



## Original Article

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# The diagnostic value of circulating tumor DNA in hepatitis B virus induced hepatocellular carcinoma: a systematic review and meta-analysis

Young Chang<sup>1</sup>, Soung Won Jeong<sup>1</sup>, Jae Young Jang<sup>1</sup>, Hyuksoo Eun<sup>2</sup>, Young-Sun Lee<sup>3</sup>, Do Seon Song<sup>4</sup>, Su Jong Yu<sup>5</sup>, Sae Hwan Lee<sup>6</sup>, Won Kim<sup>7</sup>, Hyun Woong Lee<sup>8</sup>, Sang Gyune Kim<sup>9</sup>, Seongho Ryu<sup>10</sup>, Suyeon Park<sup>11,12</sup>

<sup>1</sup>Institute for Digestive Research, Digestive Disease Center, Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul; <sup>2</sup>Department of Internal Medicine, College of Medicine, Chungnam National University, Daejeon; <sup>3</sup>Department of Internal Medicine, Guro Hospital, Korea University College of Medicine, Seoul; <sup>4</sup>Department of Internal Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Suwon; <sup>5</sup>Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul; <sup>6</sup>Department of Internal Medicine, Soonchunhyang University College of Medicine, Cheonan; <sup>7</sup>Department of Internal Medicine, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul National University College of Medicine, Seoul; <sup>8</sup>Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul; <sup>9</sup>Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon; <sup>10</sup>Soonchunhyang Institute of Medi-bio Science (SIMS), Soonchunhyang University, Cheonan; <sup>11</sup>Department of Biostatistics, Soonchunhyang University Hospital, Seoul; <sup>12</sup>Department of Applied Statistics, Chung-Ang University, Seoul, Korea

**Background/Aim:** New biomarkers are urgently needed to aid in the diagnosis of early stage hepatocellular carcinoma (HCC). We performed a meta-analysis on the diagnostic utility of circulating tumor DNA (ctDNA) levels in patients with hepatitis B virus-induced HCC.

**Methods:** We retrieved relevant articles from PubMed, Embase, and the Cochrane Library up to February 8, 2022. Two subgroups were defined; one subset of studies analyzed the ctDNA methylation status, and the other subset combined tumor markers and ctDNA assays. Pooled sensitivity (SEN), specificity (SPE), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the summary receiver operating characteristic curve (AUC) were analyzed.

**Results:** Nine articles including 2,161 participants were included. The overall SEN and SPE were 0.705 (95% confidence interval [CI], 0.629-0.771) and 0.833 (95% CI, 0.769-0.882), respectively. The DOR, PLR, and NLR were 11.759 (95% CI, 7.982-17.322), 4.285 (95% CI, 3.098-5.925), and 0.336 (0.301-0.366), respectively. The ctDNA assay subset exhibited an AUC of 0.835. The AUC of the combined tumor marker and ctDNA assay was 0.848, with an SEN of 0.761 (95% CI, 0.659-0.839) and an SPE of 0.828 (95% CI, 0.692-0.911).

**Conclusions:** Circulating tumor DNA has promising diagnostic potential for HCC. It can serve as an auxiliary tool for HCC screening and detection, especially when combined with tumor markers. (J Liver Cancer 2022;22:167-177)

**Keywords:** Carcinoma, hepatocellular; Circulating tumor DNA; Biomarkers; Diagnosis

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## Corresponding author: Soung Won Jeong

Institute for Digestive Research, Digestive Disease Center, Department of Internal Medicine, Soonchunhyang University Seoul Hospital, Soonchunhyang University College of Medicine, 59 Daesagwan-ro, Yongsan-gu, Seoul 04401, Korea  
Tel. +82-2-710-3076, Fax. +82-2-709-9696  
E-mail: jeongsw@schmc.ac.kr

## INTRODUCTION

In 2020, primary liver cancer was the sixth most common cancer and third leading cause of cancer-related deaths.<sup>1</sup> Hepatocellular carcinoma (HCC) is the most common primary

