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Utility of Liquid Biopsy Analysis in Detection of Hepatocellular Carcinoma, Determination of Prognosis, and Disease Monitoring: A Systematic Review

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Abstract

BACKGROUND & AIMS: Liquid biopsies, or blood samples, can be analyzed to detect circulating tumor cells (CTCs), cell-free DNA (cfDNA), and extracellular vesicles, which might identify patients with hepatocellular carcinoma (HCC) or help determine their prognoses. We performed a systematic review of studies of analyses of liquid biopsies from patients with HCC and their comparisons with other biomarkers.

METHODS: We performed a systematic review of original studies published before December 1, 2019. We included studies that compared liquid biopsies alone and in combination with other biomarkers for the detection of HCC, performed multivariate analyses of the accuracy of liquid biopsy analysis in determining patient prognoses, or evaluated the utility of liquid biopsy analysis in monitoring treatment response.

RESULTS: Our final analysis included 112 studies: 67 on detection, 46 on determining prognosis, and 25 on treatment monitoring or selection. Ten studies evaluated assays that characterized cfDNA for detection of HCC in combination with measurement of α-fetoprotein (AFP)—these studies found that the combined measurement of cfDNA and AFP more accurately identified patients with HCC than measurement of AFP alone. Six studies evaluated assays for extracellular vesicles and 2 studies evaluated assays for CTC in detection of HCC, with and without other biomarkers—most of these studies found that detection of CTCs or extracellular vesicles with AFP more accurately identified patients with HCC than measurement of AFP alone. Detection of CTCs before surgery was associated with HCC recurrence after resection in 13 of 14 studies; cfDNA and extracellular vesicles have been studied less frequently as prognostic factors. Changes in CTC numbers before vs after treatment more accurately identify patients with HCC

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.04.019.

Conflicts of interest

These authors disclose the following: Max Wicha is the founder of OncoMed; Anna Lok serves on the advisory board for Epigenomics; and Neehar Parikh is a consultant for Bristol-Myers Squibb, Exelixis, and Freenome, serves on the advisory boards of Eisai, Bayer, Exelixis, and Wako Diagnostics, and has received research grants from Bayer, Target Pharmasolutions, and Exact Sciences. The remaining authors disclose no conflicts.

recurrence than pretreatment counts alone, and measurements of cfDNA can identify patients with disease recurrence or progression before changes can be detected by imaging. We found little evidence that analyses of liquid biopsies can aid in the selection of treatment for HCC. Quality assessment showed risk of bias in studies of HCC detection and determination of prognosis.

CONCLUSIONS: In a systematic review of 112 studies of the accuracy of liquid biopsy analysis, we found that assays for CTCs and cfDNA might aid in determining patient prognoses and monitoring HCC, and assays for cfDNA might aid in HCC detection, but there is a risk of bias in these studies. Studies must be standardized before we can assess the clinical utility of liquid biopsy analysis in the detection and management of patients with HCC.

Keywords

Liver Cancer; Outcome; Therapy; Prediction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the third leading cause of cancer death worldwide.[1] HCC is one of the few cancers with increasing incidence in the United States and this is driven primarily by the increase in nonalcoholic fatty liver disease and the peak in hepatitis C–related complications.[2–4] Prognosis after HCC diagnosis remains poor, with a median survival of fewer than 2 years.[5]

There are several challenges in HCC management. First, current surveillance strategies using ultrasound and α-fetoprotein (AFP) are inadequate, with a sensitivity of 63% and a specificity of 84%, for the detection of early stage HCC.[6] Although other biomarkers such as AFP-L3 and des-γ-carboxyprothrombin have shown promise for early detection, they have not been validated sufficiently for routine clinical use.[7,8] Second, we lack effective therapies and biomarkers to guide personalized selection of locoregional and systemic therapies for patients with advanced-stage HCC.[9,10] A major contributor to the lack of progress in developing more effective and precise therapies for HCC is the lack of available tissue to study tumor mutations and biology because HCC can be diagnosed reliably and treated based on imaging alone and routine diagnostic biopsy is not currently recommended by guidelines.[11–13] Only recently has deep sequencing of human HCC tissue identified molecular subtypes with distinct prognoses.[14–16]

A liquid biopsy, using circulating tumor-derived markers, may address limitations in both early detection and lack of molecular data on HCC. One example is circulating tumor cells (CTCs), the presence of which represents an intermediate stage between localized disease and distant metastasis.[17] CTCs can be detected in virtually all major solid tumors in the setting of metastatic disease as well as some early and intermediate-stage cancers.[17–19] Deep sequencing of CTCs can identify mutations, some of which are distinct from those detected in primary tumor samples, as has been shown in multiple myeloma[20] and prostate cancer,[21] and may provide more comprehensive information, compared with a needle biopsy, as a result of molecular heterogeneity of tumors within the same patient and sometimes within the same tumor. Another approach is the detection of cell-free DNA (cfDNA), released into the circulation from dead cancer cells and/or secretion from viable cancer cells.[22] cfDNA can be quantified and characterized for integrity, and also can be sequenced to detect mutations, methylation, and insertions–deletions.[22] cfDNA mutational profiles have been

investigated for the detection and prognosis of HCC, and for identification of clinically actionable mutations.[23–25] A third example is extracellular vesicles (EVs), which are formed by budding of lysosomes, cell membranes, or apoptotic bodies, which subsequently can be released into the circulation.[26] EVs affect cell–cell communication and have been investigated as both targets of therapy and as therapeutic modalities themselves.[27] Furthermore, EV presence/concentration and analysis of EV contents have been studied as circulating cancer biomarkers.[28] Liquid biopsy has not been studied extensively for the early detection of other cancers,[29] however, given inadequacies in early detection of HCC and an identifiable high-risk population to target for surveillance (ie, patients with cirrhosis), liquid biopsy could fill an important gap in HCC detection.

The role of liquid biopsy in HCC detection and prognosis has been reviewed recently.[30–32] One important clinical question yet to be addressed is whether liquid biopsy outperforms or offers incremental value to existing biomarkers, most notably AFP. Another question not addressed is whether liquid biopsy is a useful method for monitoring patients receiving HCC therapy. Thus, we conducted a systematic review of the role of liquid biopsy for HCC detection, prognostication and/or prediction of response to therapy, monitoring for recurrence, and treatment selection, focusing on the comparison of liquid biopsy with existing biomarkers used in clinical practice.

Methods

Literature Search

We performed a systematic review of Medline/PubMed, EMBASE, and the Cochrane library through December 1, 2019, with no start date restriction. Search terms are detailed in Supplementary Table 1. We also screened all articles referenced in these selected studies and in several recent review articles for eligibility.[30–32] There were no language restrictions; English abstracts of non-English language articles were screened and, when applicable, full-text studies were reviewed by authors fluent in their respective languages. (Chinese was the only non-English language for which full text review was required.)

Inclusion criteria were as follows: studies of patients diagnosed with HCC in which CTCs, cfDNA, or EVs were evaluated. Studies were required to report associations between liquid biopsy and 1 of the following: (1) detection, comparing circulating markers in patients with vs those without HCC, or sensitivity of the markers for detecting HCC; (2) prognosis/prediction, evaluating the effect of levels/presence of circulating markers on clinically relevant outcomes and/or response to therapy; (3) monitoring of residual disease during or after treatment; or (4) choice of HCC therapy. Finally, detection studies had to report either a comparison between liquid biopsy and existing biomarkers or the incremental value of liquid biopsy when combined with existing biomarkers (eg, AFP), and prognostic studies had to report the effect of liquid biopsy on prognosis in multivariable analysis.

Abstracts were reviewed independently by 2 authors (V.L.C. and D.X.), with discrepancies resolved by a third author (N.D.P.).

Data Extraction

We independently abstracted the required information from eligible studies using standardized forms developed by the investigators. We collected information on inclusion/exclusion criteria and country/countries of studies. For controls, we determined whether they had chronic liver disease (CLD) or not, and, if they did, we extracted the etiology of underlying liver disease. For participants with HCC, we abstracted this information plus HCC stage and cancer treatment modalities.

Clinical End Points

For the detection component of this study, the primary outcome of interest was the diagnostic accuracy of the liquid biopsy in distinguishing HCC patients from participants without HCC. We separately analyzed the comparisons of the following: HCC vs CLD and HCC vs healthy controls. For prognosis, outcomes of interest were mortality/overall survival, and tumor progression or recurrence.

A meta-analysis was not conducted owing to the heterogeneity in which components of liquid biopsy were evaluated.

Quality Assessment

Quality assessment was performed using the QUality Assessment of Diagnostic Accuracy Studies-2 tool for diagnostic studies[33] and the QUality In Prognosis Studies tool for prognostic studies.[34]

Results

A Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart illustrating study selection is shown in Figure 1.[35] The database search identified 2225 studies, of which 79 were included in the systematic review. We also reviewed all studies referenced in the selected studies, yielding 33 additional studies for inclusion in the systematic review. In total, 112 studies were included in this systematic review: 67 on detection, 46 on prediction/prognostication, and 25 on treatment monitoring/selection. Ten studies investigated both detection and prognosis; 3 studies investigated both detection and treatment monitoring/selection; 7 studies investigated both prognosis and treatment monitoring/selection; and 3 studies investigated detection, prognosis, and treatment monitoring/selection. We identified only 3 studies on treatment selection, which was not adequate for a systematic review and therefore this application is not discussed further in this article.[36–38]

Detection

We evaluated the utility of liquid biopsy for HCC detection based on either performance vs AFP, performance in AFP-positive vs AFP-negative patients, or a combination of liquid biopsy and AFP vs AFP or liquid biopsy alone.

Circulating tumor cells.

Seventeen studies investigated the utility of CTCs for HCC detection (Table 1).[39–55] The most commonly used method to detect CTCs was positive selection for epithelial markers such as cytokeratins, epithelial cell adhesion molecule, or asialoglycoprotein receptor (8 studies). Another method used was Canpatrol (2 studies), which used filtration followed by cell staining to identify both epithelial and mesenchymal phenotypes. In general, these studies showed that CTC levels were superior to AFP in distinguishing HCC from controls (3 of 4 studies reporting test characteristics for CTCs vs AFP), that CTC levels were increased in AFP-negative HCC patients (11 of 13 studies), or that a combination of CTCs and AFP was superior to AFP alone in differentiating HCC from controls (2 of 2 studies).

