Original Paper

Circulating Tumor Cells for Predicting the Prognostic of Patients with Hepatocellular Carcinoma: A Meta Analysis

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Key Words

Hepatocellular carcinoma (HCC) • Circulating tumor cells (CTC) • Prognosis • Clinicopathologic parameter

Abstract

Background/Aims: The prognostic value of circulating tumor cells (CTC) detected in hepatocellular carcinoma (HCC) patients is currently under debate. We conducted a meta-analysis of available studies to assess its prognostic value for patients diagnosed with HCC. **Methods:** Medline, Ovid Database, Embase, The Science Citation Index, and Cochrane library, search was conducted on all studies reporting the outcomes of interest. The studies were set up according to the inclusion/exclusion criteria. Using a random-effects model, meta-analysis was performed using hazard ratio (HR), risk ratio (RR) and their 95% confidence intervals (95% CIs) as effect measures. Heterogeneity of the studies was tested for each pooled analysis. Subgroup and sensitivity analyses were also performed. **Results:** twenty-three published studies that matched the selection criteria were included in this meta-analysis. CTC positivity was significantly associated with Relapse free survival (RFS) (HR 3.03, 95% CI: [1.89–4.86]; $p < 0.00001$) and Overall survival (OS) (HR 2.45, 95% CI: [1.73–3.48]; $p < 0.00001$). CTC positivity were also significantly associated with TNM Stage (RR 1.30, 95% CI: [1.02–1.65]; $p = 0.03$), Tumor size (RR 1.36, 95% CI: [1.09–1.69]; $p = 0.006$), Vascular invasion (RR 1.99, 95% CI: [1.43–2.77]; $p < 0.0001$), Portal vein tumor thrombus (RR 1.73, 95% CI: [1.42–2.11]; $p = 0.0001$), Serum alpha-fetoprotein (AFP) level (RR 2.05, 95% CI: [1.18–3.54]; $p = 0.01$). **Conclusion:** CTC positivity indicates poor prognosis in patients with hepatocellular carcinoma, and associated with poor clinicopathologic parameters.

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Introduction

As one of the most aggressive cancers, hepatocellular carcinoma (HCC) occupies 85%–90% of primary liver cancer and it is responsible for significant morbidity and mortality in cirrhosis [1–3]. One-year survival of patients who show progress over the terminal stage is less than 10%

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[4]. Recently, international consensus was established to choose the best therapeutic method adapted for each case and expected to obtain the best prognosis, however, owing to the lack of precise markers of distant metastasis and recurrence, the prognosis of HCC patients still failed to get significant improvement. Thus, it is undeniable that early detection of tumors and metastasis is urgently needed in medicine [5].

In the field of tumor biology, different types of circulating cellular element have been identified as tumor markers [6, 7]. Circulating tumor cells (CTC) specifically refer to the tumor cells shed into blood, bone marrow or lymphatic vessels. These cells have strong potential of distant metastasis, circulating through the bloodstream, traveling to different tissues or organs of body [8]. Many attempts have been made to develop improved enrichment and identification systems to detect or enumerate CTC [9-11]. As an interesting source of biological information to evaluate dissemination, drug resistance and treatment effectiveness, CTC have been proposed as a monitoring tool in patients with solid tumors, the presence of CTC can reflect the aggressiveness of solid tumors [12-14].

Numerous studies have investigated the prognostic relevance of CTC positivity with the progression of various tumors and proved that CTC could be used to estimate prognosis and may serve as an early marker to assess antitumor activity of treatment, predict relapse free survival (RFS) or overall survival (OS) [15-18]. Although CTC detection has been applied and well documented in different types of cancer, especially breast cancer [19, 20], CTC detection remains in the experimental field and is not routinely performed in follow-up of HCC. Thus, with the aim to gain a better insight into the prognostic value of CTC in patients with HCC, we conducted a combined meta-analysis of 23 available studies [21-43] and to determine whether CTC detection indeed provide a more accurately estimate on prognosis of patients with HCC.

Materials and Methods

Literature Search

Medline, Ovid Database, Embase, The Science Citation Index, and Cochrane library were systematically searched without time and region restrictions. The following key search terms were used: "Circulating tumor cells" And "Hepatocellular carcinoma (HCC)" And "prognosis". In order to prevent missing relevant publications, the reference lists of the retrieved studies and reviews were also perused manually to check for potentially relevant studies. Cases of disagreement were resolved by discussing the title and abstract; Full-text articles (n= 57) were examined and 34 were excluded following the criteria below, and the remaining 23 available studies [21-43] were applied to determine whether CTC detection indeed provide a more accurately estimate on prognosis of patients with HCC.