liver cancer (75-90% of all the cases).<sup>2</sup> Of the several risk factors, chronic hepatitis B virus (HBV) infection is the most important, being associated with approximately 54% of HCC cases worldwide<sup>3</sup> and 62.2% of HCC cases in Korea.<sup>4</sup> Although early diagnosis is of utmost importance, HCC is often initially asymptomatic; most patients are diagnosed in the middle or late stages. Therefore, surveillance of patients with high-risk features on ultrasonography (US), with or without the measurement of  $\alpha$ -fetoprotein (AFP) levels, is important.<sup>5,6</sup> However, the recommended surveillance methods vary between studies. The sensitivity (SEN) of US-mediated early stage HCC detection in high-risk patients (with and without serum AFP measurement) was approximately 60%.<sup>7-9</sup> New biomarkers that facilitate early detection are thus required. Circulating tumor DNA (ctDNA) fragment assays (also known as ‘liquid biopsies’) may aid HCC detection<sup>10,11</sup> because ctDNA contains all of the genetic information of tumor cells and can be detected in the peripheral blood of early stage HCC patients.<sup>12,13</sup> HCC development is accompanied by genetic and epigenetic mutations in the ctDNA. HCC-specific mutations in the plasma DNA have been reported in several studies.<sup>14-16</sup> However, given the low amounts of ctDNA in patients with early stage cancers, it is difficult to distinguish true mutations from PCR and sequencing errors.<sup>17</sup> A panel of common HCC-associated mutations has been used to diagnose HCC. Genomic profiling has identified key driver mutations in *TP53*, *CTNNB1*, *PIK3CA*, *PTEN*, *AXIN1*, the promoter of *TERT*, and integrated HBV genomes.<sup>18-20</sup> Both hyper and hypomethylated ctDNA regions are used for early tumor detection, and epigenetic changes play important roles in carcinogenesis.<sup>21,22</sup> It is easier to detect, amplify, and quantify aberrant ctDNA hypermethylation than to document genomic mutations.<sup>23</sup> In humans, many 5-hydroxymethylcytosine (5hmC) epigenetic markers are generated via oxidation of 5-methylcytosine by ten-eleven translocation enzymes.<sup>24</sup> Such biomarkers aid in precision medicine because they reflect cancer gene regulation at the tissue level. Technical advances have made liquid biopsies convenient.<sup>25-28</sup> However, although many studies have reported that HCC ctDNA assays are diagnostic, they vary extensively in terms of design and results. Therefore, there is an

urgent need to assess the diagnostic utility of HCC ctDNA assays. Since the etiology of HCC could be one of the reasons for the heterogeneity among previous studies, we performed a systematic literature review and meta-analysis to assess the diagnostic utility of ctDNA assays in HBV-induced HCC.

## METHODS

### 1. Search strategy and literature selection

The meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary Table 1).<sup>29</sup> We systematically searched PubMed, Embase, and the Cochrane library. The language was limited to English and the search was limited to humans. The search terms were “circulating tumor DNA” OR “circulating DNA” OR “ctDNA” OR “plasma DNA” OR “serum DNA” OR “liquid biopsy” OR “cell free tumor DNA” OR “cell free nucleic acid” OR “circulating cell free nucleic acid” OR “circulating nucleic acid” OR “cell free DNA” AND “liver cancer” OR “hepatocellular carcinoma” OR “liver neoplasm” OR “hepatic carcinoma” OR “liver tumor” OR “liver cell carcinoma” OR “hepatocarcinoma” OR “hepatoma” OR “liver cell cancer” OR “hepatic cancer” OR “hepatic neoplasm” OR “hepatocellular cancer” OR “hepatocellular neoplasm” OR “hepatocellular tumor” OR “liver cell neoplasm” OR “HCC” AND “chronic hepatitis B” OR “chronic hepatitis B virus infection” OR “hepatitis B virus” OR “chronic HBV” OR “HBV infection” OR “hepatitis B infection.” We manually reviewed the reference lists and added the relevant articles.

### 2. Inclusion and exclusion criteria

The inclusion criteria were HBV-induced HCC, HCC ctDNA assay of serum or plasma, use of ctDNA data for initial HCC diagnosis, and availability of sensitivity (SEN) and specificity (SPE) data (either explicit or calculable). The exclusion criteria were reviews, cases, abstracts, letters, comments, meta-analyses, duplicate reports, predictions of HCC risk or prognosis, no SEN or SPE data, and/or analysis of ctDNAs that are not in the serum or plasma (rather in the liver or urine).

### 3. Data extraction and quality assessment

Two investigators independently selected relevant studies and summarized the results. We collected the following data: first author, publication year, references, study type, control modality, sample size, sampling time, sample source, detection methods, assay indicators, cutoff values, SEN, SPE, true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values. Both investigators independently assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 criteria<sup>30</sup> and any disagreement was resolved via discussion.