Most of the included studies compared the sensitivity of liquid biopsy for HCC detection in individuals with AFP levels above vs below a specific cut-off value, which varied from 4 to 400 ng/mL (most commonly 20 ng/mL).[42,43,46–55] A large study found that a CTC-derived polymerase chain reaction (PCR) score (quantifying expression of cancer-related genes in the blood) was increased in 125 of 171 (73%) patients with an AFP level less than 20 ng/mL.[44] One study using CanPatrol, a detection method that is not biased toward epithelial vs mesenchymal CTC phenotypes, reported an overall sensitivity of 78%, with lower sensitivity among patients with an AFP level less than 20 ng/mL (N = 17) than those with an increased AFP level (N = 63): 35% vs 89%, respectively ($P < .001$).[54]Another study using Cell- Search, which only identifies epithelial cells, found that CTCs were detected in 1 of 10 patients with an AFP level less than 400 ng/mL compared with 9 of 10 patients with an AFP level of 400 ng/mL or greater ($P = .008$).[47] A third study found that CD45(−) epithelial cell adhesion molecule (+) cells were detected in 6 of 12 patients with an AFP level less than 20 ng/mL vs 13 of 15 with an AFP level greater than 1000 ng/mL ($P < .05$).[43] Other studies found that CTC levels were not statistically significantly different in patients with HCC and increased vs normal AFP levels.[42,46,51,53,55]

Two studies evaluated whether CTCs provided incremental value to AFP alone for identifying HCC patients. One study using CanPatrol for the detection of CTCs in 113 HCC vs 57 CLD patients reported the area under the receiver operating characteristic curves (AUCs) for identifying HCC patients were 0.67 for AFP at a cut-off value of 400 ng/mL, 0.77 for CTCs, and 0.82 for a combination of CTCs and AFP (sensitivity not reported).[41] Another study found that CTCs, defined as the presence of *EpCAM*–messenger RNA, had a sensitivity of 42.6% and an AUC of 0.70 for differentiating HCC from controls that comprised CLD patients and healthy controls, while AFP (cut-off value, 400 ng/mL) had a sensitivity of 39.5% (AUC not reported),[45] and a combination of CTCs and AFP had a sensitivity of 73.0% and an AUC of 0.86. No studies reported that CTCs added no incremental benefit to AFP; however, the risk of publication bias exists.

Three studies compared AFP and CTCs in differentiating HCC from controls. A large study compared 395 HCC patients with 301 CLD and 210 healthy controls and found that a CTC-derived PCR score showed a sensitivity of 72.5%, a specificity of 95.0%, and an AUC of 0.88 compared with 57.0%, 90.0%, and 0.77 for AFP at a cut-off value of 20 ng/mL.[44] This score performed well in patients with early stage HCC, with an AUC of 0.92 among patients with Barcelona Clinic Liver Cancer (BCLC) stage 0, and 0.86 in patients with BCLC stage

A disease. Another study also found that a CTC-derived PCR score showed higher sensitivity than AFP (cut-off value, 20 ng/mL) in differentiating HCC vs CLD (85% vs 55%).[40] In contrast, 1 study found that CTCs (defined as CD45[−] plus either cytokeratin 19, CD90, or CD133[+]) had inferior sensitivity and specificity compared with the AFP ratio (undefined) in distinguishing HCC vs CLD.[39]

Cell-free DNA.

Several components of cfDNA may differ in patients with vs without cancer, such as total amount of cfDNA, cfDNA mutations (especially in candidate genes), or cfDNA methylation. Thirty-nine studies on cfDNA and HCC detection met inclusion criteria.[56–94] Ten studies compared diagnostic test characteristics of cfDNA properties with AFP; of these, 9 found cfDNA to be superior,[56,57,62,63,71,72,80,81,92] although 1 did not not.[82] Sixteen studies evaluated the sensitivity of cfDNA for the diagnosis of HCC among patients with normal AFP levels, results were variable, ranging from 15% to 100%, in part owing to heterogeneity in which components of cfDNA were measured.[57,59,61,69,73,77–79,84,86–91,94] Ten studies compared scores incorporating various components of cfDNA (ranging from amount of cfDNA to methylation of or mutation in specific genes) and AFP, and found that the combination had superior test characteristics than AFP level alone.[60,64–68,74,76,83,85,93] Thirteen of 14 studies comparing the combination of cfDNA and AFP with cfDNA alone found that the combination had superior sensitivity and/or specificity, [60,64–68,70,75,76,83,85,89,93] however, the incremental value of AFP was not observed in a recent large study in the United States.[74]

Extracellular vesicles.

Eleven studies on EVs in HCC detection were included (Supplementary Table 2).[95–105] Generally, EV properties added to AFP in detection capability, but the value of EVs for HCC detection is less clear than CTCs or cfDNA, in part related to greater heterogeneity in which properties of EVs were studied.

Six studies evaluated the combination measurement of EVs and AFP compared with AFP alone.[95,96,98,102–104] A large study (200 HCC patients, with 200 CLD and 200 healthy controls) found that AUCs of exosomal levels of 3 long noncoding RNAs were 0.96 and 0.53 in the training and validation cohorts, respectively, and 0.97 and 0.87 for the combination of these RNAs and AFP.[98] Another study found that exosomal miR-122, miR-148a, and AFP together showed an AUC of 0.947 for distinguishing HCC vs cirrhotic controls, vs 0.665 for AFP alone.[102]

Five studies compared EV properties with AFP without evaluating incremental value compared with AFP.[97,99–101,104] A large study with 71 HCC patients and 131 controls (37 of whom had liver disease) found that a machine learning–based score of EV long noncoding RNAs outperformed AFP for differentiating HCC vs benign liver lesions, with an AUC of 0.946 vs 0.834 ($P = .037$).[97] The utility of EVs may depend on disease etiology: 1 study reported that EV miR-212 had a higher AUC than AFP alone (0.886 vs 0.849) among HBV-related HCC, but not among non–HBV-related HCC (0.793 vs 0.840); *P* values were not provided.[105]

Quality assessment.

Results of the QUality Assessment of Diagnostic Accuracy Studies-2 analysis are shown in Supplementary Tables 3, 4, 5, and 6. The detection of HCC usually was reliable, with most studies using either cross-sectional imaging or biopsy, and in most cases the HCC patients were representative of the general HCC population. However, most studies did not specify how patients were selected (ie, consecutive patients vs convenience sampling). Furthermore, the HCC diagnosis in nearly all studies was established before the diagnostic liquid biopsy and most studies did not mention blinding, raising the possibility of bias. In addition, many studies did not separately report results for early stage HCC, or for comparisons with cirrhosis controls.

Prognosis

Liquid biopsy has the potential to serve as a prognostic biomarker or as a predictive biomarker for response to therapy. For this section, we included only studies that reported liquid biopsy properties in multivariate analysis to identify the incremental value of liquid biopsy in prognostication beyond conventional factors such as tumor stage.

Circulating tumor cells.

Twenty-one studies investigated CTCs as prognostic markers (Table 3).[36,44,45,48,49,55,106–120] Most studies (15 studies) focused on patients undergoing resection.[44,48,49,55,106–110,113–116,118,120] In this subpopulation, 13 of 14 studies found a significant association between the presence of CTC preoperatively and tumor recurrence or disease-free survival,[44,48,49,55,106–109,113–116,118,120] and 2 of 5 studies found a significant association between the presence of CTCs and overall survival.[48,107,109,117,120] The remaining studies included populations undergoing other therapies.[45,111,112,119] In all of these studies, the presence of CTC pretreatment was associated with a poorer prognosis.

Cell-free DNA.

Fifteen studies on cfDNA met inclusion criteria (Table 4).[37,56,58,63,73,77,86,92,121–127] Five studies focused on the total amount of cfDNA,[37,63,125–127] 5 studies focused on methylation either in single or multiple loci,[73,77,86,92,123] and 5 studies focused on mutations or copy number variation.[37,56,58,122,124] In general, a greater amount of cfDNA was associated with poorer overall survival, and tumor progression or recurrence.[126] Two studies reported that *RASSF1A* methylation was associated with decreased overall survival.[73,123] No other cfDNA properties were evaluated in more than 1 study.

Properties of cfDNA were associated with overall survival in multivariable analysis in most studies,[37,58,73,77,86,92,121–123,127] although 3 studies did not confirm such associations.[63,124,125]

Extracellular vesicles.

Ten studies on EVs met the inclusion criteria (Supplementary Table 6).[95,128–136] Most studies focused on exosomal microRNAs,[128–135] although 2 studies also investigated long noncoding RNAs[130,136] and another study evaluated *RAB11A* messenger RNA.[95] Six of

these studies focused on patients with early stage disease and/or undergoing surgical resection.[95,131,132,134–136] The only microRNA included in more than 1 study was miR-21, and all 3 studies found an association with increased disease progression and poorer disease-free survival.[130,133,135]

Quality assessment.

Results of the QUality In Prognosis Studies tool to assess the quality of prognostic studies are shown in Supplementary Tables 7, 8, and 9. Although measured outcomes consistently were objective (ie, based on survival or imaging), there was concern for risk of bias owing to lack of prespecified cut-off values and nonconsecutive patient selection.

Monitoring on Therapy

Thirteen studies used CTCs to monitor the response to therapy,[44–46,52,108,109,113,119,120,137–140] 12 evaluated cfDNA,[58,66,125,126,141–148] and none evaluated EVs.

Most studies on CTCs evaluated changes in CTC concentrations before vs after therapy, most commonly resection or locoregional therapy. Typically, patients who persistently had increased CTC counts after therapy or who initially had low/undetectable CTC counts who then increased posttherapy had a poorer prognosis than patients with persistently negative CTC or whose CTC concentrations decreased after therapy.[44,45,52,108,109,113,119,120,139,140] One study showed longitudinal measurements of a CTC-derived PCR score detected recurrence more accurately than AFP.[46] Another study found no association between preresection CTC count and overall or progression-free survival, but an increase in CTC count after resection was associated with poorer overall survival and progression-free survival.[140]

Few studies examined the effect of post-treatment cfDNA properties on prognosis, and those that did failed to show an association between post-treatment cfDNA and progression-free survival or overall survival.[126,141] Several studies evaluated dynamic changes in cfDNA after therapy[58,143,144,146,148] and found that cfDNA tended to increase at or before the time of disease recurrence/progression more reliably than did AFP. Indeed, 2 studies reported changes in cfDNA mutational profiles preceded radiographic documentation of tumor relapse/progression by up to 8 weeks.[143,146] It should be noted that all of the studies evaluating cfDNA for monitoring of tumor response were very small ($N < 20$), and results of some are available only in abstract form.

