | 29    | 0     | 3    | 90.6           | 100             | NA      | NA            |

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

**Notes:**
- † Fluorescent-labeled antibodies
- ‡ Cells/mL blood
- *2 10^x algorithm transformation
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 \(\geq 400\) 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.

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
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                 | 0.963 | 0.797–1.211 | .869 |
| >50                      | 46.87                 |     |            |     |
| HBsAg                    |                       |     |            |     |
| Negative                 | 50.00                 | 0.984 | 0.438–2.212 | .069 |
| 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               | 36.13                 |     |            |     |
| 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                 |     |            |     |
| Edmonton stage           |                       |     |            |     |
| Stage I–II               | 41.07                 | 0.768 | 0.443–1.330 | .345 |
| Stage II–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 II–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.

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 (EpCAM) monoclonal antigen or other monoclonal antigen. 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. 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. 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 impossible. 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-

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 subgroup 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. Therefore, we analyzed PLR and DOR further and found no significant statistical difference in
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.

References

[1] Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. J Clin Gastroenterol 2013;47(suppl):S2–6.
[2] Ryder SD. British Society of Gastroenterology Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. Gut 2003;52(suppl 3):i1–8.
[3] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;59:223–49.
[4] Benowitz S. Liver cancer biomarkers struggling to succeed. J Natl Cancer Inst 2007;99:590–1.
[5] Liao W, Yang H, Xu H, et al. Noninvasive detection of tumor-associated mutations from circulating cell-free DNA in hepatocellular carcinoma patients by targeted deep sequencing, Oncotarget 2016;7:40811–90.
[6] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011;331:1559–64.
[7] Mostert B, Steuwer AM, Martens JW, et al. Diagnostic applications of cell-free and circulating tumor cell-associated miRNAs in cancer patients. Expert Rev Mol Diagn 2011;11:529–75.
[8] Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:3213–21.
[9] Zhang L, Riehmford S, Wu G, et al. Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. Clin Cancer Res 2012;18:5701–10.
[10] Mocellin S, Hoon D, Ambrosi A, et al. The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis. Clin Cancer Res 2006;12:4605–13.
[11] 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. BMC Med Res Method 2003;3:25.
[12] Glas AS, Lijmer JG, Prins MH, et al. The diagnostic odds ratios: a single indicator of test performance. J Clin Epidemiol 2003;56:1129–35.
[13] Jaeschke R, Guyatt GH, Sackett DL. Users’ guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. JAMA 1994;271:703–7.
[14] Reitsma JB, Glas AS, Rutjes AW, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982–90.
[15] Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. Stat Med 1993;12:1293–316.
[16] Rutter CM, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations, Stat Med 2001;20:2865–84.
[17] Swets JA. Measuring the accuracy of diagnostic systems. Science 1988;240:1285–93.
[18] Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med 2002;21:1237–56.
[19] Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
[20] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
[21] Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? Stat Med 2002;21:1559–73.
[22] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
[23] Sable A, Louha M, Bonte E, et al. Efficiency of Ber-EP4 antibody for isolating circulating epithelial tumor cells before RT-PCR detection. Am J Clin Pathol 1999;112:171–8.