### 4. Statistical analysis

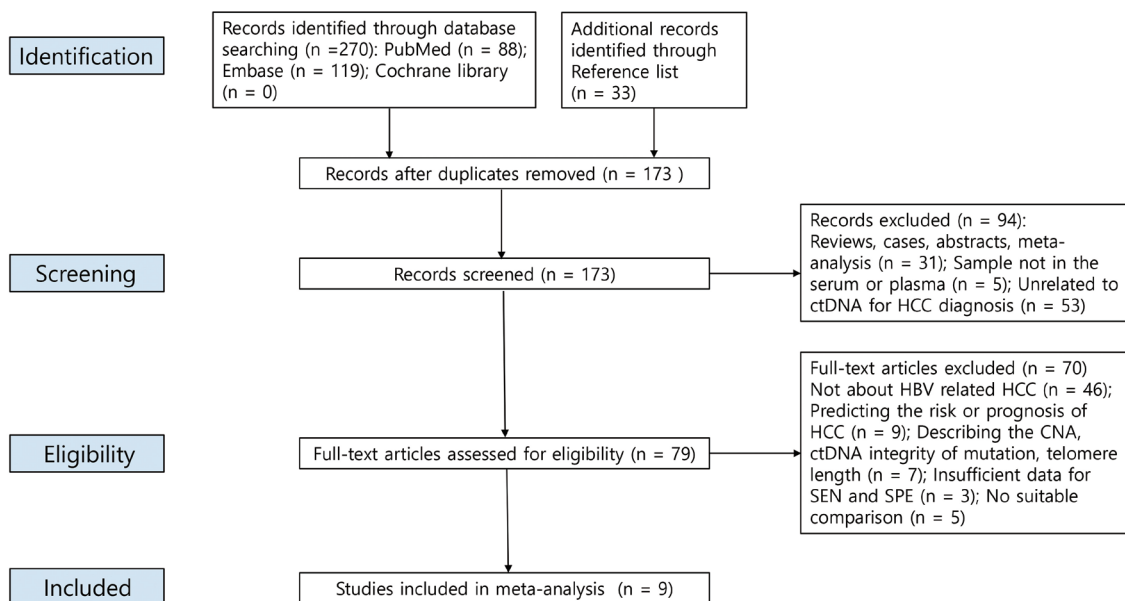
The TP, TN, FP, and FN data were extracted from all the studies. Heterogeneity was assessed by calculating the  $I^2$  statistic. An  $I^2$  value >50% reflected significant heterogeneity, and a random-effects model was then used for the analysis. Threshold effects, regression, and subgroup analyses were used to identify sources of heterogeneity. We derived the pooled SEN, SPE, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), summary receiver operating characteristic curve (SROC), and area under the curve (AUC) for all subjects and subgroups.

The Fagan Nomogram was used to validate clinical utility and the Deek funnel plot asymmetry test was used to detect publication bias ( $P < 0.10$  indicated such bias). We utilized the Rex software (version 3.6.0, Rex Soft Inc., Seoul, Korea) for analysis.

## RESULTS

### 1. Study characteristics

The PRISMA flow diagram is shown in Fig. 1. We initially retrieved 303 publications, but ultimately included nine studies<sup>19,31-38</sup> from 2013 to 2019 after excluding duplicates and studies that were poorly designed, and carefully reviewed the titles, abstracts, and full texts. The included studies enrolled 1,042 HCC patients and 1,119 controls. Most of the controls had benign liver disease (chronic hepatitis B or cirrhosis), but 149 of them were healthy. Table 1 summarizes the significant features of these studies. Of the nine studies, seven explored the diagnostic utility of ctDNA methylation<sup>32-38</sup> and two explored the utility of other genetic variants in HCC ctDNA.<sup>19,31</sup> Seven articles assessed the diagnostic utility of a combination of ctDNA and tumor marker assays.<sup>19,32-37</sup> In the six studies that reported sampling times, five



**Figure 1.** A PRISMA flow diagram of the literature search. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; CNA, copy number aberration; SEN, sensitivity; SPE, specificity.