Discussion

In this systematic review, we identified 112 studies investigating CTCs, EVs, or cfDNA as biomarkers for detection, prognostication, and monitoring for treatment response in HCC. We found that liquid biopsy has value in early HCC detection and prognosis beyond existing diagnostic tests and tumor staging, respectively. In addition, liquid biopsy may be useful for monitoring response to therapy. Overall, CTCs, EVs, and cfDNA are appealing targets of biomarker development.

For detection, cfDNA methylation scores appear to have the most favorable test characteristics of all liquid biopsy tools included in this systematic review. In particular, their sensitivity for early stage HCC compares favorably with that of AFP and ultrasound, with large studies on early detection in more than 2000 patients showing more than 75% sensitivity and more than 90% specificity for TNM stage I or BCLC stage 0 disease in phase 2 biomarker studies (case-–control comparisons).[74,92,149] In contrast, the presence of CTCs has high specificity (>90%) but generally only modest sensitivity (~60%), whereas EVs have highly variable diagnostic accuracy. We note that CTC and EV gene expression have shown greater promise for early HCC detection[46,97,98]; however, these findings require external validation. In contrast, cfDNA mutations generally have low sensitivity for early stage HCC and CTC mutational profiles have not been well studied in HCC. Validation of cfDNA methylation scores in more robust phase 2 studies in which early stage HCC is compared with appropriate controls and is shown to perform well in diverse etiologies of liver disease are required before larger phase 3 and 4 studies are appropriate.[149]

As prognostic/predictive biomarkers, CTCs have been the most extensively evaluated liquid biopsy type with numerous studies consistently showing their utility in predicting recurrence after curative therapy beyond that of conventional metrics such as cancer stage. However, whether the prognostic value of CTCs also applies to patients receiving local ablative therapies or arterial-based locoregional therapies is unclear, and the value of CTCs in predicting outcomes in patients receiving noncurative therapy is even less certain. The literature on cfDNA (especially methylation scores) and EVs and prognosis is comparatively limited and less consistent in terms of which outcomes and populations have been studied. That does not necessarily imply that they are less prognostically important however: for example, circulating tumor DNA has higher sensitivity than CTCs at detecting minimal residual disease in several different tumor types.[150] How these differences in sensitivity may translate into utility as predictive markers in HCC is not known.

Finally, we note the potential utility of a liquid biopsy for monitoring treatment response. Several small studies have shown that CTCs or cfDNA may better predict tumor recurrence or progression than AFP and changes in CTCs or cfDNA may precede changes on imaging. We caution that the studies reporting these findings are small and many are available only in abstract form. In addition, the question arises regarding how to manage abnormal liquid biopsy findings in the absence of imaging evidence of progression/recurrence. A trial in patients with metastatic breast cancer receiving first-line chemotherapy found no benefit to changing therapy based on concerning CTC trends.[151] Larger studies will be required to confirm the utility of liquid biopsy in monitoring tumor progression/recurrence and whether it can be used to guide clinical management of HCC.

Although liquid biopsy is a promising tool, we identified several limitations in the literature. First, many studies did not include appropriate study populations. For early detection, the relevant comparison is between early stage HCC and patients at risk of HCC (ie, those with cirrhosis or selected patients with chronic hepatitis B). However, many diagnostic studies included CLD patients without cirrhosis or advanced fibrosis, or even healthy controls for comparisons. Furthermore, results for early stage HCC were not always reported separately.

Finally, most studies have not separately examined the accuracy of liquid biopsy for detection of HCC owing to viral vs nonviral etiologies.

Second, prognostic studies were highly inconsistent in which outcomes were reported, which raises concern for selective reporting and limits meta-analyses. There is a risk of bias based on nonconsecutive patient selection and ad hoc or post hoc cut-off values, for example, in analysis of cfDNA methylation levels or CTC concentration. These limitations can be avoided in future studies by using prespecified cut-off values and outcome reporting. We propose minimal reporting parameters of overall survival and (depending on stage and treatment type) either progression-free survival or tumor recurrence for future studies on liquid biopsy for prognostication.

A third, more fundamental limitation is that there has been wide variation in how liquid biopsy is defined. CTCs are the best-standardized of the 3 types of liquid biopsy we analyzed in this study and there is already a Food and Drug Administration–cleared CTC detection method for use in prostate, breast, and colorectal cancers.[18] However, there are a number of alternate methods of CTC detection, some of which focus on circulating cells with epithelial phenotypes while others focus on cell size or morphology rather than cell surface markers, which make direct comparisons across methodologies difficult.[17] Similarly, multiple methylation scores have been developed from cfDNA, each of which includes different loci.[89,92] EV studies largely have focused on microRNAs, and, again, there is little consistency in which microRNAs are included in analyses. Analysis of human CTCs, EVs, and cfDNA is an evolving field, and exploratory research is critical to developing a fundamental understanding of these entities. Establishment of optimal methodologies and standards in liquid biopsy technologies is required before robust validation studies and clinical utilization.

In conclusion, liquid biopsy has the potential as a biomarker for HCC detection, prognostication, and monitoring.[152] Further^research using standardized definition and methods of detection, quantification, and characterizing of CTCs, cfDNA, and EVs; as well as standardized study design, patient selection, and outcome reporting; followed by validation of promising biomarkers will be required before liquid biopsy can be recommended in the detection and clinical management of HCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper:

AFP	α-fetoprotein
AUC	area under the receiver operating characteristic curve
BCLC	Barcelona Clinic Liver Cancer
cfDNA	cell-free DNA
CLD	chronic liver disease
CTC	circulating tumor cell
EV	extracellular vesicle
HCC	hepatocellular carcinoma
miR	microRNA
PCR	polymerase chain reaction

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What You Need to Know

Background

Liquid biopsies, or blood samples, can be analyzed to detect circulating tumor cells, cell-free DNA, and extracellular vesicles, which might identify patients with hepatocellular carcinoma (HCC) or help determine their prognoses.

Findings

In a systematic review of 112 studies of the accuracy of liquid biopsy analysis, we found that assays for circulating tumor cells and cell-free DNA might aid in the detection or monitoring of HCC, or in determining patient prognoses. However, there was a risk of bias in these studies.

Implications for patient care

Studies of liquid biopsy analysis must be standardized before we can assess its utility in the detection and management of patients with HCC.

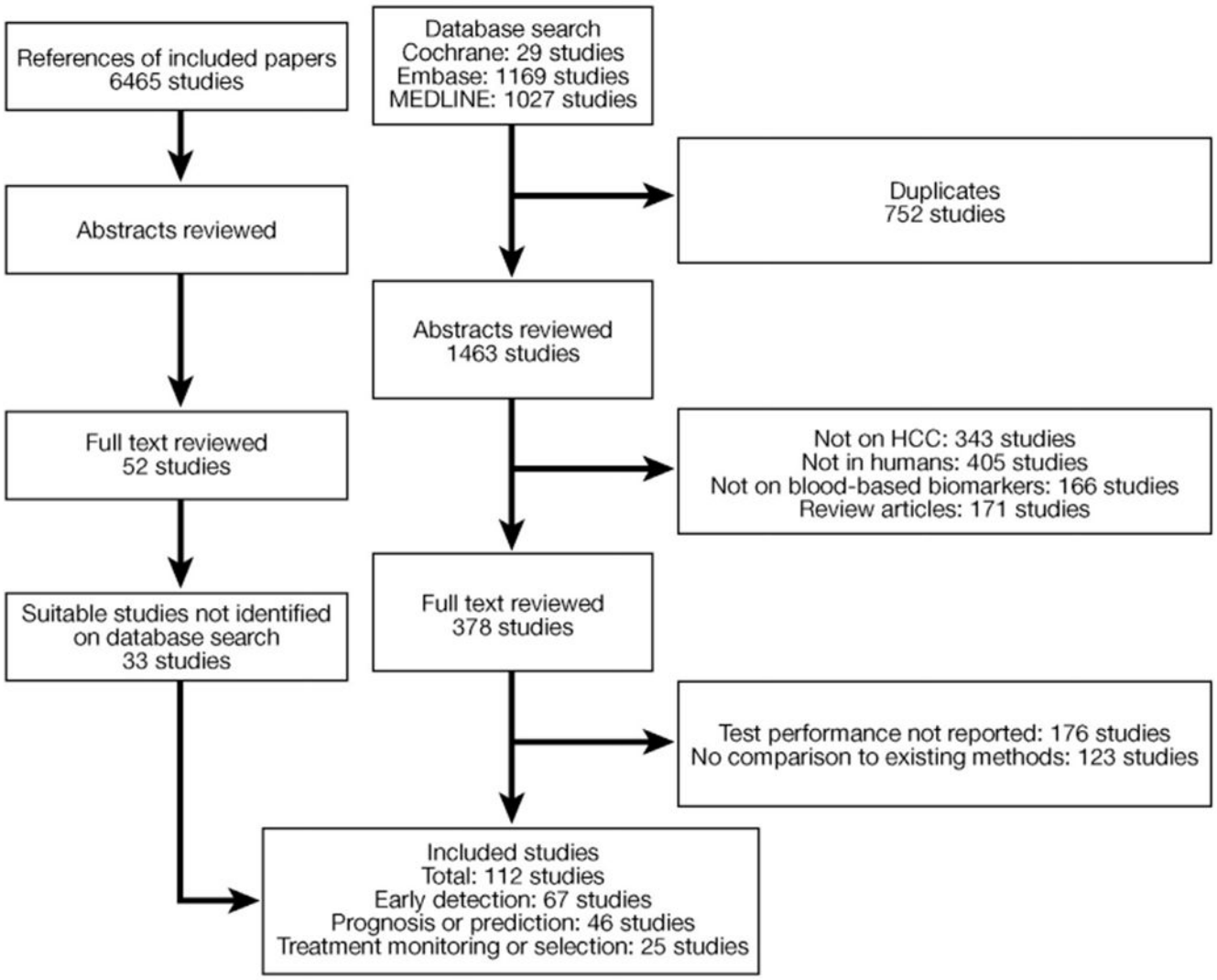

Figure 1.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart illustrating study selection. HCC, hepatocellular carcinoma.