Study eligibility criteria

Studies match all of the following inclusion criteria were considered eligible: (1) all enrolled patients were diagnosed with HCC, (2) the samples used in these studies should be peripheral blood, (3) evaluate association between the specific markers of circulating tumor cells and either relapse-free survival (RFS), overall survival (OS), or clinicopathologic parameter of hepatocellular carcinoma, (4) sufficient data to calculate a hazard ratio (HR) or a risk ratio (RR) with a 95% confidence interval (95% CI), (5) exclusion of letters to the editor, reviews, and articles published in non-English language, (6) when studies were based on the same patient population, only the most informative study was included.

Data Extraction and quality assessment

Three reviewers independently extracted data from eligible studies. The following information was extracted: the first author, the year of publication, population characteristics, detection method, the number of cases CTC positivity, prognostic values (OS and RFS), and the number of different clinical and pathological parameters (TNM stage, Tumor size, Vascular invasion, Portal vein tumor thrombus, Tumor number, Serum AFP level). Any disagreements on data extraction and quality assessment of the included studies were resolved through comprehensive discussion and checked by a fourth investigator.

The quality of the included studies was assessed with the Newcastle-Ottawa Scale (NOS) criteria for cohort and case-control studies [44]. Nine points is the perfect score, including three aspects: the definition and selection of the observation group and the control group of the study, comparable of two groups, exposed factors. More than seven points is high quality [45-47].

Statistical Analysis

Statistical analysis was performed with Review Manager (RevMan-Version5.3.). To statistically evaluate the prognostic effect of CTC, we extracted Hazard Ratio (HR) and their associated standard errors on relapse free survival (RFS) and/or overall survival (OS) from the included studies. We pooled the extracted HRs with the use of the generic inverse variance method in the Review Manager. If the HR and its 95% confidence interval (95% CI) were not provided directly, we calculated from the available data according to the method reported by Tierney J.F. et al [48]. When analyzed the association between CTCs and other parameters, Relative Risk (RR) was calculated. Heterogeneity between studies was tested with the Q test and I^2 statistic. We evaluated potential publication bias by a funnel plot, which was further examined by the Egger [49] using STATA software (Version 12.0, College Station, TX, USA). And pooled analysis of the diagnostic accuracy of CTC positivity was also calculated by STATA. A sensitivity analysis was conducted to assess the quality and consistency of results using the leave-one-out approach.

Results

Baseline Study Characteristics

387 studies were initially identified in the literature search. After screening titles and abstracts, 330 studies were excluded and 57 potential studies were reviewed further. An additional 34 studies were then excluded because they were not met the inclusion criteria. Finally, 23 studies were identified as eligible for inclusion in the meta-analysis (Fig. 1).

The included studies encompassed 1785 hepatocellular carcinoma patients and were conducted in 5 countries (China, Germany, France, Netherlands and Italy), published between 1999 and 2014. All studies analyzing peripheral blood, the number of CTC positive patients was 990 and 795 patients were CTC negative. The baseline characteristics and the quality of the included studies evaluated with the NOS are summarized in Table 1.

Fig. 1. Flowchart of the strategy used for the selection of reports used in our analysis. CTC, circulating tumor cells; HCC, hepatocellular carcinoma.

Table 1. Characteristics of studies included in the meta-analysis. Abbreviations: RT-PCR, reverse transcriptase polymerase chain reaction; NR, not report; ICC, immunocytochemistry; OS, overall survival; RFS, relapse free survival; DFS, disease-free survival; NOS, Newcastle-Ottawa Scale