**Table 1.** Characteristics of the included studies

Study	Study type	Control type	HCC/LC/CHB/HC (number)	Sampling time	Sample source	Detection methods	Assay indicators	Cutoff value	SEN (%)	SPE (%)	TP	FP	FN	TN
Cai et al. <sup>31</sup> (2019)	Case-control	CHB, LC	220/129/0	Pretreatment	Plasma	5hmC-seal technique	wd-score	27.9	82.7	67.4	182	42	38	87
Qu et al. <sup>19</sup> (2019)	Case-control	CHB	4/0/327/0	Pretreatment	Plasma	NGS	TP53, CTNNB1, AXIN1, TERT, HBV integrations	NA	100	94	4	20	0	307
Dong et al. <sup>32</sup> (2017)	Case-control	CHB, LC, HC	98/75/90/80	NA	Serum	MSP	RASSF1A	NA	52	91.5	51	21	47	224
							RASSF1A, BVES, HOXA9	NA	73.5	91.1	72	22	26	223
							RASSF1A, BVES, HOXA9, AFP	NA	83.7	78.9	82	52	16	193
Hu et al. <sup>33</sup> (2017)	Case-control	CHB, LC, HC	80/40/40/0	Pretreatment	Serum	MSP	UBE2Q1	NA	66.3	57.5	53	34	27	46
							UBE2Q1, AFP >200 ng/mL	NA	53.8	87.5	43	10	37	70
Dong et al. <sup>34</sup> (2015)	Case-control	CHB	190/0/120/0	Pretreatment	Serum	MSP	RASSF1A	NA	64.2	89.8	122	12	68	108
							RASSF1A, AFP >20 ng/mL	NA	80.9	93.4	154	8	36	112
Li et al. <sup>35</sup> (2014)	Case-control	CHB	136/0/46/0	Pretreatment	Serum	MSP	IGFBP7	NA	65.4	82.6	89	8	47	38
							IGFBP7, AFP >20 ng/mL	NA	85.3	41.3	116	27	20	19
Han et al. <sup>36</sup> (2014)	Case-control	CHB	160/0/88/0	NA	Serum	MSP	TGR5	NA	48.1	86.4	77	12	83	76
							TGR5, AFP >400 ng/mL	NA	65.0	85.2	104	13	56	75
Yang et al. <sup>37</sup> (2014)	Case-control	CHB, LC	123/28/29/0	NA	Serum	MSP	CDO1	NA	42.3	89.5	52	6	71	51
							CDO1, AFP >20 ng/mL	NA	82.9	75.4	102	14	21	43
Zhang et al. <sup>38</sup> (2013)	Case-control	HC	31/0/0/27	Pretreatment	Serum	Illumina 450 beadchip and bisulfite sequencing	DBX2, THY1	NA	88.9	87.1	28	3	3	24
							THY1	NA	85.2	80.7	26	5	5	22

HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B; HC, healthy control; SEN, sensitivity; SPE, specificity; TP, true positive; FN, false negative; FP, false positive; TN, true negative; 5hmC, 5-hydroxymethylcytosines; NA, not available; NGS, next generation sequencing; MSP, methylation-specific polymerase chain reaction; wd score, weighted diagnostic score; TP53, tumor protein P53; CTNNB1, catenin beta 1; AXIN1, Axin 1; TERT, telomerase reverse transcriptase; HBV, hepatitis B virus; AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; RASSF1A, Ras association domain family member 1; BVES, blood vessel epicaldial substance; HOXA9, homeobox A9; UBE2Q1, ubiquitin conjugating enzyme E2 Q1; IGFBP7, insulin like growth factor binding protein 7; TGR5, G-protein-coupled bile acid receptor Gpbar1; CDO1, cysteine dioxygenase type 1; DBX2, developing brain homeobox 2; THY1, Thy-1 cell surface antigen.

samples were collected before treatment, and one sample was collected before diagnosis. ctDNA was obtained from the plasma (n=2) or serum (n=7). The assay methods used included methylation-specific polymerase chain reaction and bisulfite sequencing.

## 2. Quality assessment

The quality of the nine studies is shown in Fig. 2 and Supplementary Table 2. Most were of moderate-to-high quality. However, four patients were at risk of patient selection bias because consecutive or random sampling was absent, the study design was not case-controlled, or patients were inappropriately excluded. Four studies may be associated with an unknown risk of bias in terms of the index test; it is unclear if the test results were interpreted without knowledge of the reference data or whether the threshold was predefined. All the reference standards exhibited a low risk of bias.

## 3. Diagnostic value of ctDNA assay in HBV-induced HCC patients

The SENs and SPEs of various ctDNAs in the peripheral blood of patients with HBV-induced HCC were evaluated using forest plots (Fig. 3A). Significant heterogeneity was evident in terms of both SENs ( $I^2=91.1\%$ ,  $P<0.001$ ) and SPEs ( $I^2=92.0\%$ ,  $P<0.001$ ); the SENs and false-positive rates were positively correlated ( $\rho=0.273$ ; 95% confidence interval [CI], -0.239 to 0.667). The meta-analysis employed a random-effects model. The pooled sensitivity and specificity