Table 1.

Studies on Use of Circulating Tumor Cells for Hepatocellular Carcinoma Detection

Study	CTC definition	HCC patients	Controls	Comparator, AFP cut off value, *ng/mL*	Findings: sensitivity/specificity, AUC
Bahnassy et al,[39] 2014	CD45(−) and either CK19, CD90, or CD133(+)	N = 70 Stage: 74% (late stage) Treatment: NR	33 CLD, 30 healthy	AFP ratio	CTCs had poorer test characteristics than AFP ratio; HCC vs CLD: CK19(+) CTCs: 87.1%/82.5% CD90(+) CTCs: 82.5%/89.6% CD133(+) CTCs: 40.0%/6.3% AFP ratio: 95.7%/90.5%
Bhan et al,[40] 2018	CD45(−) and hydrodynamics, followed by HCC score based on gene expression	N = 54 Stage: 39% within Milan criteria Treatment: 40% ablation 30% TACE, 28% radiation therapy, 21% resection, 19% sorafenib, 9% liver transplant, 13% other[a]	39 CLD, 10 healthy	No cut-off value provided	HCC score outperformed AFP in HCC vs CLD HCC score: 85%/95% AFP >20 ng/mL: 55%/100%
Cheng et al,[41] 2019	CanPatrol	N = 113 Stage: 65% BCLC 0/A Treatment: NR	57 CLD	400	CTCs outperformed and provided incremental benefit to AFP AFP: 44.3%/89.5%, AUC, 0.67 All CTCs (>1/5 mL): 72.6%/61.4%, AUC, 0.77 All CTCs or AFP: AUC, 0.82
Fang et al,[42] 2014	CellSearch	N = 42 Stage: 48% >7 cm maximal tumor size, 45% >3 tumors Treatment: 100% TACE	10 CLD, 10 healthy	400	CTCs: 74%/100% Sensitivity 89% among patients with high AFP and 61% with low AFP ($P = .08$)
Guo et al,[43] 2007	CD45(−) EpCAM(+) then AFP mRNA	N = 44 Stage/treatment: NR	7 healthy	20	*AFP* mRNA: sensitivity, 72.7%; overall, 50% among AFP <20 ng/mL, and 86.7% among AFP >1000 ng/mL ($P < .05$)
Guo et al,[45] 2014	Two methods: CellSearch and quantitative PCR for EpCAM in CD45-cells	N = 222 Stage: NR Treatment: 53% resection, 25% TACE, 22% radiotherapy	49 CLD, 71 healthy	No cut-off value provided	Note: cohort may overlap with Guo et al[44] 2018 *EpCAM*-mRNA(+) CTCs: 42.6%/96.7%, AUC, 0.70 *EpCAM*-mRNA(+) CTCs plus AFP: 73.0%/93.4%, AUC, 0.86
Guo et al,[44] 2018	PCR score: EpCAM, CD133, CD90, CK19	N = 395 Training: 66% BCLC 0/A, 98% resection, 2% TACE Validation: 48% BCLC 0/A, 67% resection, 33% TACE	301 CLD, 210 healthy	20	Note: cohort may overlap with Guo et al[45] 2014 PCR score: Overall: 72.5%/95.0%, AUC, 0.88 AFP low: 77.7%/95.0%, AUC, 0.89 AUC based on stage: 0.92 (stage 0), 0.86 (stage A), 0.91 (stage B) and 0.86 (stage C) AFP alone: 57.0%/90.0%, AUC, 0.77
Kalinich et al,[46] 2017	PCR score: expression of *AFP, AHSG, ALB, APOH, FABP1, FGB, FGG, RBP4*, and *TF*	N = 63 Stage: 15/25/13/46% BCLC 0/A/B/C+D Treatment: 66% ablation, 40% TACE, 28% resection, 23% liver transplant, 19% radiation therapy, 18% sorafenib, 9% SIRT, 15% other[a]	26 CLD, 31 healthy	100	15 patients with both PCR score and AFP: 4 PCR score (+) 1 AFP (+) 5 with both assays (+) 5 with both assays (−) 6 patients within Milan criteria: 2 PCR score (+) and 0 AFP (+)

Study	CTC definition	HCC patients	Controls	Comparator, AFP cut off value, *ng/mL*	Findings: sensitivity/specificity, AUC
Kelley et al,[47] 2015	CellSearch	N = 20 Stage: 100% BCLC C Treatment: NR	10 CLD	400	AFP ≤400 ng/mL: sensitivity, 90% AFP <400 ng/mL: sensitivity, 10% (*P* = .008)
Liu et al,[48] 2013	CD45(−) ICAM-1(+)	N = 60 Stage: 72% maximal tumor size >5 cm, 12% multifocal tumors Treatment: 100% surgical	N/A	20	High levels of CTCs in 56% of AFP(+) and 33% of AFP(−) patients (*P* = .14)
Sun et al,[49] 2013	CellSearch	N = 123 Stage: 82/18% BCLC 0+A/B+C Treatment: 100% surgery	5 CLD, 10 healthy	400	≥2 CTCs/7.5 mL: Overall: 41.5%/100% High AFP: sensitivity, 54.7% Low AFP: sensitivity, 31.4% (*P* = .009)
Takahashi et al,[50] 2016	Microcavity and CD45(−) EpCAM(+) CK(+)	N = 19 Stage: mixed Treatment: NR	11 CLD	4	CTCs: sensitivity, 47.3% overall With high AFP, higher numbers of CTC detected (91.9 ± 50.1 vs 3.9 ± 2.1; *P* < .05)
Xu et al,[51] 2011	ASGPR(+)	N = 85 Stage: 38/22/32/8% TNM I/II/III/IV Treatment: NR	37 CLD, 20 healthy	20 or 100	CTCs: 81 %/100% No significant differences in CTC levels based on either AFP cut-off value
Xue et al,[52] 2018	Two methods: CellSearch and either CD45(−) CK(+) DAPI(+) hybridization signal for CEP8 ≥2 or CD45(−) CK(−) DAPI(+) and hybridization signal for CEP8 >2	N = 30 Stage: 80/20 BCLC 0+A/B+C Treatment: 100% liver transplant	N/A	400	CTCs measured by hybridization in: Overall cohort: 70%/100% Low AFP: sensitivity, 90% High AFP: sensitivity, 30% (*P* = .002)
Yao et al,[53] 2005	CD45(−) EpCAM(+) then *AFP* mRNA	N = 49 Stage/treatment: NR	36 CLD, 18 healthy	20	*AFP* mRNA Overall: 72.1%/66.7% Low AFP: sensitivity, 75.0% High AFP: sensitivity, 71.0% (*P* > .05)
Yin et al,[54] 2018	CanPatrol	N = 80 Stage: 11/31/45/13% TNM I/II/III/IV Treatment: 51% surgery, 23% TACE, 26% no treatment	10 healthy	20	Overall cohort: any CTCs 77.5%/100%, Twist (+) CTCs 67.5%/100% Low AFP: sensitivity, 35.3% or 17.7% for any CTCs or Twist (+) CTCs, respectively (*P* < .001) High AFP: sensitivity, 88.9% or 71.8% for any CTCs or Twist (+) CTCs, respectively (*P* < .001)
Zhou et al,[55] 2016	CD45(−) *EpCAM*-mRNA(+)	N = 49 Stage: NR Treatment: 100% resection	N/A	400	Any CTCs: Overall: 34.6%/100% Low AFP: sensitivity, 28.2% High AFP: sensitivity, 60% (*P* = .06)

NOTE. Test characteristics are reported either as sensitivity (%)/specificity (%), area under the receiver operating characteristic curve; sensitivity (%)/specificity (%); or as individual parameters.

AFP, α-fetoprotein; ASGPR, asialoglycoprotein receptor; AUC, area under the receiver operating characteristic curve; BCLC, Barcelona Clinic Liver Cancer; CD, cluster of differentiation; CEP8,___; CK, cytokeratin; CLD, chronic liver disease; CTC, circulating tumor cell; DAPI, 4',6-diamidino-2-phenylindole (nuclear stain); EpCAM, epithelial cell adhesion molecule; HCC, hepatocellular carcinoma; ICAM, intercellular adhesion molecule; mRNA, messenger RNA; NR, not reported; TACE, transarterial chemoembolization.

[a]Patients may have received more than 1 therapy type.

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Table 2.

Studies on Use of Cell-Free DNA for Hepatocellular Carcinoma Detection

Study	cfDNA property	HCC patients	Control	Comparator, AFP cut-off value, *ng/mL*	Findings: sensitivity/specificity, AUC
Amount or integrity					
Chen et al,[60] 2013	Total amount	N = 39 Stage: multifocal disease in 13% Treatment: NR	45 healthy	NS	Sensitivity: cfDNA: 56.4% AFP: 53.8% cfDNA + AFP: 71.8% ($P < .05$ for 3-way comparison) cfDNA + AFP + α-L-fucosidase vs cfDNA + AFP: 89.7% ($P < .05$)
El-Shazly et al,[63] 2009	Total amount, integrity	N = 25 Stage: 12%/32%/48%/8% TNM I/II/III/IV Treatment: NR	25 CLD, 15 healthy	20	HCC vs CLD comparison: Total DNA amount: 72%/68%, AUC, 0.57 DNA integrity: 88%/92%, AUC, 0.75
Huang et al,[68] 2012	Total amount	N = 72 Stage: 24%/76% TNM e I+II/III +IV Treatment: NR	37 CLD, 41 healthy	400	Total cfDNA amount: HCC vs CLD: 60%/78%, AUC, 0.71 HCC vs healthy: 90%/90%, AUC, 0.95 Total DNA + AFP (HCC vs healthy): 95%/94%, AUC, 0.97
Huang et al,[66] 2016	cfDNA integrity	N = 53 Stage: 24%/76% TNM I+M/III+IV Treatment: 100% resection	15 CLD, 22 healthy	20	cfDNA integrity: 43.4%/100%/0.71 AFP: 50.9%/100%/0.61 cfDNA integrity + AFP: 79.2%/ 100%/0.85
Iizuka et al,[71] 2006	Total amount	N = 52 Stage: 46%/37%/17% TNM I/II/III Treatment: 100% resection	30 CLD, 16 healthy	AFP: 10.2 DCP: 29.5 ng/mL	AFP: 69.2%/72.7% DCP: 73.1%/75.0% cfDNA amount: 69%/93% ($P < .05$ vs both AFP and DCP)
Marchio et al,[80] 2018	Total amount, *TP53* R249S mutation by digital droplet PCR	N = 149 Stage/treatment: NR	164 CLD, 49 healthy	10	AUC: Proportion of droplets with TP53 R249S: 0.83 AFP: 0.81 ($P > .05$ vs TP53 R249S droplets) cfDNA amount: 0.59
Piciocchi et al,[82] 2013	Total amount	N = 66 Stage: 59% within Milan criteria Treatment: NR	76 CLD	14	cfDNA: 91%/43%, AUC, 0.69 AFP: 45%/83%, AUC, 0.64 ($P > .05$)
Ren et al,[84] 2006	Total amount, chromosome 8p allelic imbalance (D8S258 or D8S264)	N = 79 Stage: 62%/38% TNM I+II/III+IV Treatment: NR	20 CLD, 20 healthy	20	Total amount: HCC vs healthy, 52%/95%, AUC, 0.80 Allelic imbalance at D8S258: sensitivity, 57% for TNM stage III/IV and 22% for stage I/II High cfDNA concentration + allelic imbalance: abnormal in 8 of 24 with low AFP
Yan et al,[93] 2018	Total amount	N = 24 Stage: 58%/33%/8% BCLC A/B/C Treatment: NR	62 CLD	80.5	cfDNA amount: 62.5%/93.6%, AUC, 0.82 AFP alone: AUC, 0.67 cfDNA amount + AFP + age: 87%/ 100%, AUC, 0.98 ($P < .05$)
Mutations					
An et al,[56] 2019	cfDNA mutations	N = 26 Stage: 77%/23% TNM I/II+III Treatment: 100% resection	20 CLD	NS	AUC of different tests: cfDNA concentration: 0.917 Mutation number: 0.878 ctDNA concentration (cfDNA concentration times variant allele frequency): 0.871