[24] Vona G, Sable A, Louha M, et al. Isolation by size of epithelial tumor cells: a new method for the immunomorphological and molecular characterization of circulating tumor cells. Am J Pathol 2000;156:57–63.
[25] Liu YK, Hsu HS, Li ZL., et al. An improved strategy to detect the epithelial–mesenchymal transition process in circulating tumor cells in hepatocellular carcinoma patients. Hepatol Int 2016;10:640–6.
[26] Choi HS, Lee HM, Kim WT, et al. Detection of mycoplasma infection in circulating tumor cells in patients with hepatocellular carcinoma. Biochem Biophys Res Commun 2014;446:620–5.
[27] Kelley RK, Magbanua MJ, Butler TM, et al. Circulating tumor cells in hepatocellular carcinoma: a pilot study of detection, enumeration, and next-generation sequencing in cases and controls. BMC Cancer 2015;15:206.
[28] Yao F, Guo JM, Xu CF, et al. Detecting AFP mRNA in peripheral blood of the patients with hepatocellular carcinoma, liver cirrhosis and hepatitis. Clin Chim Acta 2005;361:119–27.
[29] Guo W, Yang XR, Sun YF, et al. Clinical significance of EpCAM mRNA-positive circulating tumor cells in hepatocellular carcinoma by an optimized negative enrichment and qRT-PCR-based platform. Clin Cancer Res 2014;20:4794–805.
[30] Xu W, Cao L, Chen L, et al. Isolation of circulating tumor cells in patients with hepatocellular carcinoma using a novel cell separation strategy. Clin Cancer Res 2011;17:3783–93.
[31] Li Y-M, Xu S-C, Li J, et al. Epithelial–mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. Cell Death Dis 2013;4:e831.
[32] Sun XF, Yu X, Yang XR, et al. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. Hepatol 2013;57:1458–68.
[33] Dent BM, Ogle LF, O’Donnell RL, et al. High-resolution imaging for the detection and characterisation of circulating tumour cells from patients with oesophageal, hepatocellular, thyroid and ovarian cancers. Int J Cancer 2016;138:206–16.
[34] Schulze K, Gasch G, Staufer K, et al. Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. Int J Cancer 2013;133:2165–71.
[35] Ogle LF, Orr JG, Willoughby CE, et al. Imagestream detection and characterisation of circulating tumour cells: a liquid biopsy for hepatocellular carcinoma? J Hepatol 2016;65:303–13.
[36] Wagurn N, Suda T, Nomoto M, et al. Sensitive and specific detection of circulating cancer cells in patients with hepatocellular carcinoma; detection of human telomerase reverse transcriptase messenger RNA after immunomagnetic separation. Clin Cancer Res 2003;9:3004–11.
[37] Wu S, Liu S, Liu Z, et al. Classification of circulating tumor cells by epithelial-mesenchymal transition markers. PLoS ONE 2015;10:e0123976.
[38] Li J, Chen L, Zhang X, et al. Detection of circulating tumor cells in hepatocellular carcinoma using antibodies against asialoglycoprotein receptor, carbamoyl phosphate synthetase 1 and pan-cytokeratin. PLoS ONE 2014;9:e96185.
[39] Cheng SW, Tsai HW, Lin YJ, et al. Lin28B is an oncotic circulating cancer stem cell-like marker associated with recurrence of hepatocellular carcinoma. PLoS ONE 2013;8:e80053.
[40] Bahnassy AA, Zeeki AK, El-Bastawisy A, et al. Circulating tumor and cancer stem cells in hepatocellular carcinoma: a systematic review. World J Gastroenterol 2014;20:18240–8.
[41] Mu H, Lin KX, Zhao H, et al. Identification of biomarkers for hepatocellular carcinoma by semiquantitative immunocytochemistry. World J Gastroenterol 2014;20:5826–38.
[42] Liu HY, Qian HH, Zhang XF, et al. Improved method increases sensitivity for circulating hepatocellular carcinoma cells. World J Gastroenterol 2015;21:2918–25.
[43] Liao W, Mao Y, Ge P, et al. Value of quantitative and qualitative analyses of circulating cell-free DNA as diagnostic tools for hepatocellular carcinoma: a meta-analysis. Medicine (Baltimore) 2015;94:e722.
[44] Littenberg B, Moses LE. Estimating diagnostic accuracy from multiple conflicting reports: a new meta-analytic method. Med Decis Making 1993;13:313–21.
[45] Irwig L, Macaskill P, Glasziou P, et al. Meta-analytic methods for diagnostic test accuracy. J Clin Epidemiol 1995;48:119–30.
[46] Fan JL, Yang YF, Yuan CH, et al. Circulating tumor cells for predicting the prognosis of patients with hepatocellular carcinoma: a meta-analysis. Cell Physiol Biochem 2015;37:629–40.
[47] Jin J, Niu X, Zou L, et al. AFP mRNA level in enriched circulating tumor cells from hepatocellular carcinoma patient blood samples is a pivotal predictive marker for metastasis. Cancer Lett 2016;378:33–7.