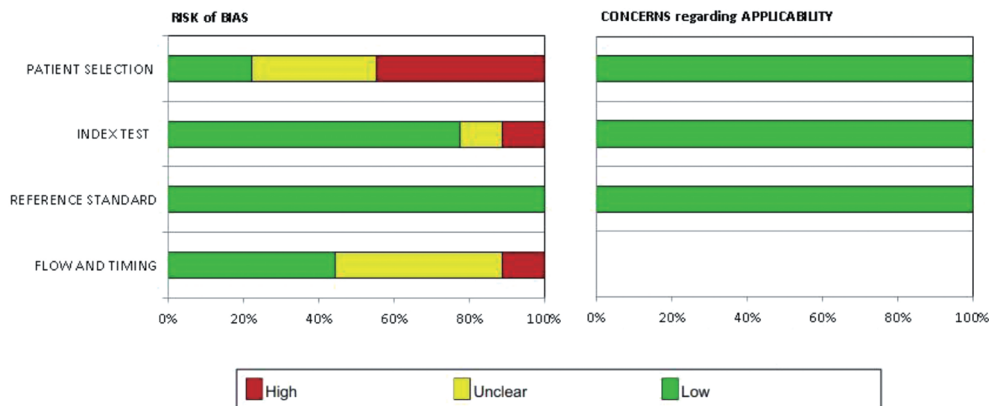
were 0.705 (95% CI, 0.629-0.771) and 0.833 (95% CI, 0.769-0.882), respectively. The DOR, PLR, and NLR were 11.759 (95% CI, 7.982-17.322), 4.285 (95% CI, 3.098-5.925), and 0.366 (95% CI, 0.301-0.336), respectively, and the SROC curve AUC was 0.835 (Fig. 4A).

## 4. Diagnostic value of ctDNA methylation assay in patients with HBV-induced HCC

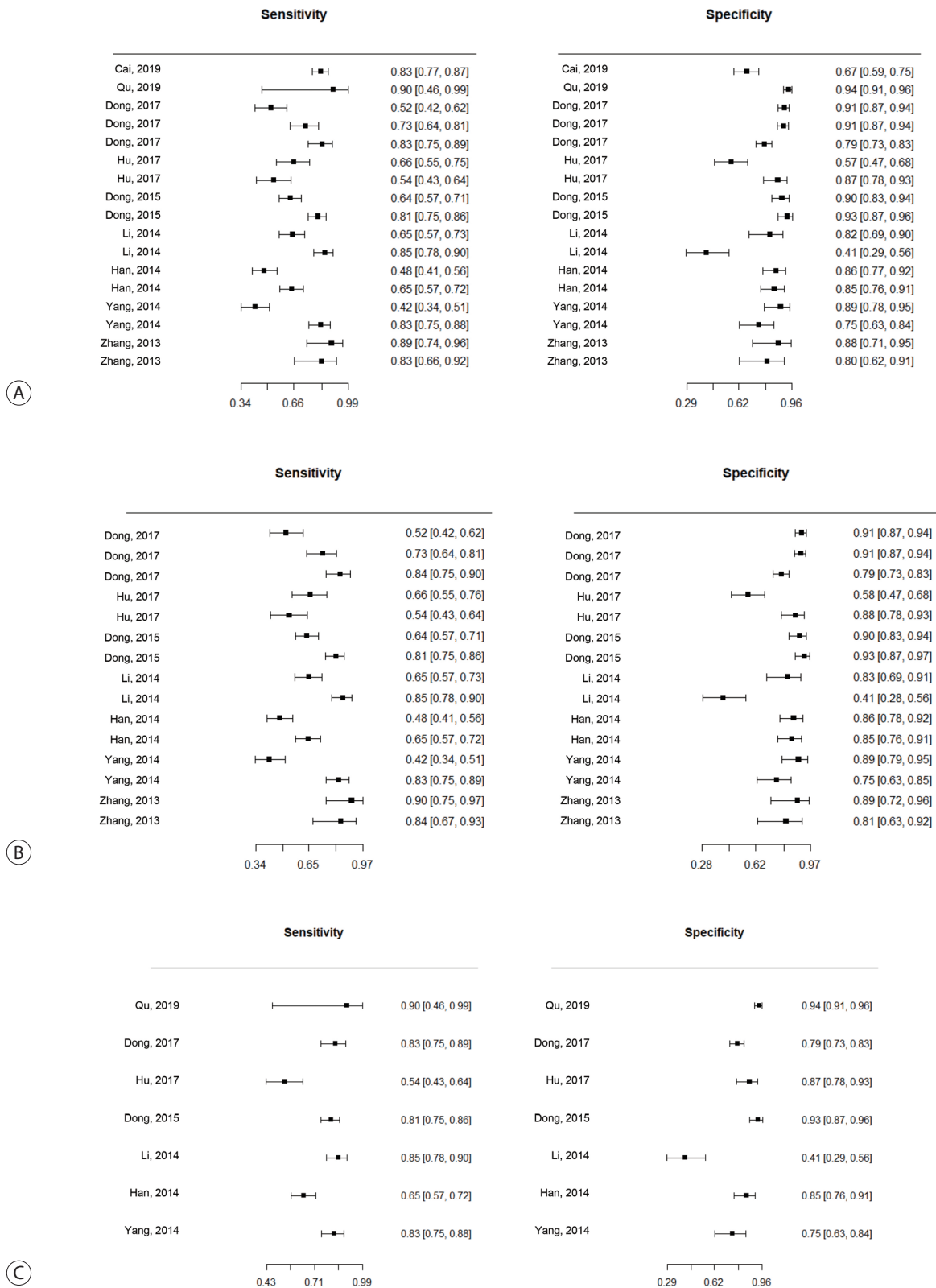
Of the nine studies, seven explored the diagnostic utility of ctDNA methylation assays. Significant heterogeneity was evident in terms of SENs ( $I^2=89.7\%$ ,  $P<0.01$ ) and SPEs ( $I^2=89.1\%$ ,  $P<0.001$ ). The pooled SEN and SPE were 0.7 (95% CI, 0.61-0.771) and 0.835 (95% CI, 0.768-0.885), respectively (Fig. 3B). The DOR, PLR, and NLR were 11.524 (95% CI, 7.539-17.616), 4.102 (95% CI, 2.925-5.752), and 0.379 (95% CI, 0.310-0.463), respectively. The SROC curve exhibited an AUC of 0.836 (Fig. 4B).