Study	cfDNA property	HCC patients	Control	Comparator, AFP cut-off value, *ng/mL*	Findings: sensitivity/specificity, AUC
					Maximal variant allele frequency: 0.802 AFP: 0.783
Cai et al,[57] 2019	Fraction of single-nucleotide or copy number variants	N = 34 Stage: NR Treatment: 100% resection	N/A	NR	ctDNA: sensitivity, 100% AFP: sensitivity, 56% AFP-L3%: sensitivity, 50% DCP: sensitivity, 82%
Igetei et al,[69] 2008	*TP53* R249S mutation	N = 85 Stage/treatment: NR	77 healthy	400	cfDNA *TP53* R249S mutation: Overall: 7.6%/100% Patients with HCC and AFP measurements: 16.7% overall, 20% without increased AFP ($P > .05$)
Liao et al,[78] 2016	*TERT, CTNNB1,* or *TP53* mutation	N = 41 Stage: 42% ≥5 cm, 27% multiple tumors, 61% vascular invasion Treatment: 100% resection	10 healthy	20	cfDNA mutations: sensitivity, 23% vs 13% in high vs low AFP ($P = .70$) Specificity, 90%
Qu et al,[83] 2019	Integrated hepatitis B virus DNA and mutations in *TP53, CTNNB1, AXIN1,* and *TERT* promoter Age, sex, AFP, and DCP also included	Training: N = 65 Validation: N = 24	Training: 70 CLD Validation: 307 CLD	None	Training cohort: patients with (+) AFP or ultrasound 93%/93%, AUC, 0.93 Both the cfDNA and protein markers contributed predictive power Validation cohort: 331 patients with (−) AFP and ultrasound screening sensitivity, 100%, specificity only 4 of 24
Xiong et al,[90] 2019	Mutations in *TP53, ARID1A, FLCN, SETD2, PTEN, BUB1B, CTNNB1, JAK1, AXIN1, EPS15*, or *CACNA2D4*	N = 37 Stage: NR Treatment: 100% resection	6 healthy	400	cfDNA mutations: Overall: 65%/100% Low AFP: 73%/100% High AFP: 53%/100%
Xu et al,[91] 2015	Copy number variation of 1q, 7q, or 19q forward strand or 1p, 9q, or 14q reverse strand	N = 31 Stage: mean maximum tumor size, 5.7 cm (SD, 3.1 cm) Treatment: 100% resection	8 CLD	10	Copy number variation score: All HCC: 83.9%/100% HCC <5 cm maximum tumor size: 68.8%/100% HCC <3 cm maximum tumor size: 57.1%/100% Low AFP: sensitivity, 7 of 10
Methylation/epigenetics					
Cai et al,[57] 2019	5hmC modifications in cfDNA	N = 1204 Stage: 12%/36%/25%/13% BCLC 0/A/B/C Treatment: NR	392 CLD, 958 healthy	20	Early stage HCC vs CLD: 5hmC-based score: 82.7%/67.4%, AUC, 0.87 in training set, 0.85 in validation set AFP: 44.8%/76.1%, AUC, 0.79 in training set, 0.69 in validation set
Chan et al,[59] 2008	*RASSF1A* methylation	N = 63 Stage: NS Treatment: 100% resection	63 CLD, 50 healthy	20	*RASSF1A* methylation detected in: 93% HCC (50% among normal AFP) 58% CLD 8% healthy controls
Chu et al,[61] 2004	*P16* methylation	N = 46 Stage/treatment: NR	23 CLD	20	*P16* methylation: Overall cohort: 48%/83% Normal AFP HCC: sensitivity, 50%
Dou et al,[62] 2016	*CDH1, DNMT3b,* or *ESR1* promoter methylation	N = 183 Stage/treatment: NR	173 CLD, 50 healthy	NS	Methylation frequency: HCC: *CDH1* 31%, *DNMT3b* 41%, *ESR1* 30%

Study	cfDNA property	HCC patients	Control	Comparator, AFP cut-off value, *ng/mL*	Findings: sensitivity/specificity, AUC
					CLD: <10% for all 3 genes Healthy controls: 0% AUC for methylation of any gene, 0.75 (0.70–0.80) AUC for AFP, 0.62 (0.55–0.68)
Han et al,[64] 2014	*TGR5* promoter methylation	N = 160 TNM stage: 59%/41% I+II/III+IV Treatment: NR	88 CLD, 45 healthy	200	HCC vs CLD: *TGR5* methylation + AFP: 68.1%/78.4% AFP alone: 30.6%/92.1% *TGR5* alone: 48.1%/86.3%
Hu et al,[65] 2017	*UBE2Q1* methylation	N = 80 TNM stage: 43%/57% I+II/III+IV Treatment: NR	80 CLD, 20 healthy	200	*UBE2Q1* methylation + AFP: 53.8%/87.5%, AUC, 0.76 *UBE2Q1* methylation alone: 66%/58%, AUC, 0.62 AFP alone: 53.8%/87.5%, AUC, 0.67
Huang et al,[67] 2014	*INK4A* methylation	N = 66 Stage: 24%/23%/27%/26% TNM I/II/III/IV Treatment: NR	43 CLD	200	*INK4A* methylation + AFP: sensitivity, 80.3% (P < .05 vs AFP alone) AFP alone: sensitivity, 45.5% *INK4A* methylation alone: sensitivity, 74.2%
Iizuka et al,[70] 2011	*SPINT2* and *SRD5A2* methylation	N = 220 Stage: 15%/34%/30%/21% TNM I/II/III/IV Treatment: NR	202 CLD	AFP: 20 DCP: 40 mAU/mL	Methylation of *SPINT2* and *SRD5A2,* AFP, and DCP: 82%/82% AUC, 0.72 for ≤5 cm HCC and 0.89 for >5 cm HCC AFP alone: 57.4%/85.7% DCP alone: 60.2%/89.3%
Ji et al,[72] 2014	*MT1M* and *MT1G* methylation	N = 121 Stage: 53%/47% TNM I+II/III+IV Treatment: 100% noncurative	37 CLD, 31 healthy	20	*MT1M* methylation: 49% HCC, 5% CLD, 7% healthy controls *MT1G* methylation: 70% HCC, 16% CLD, 13% healthy controls *MT1M* or *MT1G* methylation: HCC vs CLD: 90%/81%, AUC, 0.86 HCC vs healthy: 91%/84%, AUC, NR AFP alone: HCC vs healthy: 56.0%/62.1%
Kanekiyo et al,[73] 2015	*RASSF1A, CCND2, CFTR, SPINT2, SRD5A2*, and/or *BASP1* methylation	N = 125 Stage: 46%/54% TNM I+II/III+IV Treatment: 100% resection	N/A	AFP: 20 DCP: 40 ng/mL	Serum methylation score: Positive in 41% vs 48% of high vs low AFP Positive in 42% vs 46% of high vs low DCP (P > .05 for both)
Kisiel et al,[74] 2018	Methylation score: *HOXA1, EMX1, ECE1, AK055957, PFKP, CLEC11A*	N = 116 Stage: 4%/44%/15%/30%/7% BCLC 0/A/B/C/D Treatment: NR	80 CLD, 98 healthy	10	Methylation score: HCC vs cirrhosis: 95%/86%, AUC, 0.93–no improvement after adding AFP HCC vs healthy: 95%/95% Sensitivity based on cancer stage: 75% (BCLC stage 0), 93% (stage A/B), and 100% (stage C/D)
Kuo et al,[75] 2014	*HOXA9* methylation	N = 40 Stage/treatment: NR	34 healthy	10	*HOXA9* methylation: 73%/97% *HOXA9* methylation + AFP: 95%/97%
Li et al,[76] 2014	*IGFBP7* promoter methylation	N = 136 Stage: 51%/49% TNM I+II/III+IV Treatment: NR	46 CLD, 35 healthy	20	*IGFBP7* promoter methylation + AFP: 85%/41% (P < .05 for both sensitivity and specificity vs AFP) *IGFBP7* methylation alone: 65%/83% AFP alone: 57%/52%