Reference	Year	Number of patients (M/F)	Methods	Positive(%)	Tumor Stage/Grade	Followup (months)	Outcome	NOS
Choi,G.h.[21]	2015	63(48/15)	RT-PCR	49 (77.7%)	I-IV (Edmondson)	5-31	RFS	5
Cillo,U.[22]	2004	50(41/9)	RT-PCR	20 (40%)	I-IV (TNM)	1-32	RFS/OS	6
Fan,S.T.[23]	2011	82 (67/15)	CellSearch	56 (68.3%)	I-IV (TNM)	1.3-57.1	RFS/OS	5
Jeng, K.S.[24]	2004	81(42/39)	Other ICC	19 (23.45%)	I-IV (Edmondson)	24-60	RFS	6
Rahbari, N.N.[25]	2014	63(NR)	RT-PCR	36 (57%)	I-IV (UICC)	2.9-63.4	RFS	7
Hinz, S.[26]	2012	108(68/40)	RT-PCR	42 (38.9%)	I-IV (UICC)	1-111	OS	6
Kong, S.Y.[27]	2009	343(272/71)	RT-PCR	204 (59.4%)	I-IV (UICC)	12-60	OS	7
Matsumura,M[28]	1999	88(66/22)	RT-PCR	55 (63%)	I-IV (TNM)	13-46	OS	5
Pilati, P.[29]	2012	50(NR)	RT-PCR	25 (50%)	NR	4-37	OS	6
Kelley,R.K.[30]	2015	20(0/20)	CellSearch	8 (40%)	0-C (BCLC)	2-25	OS	7
Morris,K.L.[31]	2014	52 (46/6)	CellSearch	14 (28%)	II-IV (TNM)	16(Mean)	TTP/OS	5
Mu,H.[32]	2014	62(53/9)	CellSearch	30 (48.3%)	I-IV (AJCC)	24.1-48.5	OS	7
Schulze, K.[33]	2013	59(NR)	CellSearch	18 (30.5%)	A-C (BCLC)	2-36.1	OS	6
Sun, Y.F.[34]	2013	123(115/8)	CellSearch	51 (41.46%)	0-C (BCLC)	12.3-232	TTR	7
Liu, S.[35]	2013	60(53/7)	Flow cytometry	30 (50%)	II-IV (Edmondson)	3-24	DFS/OS	6
Vona, G.[36]	2009	44(32/12)	ISSET	23 (52.27%)	NR	1-40	OS	7
Fang, Z.T.[37]	2014	42(9/33)	CellSearch	22 (52.38%)	NR	4-10	TTP	6
Xu, W.[38]	2011	85(69/16)	CellSearch	69 (81%)	I-IV (TNM)	NR	NR	6
Li, Y.M.[39]	2013	60(NR)	CellSearch	46 (76.7%)	I-IV (TNM)	NR	NR	6
Yao,M[40]	2013	123(103/20)	RT-PCR	87 (70.7%)	I-IV (TNM)	NR	NR	6
Li, J.[41]	2014	27(21/6)	CellSearch	24 (88.9%)	I-IV (TNM)	NR	NR	7
Mou,D.C.[42]	2001	30(21/9)	RT-PCR	13 (43.3%)	I-IV (TNM)	1-33	OS	6
Bahnassy, A.A.[43]	2014	70(67/3)	Flow cytometry	39 (55.71%)	I-IV (TNM)	NR	NR	5

Fig. 2. Summary estimates of hazard ratio (HR) for relapse free survival (RFS) between CTCs-positive and CTCs-negative HCC patients.

Overall analysis of CTC effects on survival of patients with hepatocellular carcinoma

We extracted Hazard Ratio (HR) and their associated standard errors on relapse free survival (RFS) and/or overall survival (OS) from the included studies. The HR was measured by comparing the CTC positive and CTC negative. HR > 1 implies a poor prognosis in the CTC positive groups. Data on RFS were available in 5 studies [21-25], the pooled analysis showed that the presence of CTC significantly increased the risk of disease recurrence in HCC patients (HR 3.03, 95% CI: [1.89-4.86]; $p < 0.00001$) (Fig. 2), as the heterogeneity among studies was moderate ($p = 0.04$, $I^2 = 60\%$). The HRs for OS were available in 13 studies [21, 24-35]. Pooled analysis showed that CTC positivity was significantly associated with poor OS and increased

Fig. 3. Subgroup analysis by detection method for the difference in OS between CTCs-positive and CTCs-negative HCC patients.

risk of death (HR 2.45, 95% CI: [1.73–3.48]; $p < 0.0001$) (Fig. 3), and the heterogeneity among studies was significant ($I^2 = 62\%$, $p = 0.002$). We stratified studies of positive CTC detected by RT-PCR, Cell-search system and other ICC for subgroup. A similar trend was observed in the pooled analysis using the different methods (RT-PCR: HR 2.12, 95% CI: [1.24–3.62], $p = 0.006$; Cell-search system: HR 2.92, 95% CI: [1.85–4.63], $p < 0.00001$; other ICC: HR 2.45, 95% CI: [1.12–5.35], $p = 0.02$).