## 5. Diagnostic value of the ctDNA assay combined with tumor markers in patients with HBV-induced HCC

Seven studies explored the diagnostic utility of combined ctDNA and tumor marker assays. One study measured both  $\alpha$ -fetoprotein (AFP) and des-gamma-carboxy-prothrombin (DCP) levels, while the others considered only AFP levels. The  $I^2$  values were 66.9% for SENs and 85.0% for SPEs. The pooled SEN and SPE were 0.760 (95% CI, 0.659-0.839) and 0.828 (95% CI, 0.692-0.911), respectively (Fig. 3C), and the



**Figure 2.** Quality assessment of the included studies using the revised QUADAS-2. QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies-2.



**Figure 3.** Forest plots of sensitivity and specificity of ctDNA assay for diagnosing HBV-related HCC. (A) Overall ctDNA assay. (B) ctDNA methylation. (C) ctDNA assay combined with tumor markers. ctDNA, circulating tumor DNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

AUC of the SROC curve was 0.848 (Fig. 4C). The DOR, PLR, and NLR values were 14.273 (95% CI, 7.299-27.910), 4.641 (95% CI, 2.563-8.402), and 0.304 (95% CI, 0.215-0.430), respectively.

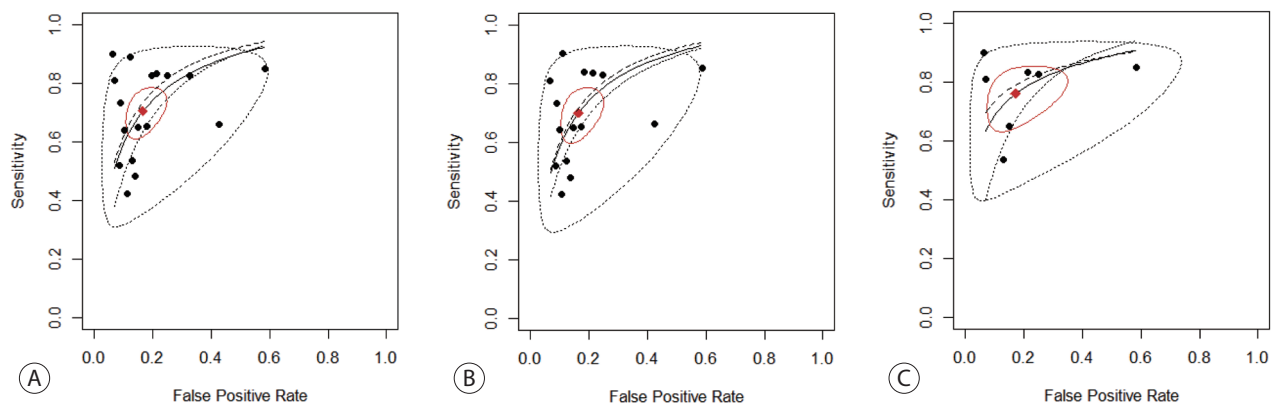
## 6. Meta-regression analysis and publication bias

Meta-regression analysis was performed to explore the potential causes of heterogeneity. Sample sources, assay methods, number of genes studied, and inclusion of tumor markers did not significantly contribute to heterogeneity (Table 2). Publication bias was assessed using the Deek funnel plot asymmetry test. No significant overall bias was evident ( $P=0.985$ ) (Fig. 5A), and no bias was apparent in either the ctDNA methylation group ( $P=0.428$ ) (Fig. 5B), or the ctDNA with tumor markers group ( $P=0.838$ ) (Fig. 5C).