Study	cfDNA property	HCC patients	Control	Comparator, AFP cut-off value, *ng/mL*	Findings: sensitivity/specificity, AUC
Li et al,[77] 2018	*IGFBP7* promoter methylation	N = 155 Stage: 63%/37% TNM I+II/III+IV Treatment: 100% resection	60 CLD, 20 healthy	200	Serum *IGFBP7* promoter methylation: sensitivity, 69% vs 67% in high vs low AFP (*P* = .81)
Lu et al,[79] 2017	Methylation score: *APC, COX2, RASSF1A, miR-203*	N = 203 Stage: 89%/11% TNM I+II/III+IV Treatment: 100% resection	104 CLD, 50 healthy	20	HCC vs controls: 84%/83%, AUC, 0.87 Sensitivity, 75% of patients with low AFP
Oussalah et al,[81] 2018	*SEPT9* methylation	Derivation cohort (France): N = 51, BCLC 25%/39%/31%/2% A/B/C/D Validation cohort (Germany): N = 47, BCLC stage 39%/22%/15%/24% A/B/C/D	Derivation cohort: 135 CLD Validation cohort: 56 CLD	NS	*SEPT9* methylation: 85%/91%, AUC, 0.96 AFP alone: AUC, 0.85 (*P* = .002 vs *SEPT9* methylation)
Sun et al,[85] 2013	*TFPI2* methylation	N = 43 Stage: 40%/19%/33%/9% TNM I/II/III/IV Treatment: NR	24 CLD, 26 healthy	400	*TFPI2* methylation or AFP alone: similar sensitivity (47% vs 54%) *TFPI2* methylation + AFP: sensitivity, 61% (*P* < .05)
Tangkijvanich et al,[86] 2007	*LINE-1* hypomethylation	N = 85 Stage: 82% >5 cm, 36% multiple tumors Treatment: various	93 CLD, 30 healthy	400	*LINE-1* hypomethylation: sensitivity in high vs low AFP (61.7% vs 52.6%), *P* > .05
Wang et al,[87] 2006	*GSTP1* methylation	N = 32 Stage: NR Treatment: 100% resection	8 CLD	N/A	Methylated cfDNA *GSTP:* sensitivity, 50% (61% among patients with tissue *GSTP1* methylation), specificity, 100% Methylation detected in 4 of 9 patients with low AFP
Wei et al,[88] 2018	*SOCS3* promoter methylation	N = 119 Stage: 34% tumor >5 cm, 17% >1 tumor, 17% portal vein involvement Treatment: 100% resection	157 CLD, 50 healthy	400	*SOCS3* cfDNA methylation: Overall: 28.6%/95% High vs low AFP (52.9% vs 10.3%), *P* < .001
Wen et al,[89] 2015	Methylation score: *RGS10, ST8SIA6, RUNX2, VIM, CACNA1C, TBX2, SOX9* (5' *end*), *NEDD4L (intron), ALX3, ZNF683* (3' *end*), *KCNQ4* (i), *ERG, PTPN18 (intron), SYN2, LINC00682* (3' *end*), *CPLX1 (intron), FLJ42709, UBD* (3' *end*), *SNX10* (3' *end*), *TRPS1* (*intron*)	N = 36 Stage: 36%/25%/22%/17% TNM I/II/III/IV Treatment: NR	17 CLD, 38 healthy	20	Two cfDNA methylation scores, either score positive: Training set: 93%/91% Validation set: 100%/80% Combined cohort: 94%/89% Sensitivity, 100% in patients with low AFP (N = 10)
Xu et al,[92] 2017	Methylation score: cg10428836, cg26668608, cg25754195, cg05205842, cg11606215, cg24067911, cg18196829, cg23211949, cg17213048, cg25459300	N = 1098 Stage: 16%/16%/52%/12% TNM I/II/III/IV Treatment: NR	835 healthy	25	cfDNA levels: Training set: 85.7%/94.3%, AUC, 0.97 Validation set: 83.3%/90.5%, AUC, 0.94 AFP: AUC, 0.82 (*P* < .05 vs cfDNA)
Yeo et al,[94] 2005	*RASSF1A* methylation	N = 40 Stage: 30% ≥5 cm Treatment: 100% resection	10 healthy	20	*RASSF1A* methylation Overall: 43%/100% Low AFP: sensitivity, 36%

NOTE. Test characteristics are reported either as sensitivity (%)/specificity (%), area under the receiver operating characteristic curve; sensitivity (%)/specificity (%); or as individual parameters.

5hmC, 5-hydroxymethylcytosine; AFP, α-fetoprotein; AUC, area under the receiver operating characteristic curve; BCLC, Barcelona Clinic Liver Cancer; CLD, chronic liver disease; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; DCP, des-carboxyprothrombin; HCC, hepatocellular carcinoma; NR, not reported; PCR, polymerase chain reaction.

Table 3.

Studies On Circulating Tumor Cell as Predictive or Prognostic Markers

Study	CTC definition	HCC patients	Outcome, adjusted analysis
Dong et al,[106] 2015	CellSearch	N = 156 Stage: NR Treatment: 88% resection	Any CTCs: increased recurrence (P = .001) Consistent lack of CTCs: greater time to relapse (P = .015) Adjusted for NR
Fan et al,[107] 2011	CD44(+) CD90(+) CD45(−)	N = 82 Stage: 5%/34%/34%/27% TNM I/II/III/IV Treatment: 100% resection	CTCs associated with poorer: Median recurrence-free survival (6.0 vs >46.5 mo) 2-year recurrence-free survival (22.7% vs 64.2%) 2-year overall survival (58.5% vs 94.1%); P < .001 for all Adjusted for tumor size, tumor number, TNM stage, or blood transfusions
Fu et al,[108] 2019	CanPatrol	N = 25 Stage: NR Treatment: 100% liver transplant	Number of interstitial CTCs associated with recurrence post-transplant Adjusted for NR
Guo et al,[45] 2014	Two methods: CellSearch or quantitative PCR for *EpCAM* in CD45(−) cells	N = 299 Stage: NR Treatment: 53% resection, 25% TACE, 22% radiotherapy	*EpCAM*(+) mRNA pretreatment associated with poorer outcomes: Surgery: recurrence HR, 2.71 (1.52–4.86) Adjusted: satellite lesions TACE: progression HR, 3.75 (1.41–9.97) Unadjusted Radiotherapy: progression HR, 5.07 (1.39–18.47) Unadjusted
Guo et al,[44] 2018	PCR score: *EpCAM, CD133, CD90, CK19*	N = 395 Training: Stage: 66% BCLC 0/A Treatment: 98% resection, 2% TACE Validation: Stage: 48% BCLC 0/A Treatment: 67% resection, 33% TACE	Increased recurrence with increased PCR score: Training: HR, 2.69 (1.62–4.48) Adjusted for tumor size and vascular invasion Validation: HR, 3.13 (1.36–7.19) Adjusted for tumor encapsulation, tumor size, satellite lesions, and vascular invasion Associations were significant among BCLC stage 0/A cancers and with AFP ≤20 ng/mL
Ha et al,[109] 2019	Tapered slit filter	N = 105 Stage: 19%/81% BCLC 0/A Treatment: 100% resection	Increase in CTC count after surgery: Increased recurrence: HR, 2.28 (1.06–4.90), adjusted for HBV etiology, tumor size, AST, ALT, and satellite lesions No difference in overall survival Presence of preoperative CTCs: no association with recurrence Presence of postoperative CTCs: no association with recurrence
Hamaoka et al,[110] 2019	Glypican-3(+)	N = 85 Stage: NR Treatment: 100% resection	Presence of CTCs associated with higher risk of microscopic portal vein invasion Adjusted for AFP, AFP-L3, multifocal disease, prothrombin time, and albumin
Li et al,[36] 2016	Density-based, CD45(−), pan-CK(+), and either pAkt1/2/3 or pERK1/2(+)	N = 59 Stage: NR Treatment: 100% sorafenib	High proportion of pERK (+) pAkt (−) CTCs: Superior progression-free survival: HR, 9.39 (3.24–27.19) Adjusted for Child-Pugh class, TNM stage, and presence of pERK(−) pAkt(+) CTCs
Liu et al,[48] 2013	CD45(−) ICAM-1(+)	N = 60 Stage: 72% maximal tumor size >5 cm, 12% multifocal tumors Treatment: 100% resection	High proportion of ICAM-1 cells associated with: Poorer disease-free survival (HR, 7.15; 2.99–17.05), adjusted for AFP, tumor size, tumor number, portal vein tumor thrombus, and ascites No association with overall survival (HR, 2.28; 0.95–7.82), adjusted: portal vein tumor thrombus, ascites, and prealbumin

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Study	CTC definition	HCC patients	Outcome, adjusted analysis
Nel et al,[111] 2014	CD45(−) and either EpCAM, panCK, or CD133(+)	N = 10 Stage: NR Treatment: 100% SIRT	Low CD133+ cell ratio associated with increased progression (P = .02) Adjusted for AFP (P = .02)
Ogle et al,[112] 2016	CD45(−), size	N = 69 Stage: NR Treatment: 39% arterial therapy, 13% sorafenib, 6% liver transplant, 4% resection, 28% supportive care only	Presence of CTCs at any time (N = 69): Poorer overall survival: HR, 2.34 (1.01–5.43) Adjusted for tumor size, portal vein thrombus, and extrahepatic disease Decreased time to progression (P = .006) Presence of CTCs post-treatment (N = 40): Poorer overall survival: HR, 6.16 (1.71–22.33) Adjusted for tumor size, portal vein thrombus, and extrahepatic disease Decreased time to progression (P = .002)
Qi et al,[113] 2018	CanPatrol	N = 112 Stage: 39/21/30 BCLC 0+A/B/C Treatment: 100% resection	CTCs associated with HCC recurrence: CTC count: HR, 1.04 (1.03–1.05)[a] Mesenchymal CTC percentage: HR, 1.02 (1.01–1.03)[a] Mesenchymal > epithelial CTC percentage: HR, 1.03 (1.01–1.03)[a] Mesenchymal = epithelial CTC percentage: HR, 1.00 (0.99–1.03) Mesenchymal < epithelial CTC percentage: HR, 1.00 (0.99–1.01) Epithelial CTC percentage: HR, 1.00 (0.99–1.01)
Schulze et al,[114] 2017	CellSearch	N = 57 Stage: NR Treatment: 100% resection	CTCs associated with poorer: Recurrence: HR, 2.3 (P = .027) Recurrence-free survival: 5.0 ± 1.5 vs 12.0 ± 2.5 mo (P = .039) Adjusted for incomplete resection
Sun et al,[49] 2013	CellSearch	N = 123 Stage: 82%/18% BCLC 0+A/B+C Treatment: 100% resection	Presence of CTCs associated with: Increased recurrence: HR, 5.20 (2.65–10.21) Adjusted for AFP, tumor size, tumor encapsulation, satellite lesions, and vascular invasion
Sun et al,[115] 2018	CellSearch	N = 73 Stage: 77%/23% BCLC 0+A/B+C Treatment: 100% resection	Presence of CTCs from different vascular sites associated with: Intrahepatic recurrence: Peripheral vein: HR, 0.77 (0.14–5.19) Peripheral artery: HR, 2.54 (0.87–7.42) Peripheral vein CTC or clusters: HR, 3.48 (1.40–8.61) Adjusted for CTC presence in the above locations, AFP, vascular invasion Lung metastasis: Hepatic vein CTC: HR, 0.59 (0.04–9.54) Intrahepatic inferior vena cava CTC: HR, 0.67 (0.10–4.40) Hepatic vein CTC or clusters: HR, 42.20 (3.73–477.80) Adjusted for CTC presence in the above locations
von Feldon et al,[116] 2017	CellSearch	N = 57 Stage: 46%/32%/21% T1/T2/T3, vascular invasion in 38% Treatment: 100% resection	CTCs associated with increased recurrence: HR, 3.1 (1.0–9.4) Adjusted for resection margin
Vona et al,[117] 2004	Filter (diameter > 25 mm)	N = 44 Stage: no extrahepatic metastasis, 39% multinodular, 39% maximum tumor ≤3 cm, 45% portal vein thrombus Treatment: NR	Presence of CTCs or clusters associated with poorer overall survival (HR, NR; P = .02). NS after adjustment for eligibility for surgery, Child–Pugh class, unifocal disease, or portal vein thrombus