Correlation of Circulating Tumor Cells with Clinicopathologic Parameters

When analyzed the association between CTC and other parameters, Relative Risk (RR) was calculated, $RR > 1$ implied that CTC was associated with parameter. 9 studies [22, 28, 31, 38–43] reported the relationship between CTC positivity and TNM stage, the overall positive rate of CTC in stage I and II group was 43.71% compared with 56.28% of stage III and IV group. Pooled analysis showed that CTC positivity in stage III and IV is greater than that in stage I and II ($RR = 1.30$, 95% CI: [1.02–1.65]; $p = 0.03$; random effects), with significant heterogeneity between studies ($I^2 = 73\%$, $p = 0.0002$) as shown in Fig. 4A. Tumor size [27, 28, 34–39, 41] ($RR = 1.36$, 95% CI: [1.09–1.69]; $p = 0.006$; random effects) was associated with CTC positivity (Fig. 4B), the between-study heterogeneity was moderate ($I^2 = 33\%$, $p = 0.16$). 8 studies [22, 27, 30, 33–35, 38, 39] analysis showed that positive CTC were associated with Vascular invasion ($RR = 1.99$, 95% CI: [1.43–2.77]; $p < 0.0001$; random effects) (Fig. 4C), the between-study heterogeneity was significant ($I^2 = 49\%$, $p = 0.06$). 8 studies [27, 36–41] assessed the relationship between CTC positivity and portal vein tumor thrombus ($RR = 1.73$, 95% CI: [1.42–2.11]; $p < 0.0001$; random effects) (Fig. 4D), the heterogeneity among studies was moderate ($I^2 = 28\%$, $p = 0.20$). The RRs for Tumor number were available in 6 studies [22, 27, 34–36, 40], the estimated pooled RR showed a trend towards greater numbers of CTC with more tumor number, but they did not achieve statistical significance ($p = 0.07$, Fig. 4E). RRs for Serum AFP level were available in 8 studies [24, 27, 30, 31, 34, 37, 40, 42]. The estimated pooled RRs indicated that CTC was associated with $AFP \geq 400$ ng/mL ($RR = 2.05$,

Fig. 4. Meta-analysis of the association between CTC and tumor-related parameters in HCC patients. (A), TNM stage; (B), Tumor size; (C), Vascular invasion; (D), Portal vein tumor thrombus; (E), Tumor number; (F), AFP level.

95% CI: [1.18–3.54]; $p=0.01$, the between-study heterogeneity was significant ($I^2=84\%$, $p<0.000001$) (Fig. 4F).

Fig. 5. Sensitivity analysis of the influence of each individual study on the pooled HRs by omitting individual studies.

Fig. 6. The publication bias of this meta-analysis were assessed by Egger's linear regression test and Begg's funnel plots. Egger's linear regression test (A) and Begg's funnel plots (B) of OS in patients with HCC. Log[HR] natural logarithm of HR; horizontal line mean magnitude of the effect. Each point represents a separate study for the indicated association. Note: A funnel plot with pseudo 95 % confidence limits was used.

Sensitivity analysis and publication bias

Sensitivity analysis was conducted by deleting one single study from the overall pooled analysis each time, the result showed that neither the direction nor the magnitude of the estimated pooled results for OS was obviously affected, indicating that no single study dominated our results (Fig. 5). No significant publication bias was found in the pooled analysis of RFS tested by Begg's test ($p=0.541$, Fig. 6B). Furthermore, Egger's test (Fig. 6A)

was performed to assess the publication bias of OS in this meta-analysis. The P value of the Egger's test was 0.015, which was > 0.01 but < 0.05 , it indicates that there is publication bias existed in this meta-analysis.

Discussion

Numerous studies have showed that the presence of CTC was significantly associated with prognosis or other clinicopathologic parameters in hepatocellular carcinoma. While, the lack of statistical power together with their different studies design and results limited the individual clinical value with the prognostic effect of CTC positivity, the clinical significance of CTC in HCC patients has not yet been confirmed, and whether CTC can be used as a predictive marker for prognosis is controversial.