## DISCUSSION

HBV infection remains the principal risk factor for HCC and, thereby, for cancer-associated deaths. One of the factors primarily responsible for the poor prognosis of HCC is its low early diagnosis rate. Despite various surveillance programs, many patients are still diagnosed at advanced stages, when optimal treatment is often impossible. It is important to detect HCC at an early stage to increase the survival rate of patients.

With the increasing advances in sequencing technology, novel tools for diagnosing liver diseases are emerging. Liquid biopsy is attracting attention as a noninvasive and reliable biomarker. Circulating extracellular vesicles, DNA, RNA, and tumor cells have emerged as attractive liquid biopsy candidates because they fulfil the key requirements of ideal biomarkers.<sup>39</sup> The application of novel molecular techniques to liquid biopsies has improved our understanding of the impact of ctDNA detection on HCC diagnosis.<sup>40</sup> We conducted

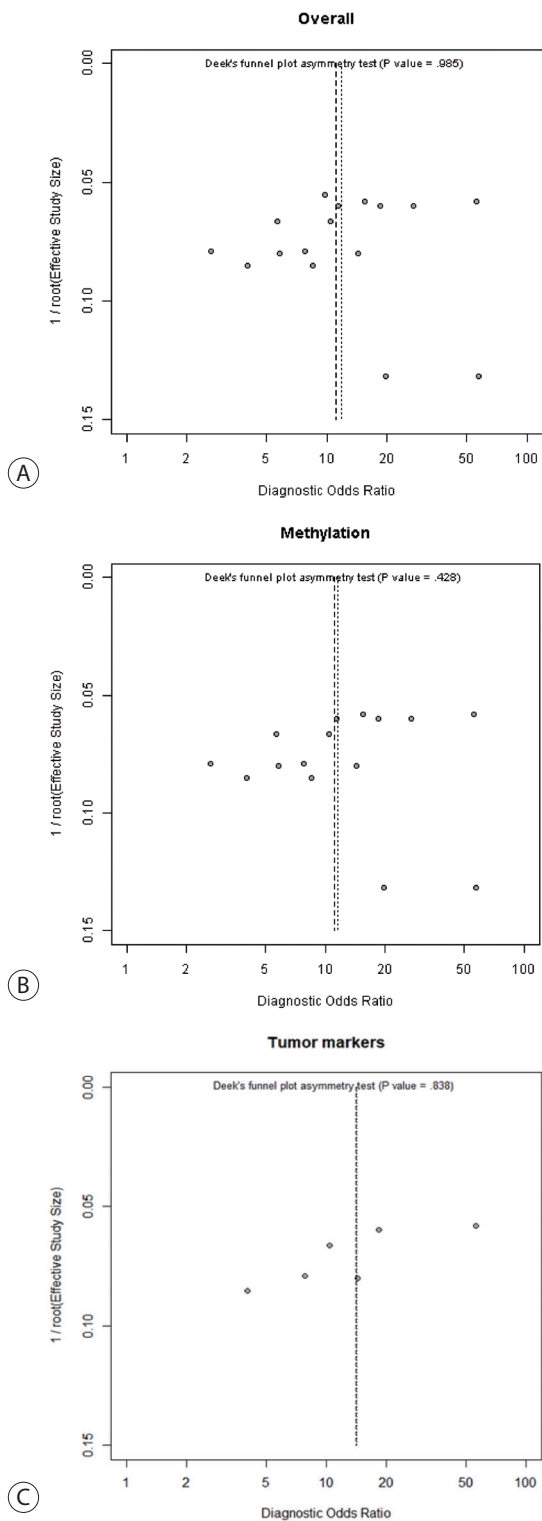


**Figure 4.** SROC curves of diagnostic value for ctDNA assay for diagnosing HBV-related HCC. (A) Overall ctDNA assay. (B) ctDNA methylation. (C) ctDNA assay combined with tumor markers. SROC, summary receiver operating characteristic; ctDNA, circulating tumor DNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

**Table 2.** Meta-regression of impacts of study features on diagnostic value of ctDNA for HCC

Covariates	Coefficient	Standard error	P-value	95% CI
Sample source	-0.272	0.716	0.704	-1.677 to 1.132
Assay methods	-0.272	0.716	0.704	-1.677 to 1.132
Number of genes	0.526	0.324	0.104	-0.109 to 1.160
Tumor markers	0.512	0.481	0.287	-0.431 to 1.454

ctDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; CI, confidence interval.



**Figure 5.** Funnel plots to evaluate the publication bias for ctDNA assay for diagnosing HBV-related HCC. (A) Overall ctDNA assay. (B) ctDNA methylation. (C) ctDNA assay combined with tumor markers. ctDNA, circulating tumor DNA; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

a systematic meta-analysis of studies on ctDNA-based HCC diagnosis, particularly in patients with chronic hepatitis B.