Study	CTC definition	HCC patients	Outcome, adjusted analysis
Wang et al,[118] 2018	CanPatrol	N = 62 Stage: 37%/63% BCLC 0+A/B+C Treatment: 100% resection	CTCs associated with recurrence: Total CTCs: unadjusted HR, 2.95 (1.18–7.35), NS after adjustment Mesenchymal CTCs: unadjusted HR, 4.74 (2.04–11.01), adjusted HR, 3.45 (1.39–8.56) Mixed CTCs: unadjusted HR, 2.94 (1.31–6.59), NS after adjustment Adjusted for tumor size, portal vein tumor thrombus, AFP, Edmondson stage, alkaline phosphatase, and the 3 categories of CTCs
Wu et al,[119] 2019	CD45(−) and abnormal chromosome 8 amplification by FISH	N = 155 Stage: 38%/14%/48% BCLC A/B/C, 34%/35%/28%/4% TNM I/II/III/IV Treatment: 100% TACE	Pre-TACE CTCs associated with poorer overall survival: HR, 2.84 (1.41–5.73) Adjusted for ECOG score and serum Dickkopf-1 concentration
Yu et al,[120] 2018	CellSearch	N = 139 Stage: 40%/60% BCLC 0+A/B+C Treatment: 100% resection	4 categories: (1) persistent (+), (2) preoperatively (+) but postoperatively (−), (3) preoperatively (−) but postoperatively (+), (4) persistently (−). For a 1-point increase in category: Disease-free survival: HR, 0.53 (0.41–0.68) Overall survival: HR, 0.48 (0.36–0.66) Adjusted: multifocal vs unifocal disease, tumor size >5 cm, macroscopic tumor thrombosis, and microscopic vascular invasion
Zhou et al,[55] 2016	*EpCAM* mRNA(+)	N = 49 Stage: 90% BCLC 0/A Treatment: 100% resection	High *EpCAM* mRNA increased recurrence: HR, 6.67 (1.94–22.88) Adjusted for satellite lesion presence and Treg/CD4+ T-cell proportion

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; CD, cluster of differentiation; CK, cytokeratin; CTC, circulating tumor cell; ECOG,____; EpCAM, epithelial cell adhesion molecule; FISH, fluorescence in situ hybridization; HR, hazard ratio; ICAM, intercellular adhesion molecule; mRNA, messenger RNA; pAkt,_____; panCK,_____; PCR, polymerase chain reaction; pERK,_____; NR, not reported; SIRT, selective internal radiation therapy; TACE, transarterial chemoembolization; Treg, regulatory T cell.

[a]Significant after adjustment for AFP, cirrhosis, tumor size, BCLC stage, number of nodes, microvascular invasion, portal vein thrombus, and tumor capsule.

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Table 4.

Studies on Cell-Free DNA as Predictive or Prognostic Markers

Study	cfDNA property	Cancer stage/treatment	Outcome, adjusted analysis
An et al,[56] 2019	Any mutation	N = 26 Stage: 77%/23% TNM I/II+III Treatment: 100% resection	Presence of cfDNA mutations postresection: shorter disease-free survival (median, 8.3 mo vs unreached) Adjusted for portal vein tumor thrombus
Cai et al,[57] 2019	Fraction of single-nucleotide or copy number variants	N = 34 Stage: NR Treatment: 100% resection	Presence of ctDNA (mutated cfDNA) postoperatively: Decreased relapse-free survival Decreased overall survival (P = .001 for both) Combining ctDNA and des-carboxyprothrombin further increased predictive power
Chelis et al,[121] 2013	*SOX17* promoter methylation	N = 87 Stage: 6%/17%/77% BCLC A/B/C Treatment: NR	Methylated vs nonmethylated: overall survival 5 vs 14 mo, P = .006 Adjusted for performance status (P = .04)
El-Shazly et al,[63] 2010	Total amount, integrity	N = 25 Stage/treatment: NR	Overall survival: cfDNA amount: HR, 0.54 (0.20–1.60) cfDNA integrity: HR, 1.86 (1.20–2.88) Adjusted for tumor size, TNM stage, vascular invasion, nodal involvement, metastatic disease
Howell et al,[122] 2017	*CSMD3* mutations	N = 51 Stage/treatment: NR	*CSMD3* mutations, increased mortality: HR, 4.56 (1.17–17.69) Adjusted for age, Child–Pugh score, BCLC stage
Huang et al,[123] 2011	*APC* or *RASSF1A* methylation	N = 72 Stage: 24%/76% UICC I+II/III+IV Treatment: NR	Overall survival poorer with *RASSF1A* or *APC* methylation: *RASSF1A* methylation: HR, 3.26 (1.48–7.21), adjusted for age, sex, tumor size, TNM stage, α-fetoprotein *APC* methylation: poorer on unadjusted analysis, NS after adjustment
Jiao et al,[124] 2018	*TERT* mutations	N = 218 Stage: 39%/22%/33% TNM I/II/III Treatment: NR	Presence of *TERT* mutations: All HCC patients, decreased overall survival (P = .006) but NS after adjustment for tumor stage (P = .19) HCC patients with cirrhosis: trend toward significance after adjustment for tumor stage (P = .051)
Kanekiyo et al,[73] 2015	Methylation of *RASSF1A, CCND2, CFTR, SPINT2, SRD5A2*, and/or *BASP1*	N = 125 Stage: 46%/54% TNM I+II/III+IV Treatment: surgical	Methylation of ≥3 of 6 genes: Disease-free survival: HR, 2.18, P < .0001 Overall survival: HR, 4.20, P < .001 Adjusted for tumor size, tumor differentiation, venous invasion stage, cirrhosis, AFP, DCP, methylation of all 6 individual genes
Li et al,[77] 2018	Methylation of *IFGBP7*	N = 155 Stage: 63%/37% TNM I+II/III/IV Treatment: 100% resection	Methylation of *IFGBP7* associated with: Increased recurrence: HR, 4.99 (1.51–16.47), adjusted for tumor size, vascular invasion, TNM stage Poorer overall survival: HR, 3.86 (2.07–7.20), adjusted for tumor size, vascular invasion, TNM stage, early tumor recurrence
Oh et al,[37] 2019	Total amount, genomic instability, and *VEGFA* amplification	N = 151 Stage: 97% BCLC C Treatment: 100% sorafenib	Higher amount of cfDNA associated with: Shorter time to progression: HR, 1.17 (1.20–2.44), P = .002, adjusted for AFP Shorter overall survival: HR, 3.50 (2.36–5.20), P < .0001, adjusted for macroscopic vascular invasion and AFP Genomic instability associated with: Shorter time to progression: HR, 2.09 (1.46–3.00), P < .0001, adjusted for AFP Shorter overall survival: HR, 3.35 (2.24–5.01), P < .0001), adjusted for macroscopic vascular invasion and AFP
Ono et al,[125] 2015	Total amount	N = 46 Stage: 24%/39%/33%/4% T1/T2/T3/T4, all N0/M0	Presence of cfDNA associated with: Increased recurrence (P = .01), unadjusted Increased extrahepatic metastasis (P = .04), unadjusted

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Study	cfDNA property	Cancer stage/treatment	Outcome, adjusted analysis
		Treatment: 100% surgical (resection or liver transplant)	Similar overall survival (P = .07), unadjusted Increased microscopic vascular invasion: HR, 6.10 (1.11–33.33), adjusted for AFP, DCP, tumor size Among patients with serial cfDNA measurements, cfDNA levels correlated with clinical course (eg, decreased after therapy and increased with recurrence)
Park et al,[126] 2018	Total amount	N = 55 Stage: 23%/23%/27%/27% TNM I/II/III/IV Treatment: 100% radiotherapy	cfDNA levels decreased after successful but not unsuccessful therapy Higher post-therapy cfDNA was associated with: Similar overall survival (P = .15) Similar progression-free survival (P = .26) Increased intrahepatic failure: HR, 2.41 (1.06–5.46), adjusted for cirrhosis, multifocal tumors, TNM stage Decreased local control: HR, 1.96 (0.57–6.81), adjusted for cirrhosis, multifocal tumors, TNM stage
Tangkijvanich et al,[86] 2007	*LINE-1* hypomethylation	N = 85 Stage: 82% >5 cm, 36% multiple tumors Treatment: NR	*LINE-1* hypomethylation associated with poorer overall survival: HR, 1.74 (1.09–2.79) Adjusted for age, sex, AFP, hepatitis B virus etiology, Child–Pugh class, tumor size, tumor number, venous invasion, extrahepatic metastasis, CLIP score, and HCC therapy
Tokuhisa et al,[127] 2007	Total amount	N = 87 Stage: 46%/44%/10% TNM I/II/III Treatment: 100% resection	High cfDNA associated with: Poorer overall survival: HR, 3.4 (1.5–7.6), adjusted for tumor size Increased distant metastasis: HR, 4.5 (1.3–14.9), adjusted for tumor grade Similar disease-free survival (P = .19)
Xu et al,[92] 2017	Methylation of 8 genes: *SH3PXD2A, C11orf9, PPFIA1*, chromosome 17:78, *SERPINB5, NOTCH3, GRHL2*, and *TMEM8B*	N = 1049 Stage: 16/16/52/12 TNM I/II/III/IV Treatment: NR	Overall survival based on high risk score: Training set (n = 680): HR, 2.41 (1.90–3.03) Validation set (n = 369): HR, 1.55 (1.25–1.92) Adjusted for AFP, TNM stage, sex, age

AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; cfDNA, cell-free DNA; CLIP, Cancer of the Liver Italian Program; ctDNA, circulating tumor DNA; DCP, des-carboxyprothrombin; HR, hazard ratio; NR, not reported; TACE, transarterial chemoembolization; UICC, Union for International Cancer Control.