The results of our meta-analysis show that CTC positivity was associated with poor RFS and OS, significantly increased risk of disease recurrence and death. Moreover, we evaluated the correlation of CTC with the main HCC clinical pathological parameters. Significant correlations were observed between CTC detected in the blood and TNM stage, tumor size, vascular invasion, Portal vein tumor thrombus, tumor number or serum AFP level, but not with tumor number. Thus, CTC positivity in peripheral blood of HCC patients should indeed be considered as a prognostic marker. A sensitivity analysis, performed by removing each study individually, confirmed the stability of our results.

However, during the process of our meta-analysis, several limitations must be noted. We noticed a certain degree of heterogeneity, and potential sources of heterogeneity may derive from the follows: Firstly, different in demographic, clinicopathologic data or characteristics (i.e. age, sex and race) of included patients, particularly, the etiological factors of HCC. The different etiologies include hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, chronic alcohol abuse, non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, liver metastases from other types of cancer and so on. Hepatitis is the leading cause of HCC in Asia, while in Europe, the majority of cases was based on heavy chronic alcohol abuse. Therefore, the integration of CTC detection in homogeneous patients may more accurately predict the tumor response. Secondly, types of target tumor markers, standard of CTC positivity and detection method. The hepatocellular carcinomas can synthesize various tumor-related proteins, polypeptides, and isoenzymes more or less specific of the hepatoma tissues as well as the corresponding mRNA [50, 51]. We observed that many specific HCC markers are available and useful for the detection of the CTC, duo to the heterogeneity of the hepatocellular carcinoma, at least partly. About the standard of CTC positivity, the cutoff value and blood collection were different among studies. Different methods have different definitions, Furthermore, the definitions of CTC positivity and blood collection in the studies that using the same method such as ICC were also different. Various methods have been developed and optimized for CTC detection. Immunocytochemistry (ICC) and reverse transcriptase polymerase chain reaction (RT-PCR) are the two main approaches currently. Real-time polymerase chain reaction is a method that in addition to be specific by the nature of the primers used which can specifically amplify the number of copies of mRNA originally presents in the sample, such as AFP mRNA. Among various ICC methods, the Cell-search system is the only assay authorized by the U.S. Food and Drug Administration (FDA) for clinical use. This system is mainly based on semiautomatic isolation of epithelial tumor cells using immunomagnetic separation technology, and has been widely used in a number of malignancies, mainly is breast cancer [52-54]. In our analysis, the number of studies using RT-PCR to detect CTC is nearly equal to the number of studies using ICC. Subgroup analyses for OS stratified by detection method were performed. Subgroup analysis pooled HRs for OS are stably statistically significant in RT-PCR, Cell-search and other ICC subgroups with no heterogeneity. The pooled results are fairly stable and not influenced by the CTC detection method firmly provides evidence that the presence of CTC in peripheral blood indicates poor prognosis in patients with HCC. In addition, sampling time, also an important factor that

leads to heterogeneity and interferes the prognostic value of CTC positivity. Huang, et al [55] found that the prognostic and predictive significance of CTC was relevant to CTC sampling time through analyzed the effect of CTC as a predictive biomarker according to different sampling times.

We addressed the issue of heterogeneity by a rigorous methodological approach that used the random-effects model for more conservative estimates. However, heterogeneity could be decreased but not eliminated. Something else was also responsible for the limitations. Firstly, it is the inability to access primary data of the included studies. Prognostic factors of hepatocellular carcinoma are complicated, while there is very limited data on the clinical relevance of CTC positivity in HCC patients. Our data for meta-analysis was from the included studies and primary data was inaccessible, thus, we were unable to exclude every possible confounding factor. Moreover, several studies did not provide HRs directly and we must estimate them from the available data according to the method reported by Tierney J.F. [48]. Furthermore, studies introduced to pooled analysis have relatively small sample size and language restriction of our analysis to published studies written in English, may also result in publication bias. Our meta-analysis also indicates that CTC were associated with the RFS and OS and clinical pathological parameters (TNM stage, tumor size, vascular invasion, portal vein tumor thrombus, AFP level) of HCC patients, although we were unable to conduct analyses considering certain potentially relevant factors.

In conclusion, the present results support the notion of a strong prognostic value of CTC in HCC. CTC could be useful as an effective indicator to evaluate the poor clinicopathological prognostic factors in the progression of HCC. In future, well-designed, large-scale, detailed and accurate studies are required to explore CTC predictive value for the prognosis of patients with HCC.

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Disclosure Statement

No potential conflicts of interest were disclosed.

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