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ORIGINAL RESEARCH

# Correlation Analysis Between Circulating Tumor DNA and Prognosis in Patients with Hepatocellular Carcinoma

Xiaofei Ma[1,*], Wenhao Yuan[1,2,*], Siying Liu[3,4], Qingqi Ren[1], Zewei Lin[1]

[1]Department of Hepatobiliary and Pancreatic Surgery, Peking University Shenzhen Hospital, Shenzhen, Guangdong, People's Republic of China; [2]Medical School, Shenzhen University, Shenzhen, Guangdong, People's Republic of China; [3]Department of Ultrasound, Peking University Shenzhen Hospital, Shenzhen, Guangdong, People's Republic of China; [4]The First Clinical Medical School, Guangdong Medical University, Zhanjiang, Guangdong, People's Republic of China

*These authors contributed equally to this work

Correspondence: Qingqi Ren; Zewei Lin, Department of Hepatobiliary and Pancreatic Surgery, Peking University Shenzhen Hospital, Shenzhen, Guangdong, 518036, People's Republic of China, Email renqq@alumni.sysu.edu.cn; szlinzw@126.com



**Purpose:** Circulating tumor DNA (ctDNA) is a noninvasive biomarker for tumor burden. Blood-based assays utilizing ctDNA copy number aberrations and fragment size show promise in hepatocellular carcinoma (HCC), but their prognostic value is unclear. This study evaluated the clinical relevance of a ctDNA-based score in predicting HCC prognosis.

**Patients and Methods:** Clinical and ctDNA data from 93 HCC patients were analyzed. Patients were stratified by a predefined ctDNA score cutoff. Group comparisons used chi-square tests. Kaplan-Meier and multivariate Cox regression analyses assessed prognostic relevance.

**Results:** Elevated ctDNA scores were associated with aggressive features: higher AFP and PIVKA-II levels, larger tumor size, macrovascular invasion, and advanced TNM stage (all $P < 0.001$). Patients with ctDNA scores > 0.61199 had significantly worse overall survival (OS). Univariate analysis identified 19 OS-associated variables, including inflammatory markers, biochemical indices, and tumor burden parameters. Multivariate analysis confirmed ctDNA score > 0.61199 (HR = 6.99, 95% CI 2.34–20.85, $P < 0.001$), AFP > 400 ng/mL (HR = 0.28, 95% CI 0.13–0.61, $P = 0.001$), ALB < 35.6 g/L (HR = 3.29, 95% CI 1.34–8.06, $P = 0.009$), and tumor number > 3 (HR = 3.85, 95% CI 1.58–9.40, $P = 0.003$) as independent prognostic determinants.

**Conclusion:** Elevated ctDNA scores are strongly associated with adverse outcomes in HCC, highlighting their potential as a noninvasive prognostic biomarker. Integrating ctDNA assessment could improve risk stratification and treatment decisions, warranting validation in prospective studies.

**Keywords:** circulating tumor DNA, hepatocellular carcinoma, prognosis, copy number aberrations, fragment size

## Introduction

Hepatocellular carcinoma (HCC) is the fourth most common cancer and the second leading cause of cancer death worldwide. In China, the burden of HCC is believed to represent more than 50% of the world incidence.[1,2] Although surgical treatments like hepatectomy and liver transplantation can be curative, long-term outcomes remain poor due to high recurrence rates. Epidemiological statistics show that HCC has a high recurrence rate after surgery: more than 50% of patients recur within 3 years, and 70–80% recur within 5 years.[3,4] Notably, the recurrence rate is highest in the first year post-resection or transplantation. The 5-year survival rate of HCC patients is approximately 40–70%.[5–7] Thus, this clinical scenario highlights the urgent need for more efficient early detection beyond the current standard of Alpha-Fetoprotein (AFP) in combination with ultrasonography, with an estimated sensitivity of 63%.[8] Promisingly, novel serum and tissue biomarkers have been identified, but their practical application has been limited by poor sensitivity and high costs.[9]

New developments in liquid biopsy with the promising biomarker ctDNA in oncological treatment.[10,11] Being one of the subtypes of cfDNA which accounts for <1% of the total cfDNA, ctDNA is released into circulation from tumor cells