Table 5.

Studies on Liquid Biopsy to Monitor Disease or Guide Treatment Selection

Study	Liquid biopsy	HCC patients	Finding
Circulating tumor cells			
Chen et al,[137] 2019	CanPatrol	N = 113 Stage: 35%/65% BCLC 0+A/B+C Treatment: 100% resection	No significant changes postresection in total CTC count or CTC subtype counts
Fu et al,[108] 2019	CanPatrol	N = 25 Stage: NR Treatment: 100% liver transplant	19 of 22 patients with pretransplant CTC had lower numbers of CTCs post-transplant CTC counts increased in 6 of 8 with post-transplant recurrence
Guo et al,[45] 2014	PCR for *EpCAM* in CD45(−) cells	N = 77 Stage: NR Treatment: 45% resection, 55% TACE or radiotherapy	Surgery: *EpCAM* expression Decreased postresection: 48.6% vs 17.1% positive preresection vs postresection Persistent expression or initially negative then positive expression higher recurrence: 50%-75% vs 7.7%-12.5% TACE/radiotherapy: *EpCAM* expression Decreased post-treatment: 50.0% vs 10.0% positive pretreatment vs post-treatment All patients with progressive disease had increased *EpCAM* expression post-treatment ($P < .01$)
Guo et al,[44] 2018	PCR score: EpCAM, CD133, CD90, CK19	N = 60 Stage: NR Treatment: 100% resection	PCR scores decreased postresection: 70.0%-31.7% positive Persistently positive scores and scores that became positive postresection were associated with higher recurrence
Ha et al,[109] 2019	Tapered slit filter	N = 105 Stage: 19%/81% BCLC stage 0/A Treatment: 100% resection	Patients with AFP <200 ng/mL: increase in postoperative CTCs associated with: Poorer overall survival ($P = .047$) Poorer recurrence-free survival ($P < .001$) Similar findings in subgroup with HCC and cirrhosis
Kalinich et al,[46] 2017	PCR score	N = 5 Stage: 80% BCLC C/D Treatment: NR	PCR scores post-therapy correlated with recurrence
Lee et al,[138] 2017	CellSearch	N = 10 Stage: NR Treatment: 100% SBRT	Objective response at 3 months after SBRT correlated with percentage change in CTC (r = 0.703, $P = .023$), but not change in AFP
Qi et al,[113] 2018	CanPatrol	N = 112 Stage: 39%/21%/30% BCLC stage 0+A/B/C Treatment: 100% resection	8–10 days after resection, total CTC counts decreased whereas the proportion of mesenchymal-type CTCs increased 8 patients with recurrence had increased CTC count 2 months post-resection Detected earlier than the recurrence
Wang et al,[139] 2018	CanPatrol	N = 47 Stage: 30%/70% T1+2/T3+4 Treatment: 100% liver transplant	CTCs measured either pretransplant and 1 month post-transplant (N = 20) or 1 and 3 months post-transplant (N = 27) Trends in number of all CTCs or specific CTC subgroups was not associated with recurrence post-transplant in either group
Wu et al,[119] 2019	CD45(−) and abnormal chromosome 8 amplification by FISH	N = 155 Stage: 38%/14%/48% BCLC stage A/B/C, 34%/35%/28%/4% TNM stage I/II/III/IV Treatment: 100% TACE	CTCs measured before TACE and 1 and 4 weeks after TACE Responders to TACE: numbers of CTCs decreased 1 and 4 weeks post-TACE Nonresponders to TACE: CTC counts higher at week 4 than pre-TACE; pre-TACE CTC counts also were higher in nonresponders
Xue et al,[52] 2018	Two methods: CellSearch and either CD45(−) CK(+)	N = 23 (subset of larger study) Stage: NR Treatment: 100% liver transplant	iFISH CTC counts were measured 3 months post-transplant Compared with pretransplant levels, decreased in 65.2%, did not change in 21.7%, and increased in 13.0% of patients

Study	Liquid biopsy	HCC patients	Finding
	DAPI(+) hybridization signal for CEP8 ≥2 or CD45(−) CK(−) DAPI(+) and hybridization signal for CEP8 >2		1 patient with a marked increase in CTC count from 2/7.5 to 12/7.5 mL had extrahepatic recurrence 4 months later Outcomes in the remaining patients were not reported
Ye et al,[140] 2018	CanPatrol	N = 42 Stage: 88%/12% TNM stage I+II/III+IV, 81%/19% BCLC stage A+B/C+D Treatment: 100% resection	Preoperative CTC counts: not associated with overall survival or progression-free survival Postoperative CTC count >5: Poorer progression-free survival: HR, 6.89 (1.64–29.00) No association with overall survival: HR, 15.65 (0.80–304.64) Increase in CTC counts postoperatively: Poorer progression-free survival: HR, 39.58 (4.22–371.64) All HRs were adjusted for tumor stage, number of tumors, tumor size, age, and sex
Yu et al,[120] 2018	CellSearch	N = 139 Stage: 40%/60% BCLC stage 0+A/B+C Treatment: 100% resection	CTC counts measured 1 day before and 3 days after surgery CTC counts increased postoperatively in 42%, were unchanged in 33%, and decreased in 33% Patients with persistently increased CTC counts had the poorest overall survival and disease-free survival
Cell-free DNA			
Alunni-Fabroni et al,[141] 2019	cfDNA amount	N = 13 Stage: 23%/31%/46% BCLC stage A/B/C Treatment: TARE plus sorafenib (77%), RFA + sorafenib (15%), RFA alone (8%)	cfDNA was measured after locoregional therapy, and every 8 weeks thereafter cfDNA levels at 16 and 24 weeks postintervention trended toward an association with poorer overall survival (P = .057 and .095, respectively) 3 patients with reported data: cfDNA concentration trends corresponded to disease recurrence and provided information beyond AFP trends alone
Cai et al,[57] 2019	Fraction of single-nucleotide or copy number variants	N = 34 Stage: NR Treatment: 100% resection	Four patients with data reported: all had initial reduction in SNV/CNV fraction after initial surgery Three patients with recurrence: cfDNA amount and SNV/CNV fraction dynamics changed dynamically with recurrent cancer
Chen et al,[142] 2018	cfDNA sequencing	N = 11 Stage: NR Treatment: 100% resection	1 patient: *CHD4* A1789T and *NCOA2* A2777T allele frequency corresponded to tumor volume Another patient, *FOXA1* T254G and *ABL1* T642G allele frequency corresponded to tumor burden
Du et al,[143] 2018	Mutations in cfDNA *NRAS, BRAF, PIK3CA, KRAS, ARID1A, AXIN1, ARID2, TERT, TP53*, and *CTNNB1*	N = 3 Stage: advanced stage Treatment: TACE	Mutational burden identified relapse weeks before changes in CT or AFP
Evans et al,[144] 2019	cfDNA amount	N = 18 Stage: NR Treatment: TACE	All patients, cfDNA levels decreased after TACE and then increased at time of progression True in both AFP-positive and AFP-negative tumors
Higuera et al,[145] 2019	cfDNA mutations	N = 12 Stage/treatment: NR	Two patients undergoing resection, follow-up samples >20 months later showed no mutations One patient, a *TERT* mutation with 8% frequency was found 10 months pre-HCC diagnosis
Huang et al,[66] 2016	DNA integrity	N = 13 (subset of larger study) Stage: 24%/76% TNM stage I+II/III+IV Treatment: 100% resection	cfDNA integrity increased postresection in 11 of 13 patients with longitudinal measurements (P = .0003) NR whether this correlated to long-term outcomes
Li et al,[146] 2019	cfDNA mutations	N = 3 Stage: 33%/67% BCLC A/C Treatment: TACE	All 3 patients had week 4 CT with treatment response while week 10 CT showed disease recurrence Two patients: cfDNA mutational burden remained stable at week 1 but increased at week 4 after TACE; AFP continued to decrease during this time

Study	Liquid biopsy	HCC patients	Finding
			Third patient: mutational burden was lower at week 1 then increased at week 4; AFP increased at both weeks 1 and 4
Ono et al,[125] 2015	Total amount	N = 46 Stage: 24%/39%/33%/4% T1/T2/T3/T4, all N0/M0 Treatment: 100% surgical (resection vs liver transplant)	Among patients with serial cfDNA measurements, cfDNA levels correlated with clinical course (eg, decreased after therapy and increased with recurrence)
Park et al,[126] 2018	Total amount	N = 55 Stage: 23%/23%/27%/27% TNM stage I/II/III/IV Treatment: radiotherapy	cfDNA levels decreased after successful but not unsuccessful radiotherapy
Wong et al,[147] 2003	*p16INK4a* methylation	N = 10 Stage: NR Treatment: 100% resection	Plasma *p16INK4a* median methylation decreased in 5 of 8 patients after resection ($P = .07$) Buffy coat *p16INK4a* median methylation decreased in 10 of 10 patients after resection ($P = .01$)
Yang et al,[148] 2019	cfDNA mutations	N = 12 Stage: NR Treatment: 100% sorafenib	Dynamic changes in cfDNA mutations in 19 genes were associated with therapeutic efficacy

AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CD, cluster of differentiation; cfDNA, cell-free DNA; CK, cytokeratin; CNV,____; CT, computed tomography; CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; FISH, fluorescence in situ hybridization; HR, hazard ratio; iFISH,____; NR, not reported; PCR, polymerase chain reaction; RFA,____; SBRT,____; SNV,____; TACE, transarterial chemo-embolization. TARE, transarterial radioembolization.