In this meta-analysis, various ctDNA assays, including somatic mutations and methylation, had a fair diagnostic performance for HBV-related HCC with an AUC of 0.835. The ctDNA methylation assay group showed a similar performance, with an AUC of 0.836. Notably, when ctDNA assays were combined with assays of tumor markers such as AFP and DCP, the diagnostic performance improved (AUCs of 0.838 and 0.829 with and without tumor marker assays, respectively; data not shown) in terms of discriminating HCC patients from control individuals. Previous studies have demonstrated that AFP exhibits unsatisfactory diagnostic performance (SENs, 0.478-0.540).<sup>41,42</sup> When combined, detection of ctDNA and AFP assay showed an excellent diagnostic performance (AUC, 0.944).<sup>42</sup> Along with our results, these results highlight that combinations of ctDNA and tumor markers have the potential to be novel adjuvant noninvasive tools for tumor markers in the screening and detection of HCC, regardless of its etiology.

We measured the DORs, wherein a DOR >10 indicated that the test discrimination was satisfactory.<sup>43</sup> The pooled DOR for the ctDNA assay that discriminated HCC patients from controls was 11.759, but this increased to 14.273 when the assay was combined with tumor markers, indicating the capability of integrating ctDNA assay with tumor markers to screen and detect HCC. The PLR and NLR values were also derived. The PLRs of the ctDNA assay alone and in combination with tumor marker assays were 4.285 and 4.641, respectively, indicating that HCC cases were 4- to 5-fold more likely to be ctDNA-positive than controls. The NLRs of the ctDNA assay alone and in combination with tumor marker assays were 0.366 and 0.304, respectively, indicating that the probability that individuals who tested negative in the ctDNA assay would in fact have HCC was 36.6% and 30.4%, respectively. Therefore, the negative ctDNA assay results should be interpreted with caution.

This study had several limitations. First, despite a thorough literature search, several valuable articles might not have been included by the search strategy. Although we added these articles manually as much as possible, there is still the



possibility that suitable publications were not included. Second, a significant among-study heterogeneity was observed. We performed a meta-regression to explore the potential causes of heterogeneity, but the sample source, assay method, number of genes studied, and selected tumor markers did not explain the heterogeneity. Heterogeneity might have arisen for other reasons that could not be assessed in this study because of a partial lack of data. Lastly, there are no data comparing the accuracy of ctDNA with surveillance versus surveillance alone for early HCC diagnosis. Further large-scale prospective clinical studies are needed to confirm the diagnostic utility of ctDNA assays in HCC. In conclusion, ctDNA assays may aid in HCC screening and detection, particularly when combined with tumor marker assays.

### Conflict of Interest

Young Chang, Hyuksoo Eun and Do Seon Song currently serve as editorial board members for *J Liver Cancer*, and they were not involved in the review process of this article. Otherwise, the authors have no conflicts of interest to disclose.

### Ethics Statement

This systemic review and meta-analysis is fully based on the articles which was already published and did not involve additional patient participants. Therefore, IRB approval is not necessary.

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### Data Availability

The data presented in this study are available upon reasonable request from the corresponding author.

### ORCID

Young Chang <https://orcid.org/0000-0001-7752-7618>  
Soung Won Jeong <https://orcid.org/0000-0003-2855-6011>

Jae Young Jang <https://orcid.org/0000-0001-5335-752X>  
Hyuksoo Eun <https://orcid.org/0000-0003-0485-0072>  
Young-Sun Lee <https://orcid.org/0000-0001-6396-0859>  
Do Seon Song <https://orcid.org/0000-0002-8278-4878>  
Su Jong Yu <https://orcid.org/0000-0001-8888-7977>  
Sae Hwan Lee <https://orcid.org/0000-0001-8320-5914>  
Won Kim <https://orcid.org/0000-0002-2926-1007>  
Hyun Woong Lee <https://orcid.org/0000-0002-6958-3035>  
Sang Gyune Kim <https://orcid.org/0000-0001-8694-777X>  
Seongho Ryu <https://orcid.org/0000-0001-9697-7972>  
Suyeon Park <https://orcid.org/0000-0002-6391-557X>

### Author Contribution

Study concept and design: JSW, CY, PS

Acquisition of data: EH, SDS, LYS, CY, JSW

Analysis and interpretation of data: LHW, KSG, LSH, KW, YSJ, JJY, RS, CY, JSW

Drafting of the manuscript: CY, JSW

Statistical analysis: PS, CY, JSW

Study supervision: JSW, CY

Writing review & editing: all authors

Approval of final manuscript: all authors

### Supplementary Material

Supplementary data can be found with this article online <https://doi.org/10.17998/jlc.2022.09.19>.

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