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mediated by inflammatory response and apoptosis.[12–14] Compared with normal cfDNA (usually >167 bp), tumor-derived DNA (ctDNA) carries genetic variations of tumor cells, such as point mutations, DNA methylation profiles, CNAs, and FS profiles (FS profiles represent the primary tumor heterogeneity when detecting ctDNA by NGS or targeted PCR platforms).[15–19] These molecular profiles make ctDNA become a promising biomarker for the non-invasive monitoring of tumor evolution and treatment response.[20]

Although most prior studies focused on ctDNA methylation and mutation analysis, limited methylation signal abundance in circulation and tumor mutational heterogeneity restricted their clinical translation.[21–24] Notably, our team successfully constructed a blood-based assay combining CNA and FS profiling with 50% detection rates in AFP-negative HCC patients.[25] However, the prognostic power of this integrated approach—combining both CNA and FS features into a single composite score—remains to be fully elucidated.[26]

In this retrospective study, we examined the clinical significance of our blood-based ctDNA genomic classifier in predicting HCC outcomes using pre-operative cfDNA samples from 93 longitudinally followed HCC patients. By constructing a dual-parameter model using CNA pattern and FS features to compute a ctDNA composite score, we sequentially explored the association between ctDNA genomic classifier and disease progression and survival.

# Patients and Methods

## Study Population and Design

In this retrospective cohort study, we included 93 HCC patients treated in Peking University Shenzhen Hospital from June 2018 to September 2021. HCC was diagnosed according to National Health Commission of People's Republic of China (2017). The inclusion criteria comprised pathologically confirmed HCC, age ≥ 18 years, availability of preoperative blood samples, and complete clinical and follow-up data. Patients with extrahepatic metastases, concurrent malignancies and incomplete clinical data or follow-up were excluded. The retrospective study was approved by the medical ethic committee of Peking University Shenzhen Hospital followed by Declaration of Helsinki. The ethic approval number was 2020(1). Due to the retrospective design, the written informed consent was waived. Before the preoperative, peripheral blood sample was collected according to standard operation for ctDNA analysis.

## Therapeutic Interventions and Monitoring

Sixty-five patients underwent radical hepatectomy according to Chinese HCC management guidelines, while 28 received multimodal therapy including radiofrequency ablation (RFA), transarterial chemoembolization (TACE), molecular-targeted therapy, and/or systemic chemotherapy. Post-therapeutic surveillance protocols included quarterly monitoring of AFP and protein induced by PIVKA-II levels combined with abdominal ultrasonography, supplemented by contrast-enhanced CT imaging every 4 to 12 months to detect recurrence according to RECIST 1.1 guidelines. Radiologically confirmed disease progression triggered personalized salvage therapies tailored to tumor characteristics and hepatic functional reserve. Survival outcomes were clearly defined. Disease-free survival (DFS) was calculated from the date of initial treatment to either radiologic or biochemical recurrence, or the last follow-up. Overall survival (OS) was measured from histopathologic diagnosis to death from any cause or censoring at the final follow-up. This approach ensured standardized longitudinal tracking across treatment modalities.

## Clinical Parameter Acquisition

A longitudinal biorepository was created by retrospectively collecting comprehensive clinical data and whole-blood specimens from all participants. This repository systematically records multidimensional clinical parameters across five key domains: demographic characteristics (age, sex, hepatitis B serological status); hepatobiliary function (Child-Pugh classification, alanine aminotransferase [ALT, U/L], albumin [ALB, g/dL], total bilirubin [TBIL, μmol/L]); renal homeostasis (serum creatinine [μmol/L], uric acid [μmol/L], estimated glomerular filtration rate [eGFR]); coagulation profiles (prothrombin time [PT, s], international normalized ratio [INR]); and tumor pathobiology (maximum lesion diameter [cm], multifocality, hepatic macrovascular invasion, and validated serum biomarkers such as α-fetoprotein [AFP, ng/mL]

and protein induced by vitamin K absence-II [PIVKA-II, mAU/mL]). Pretherapeutic interventions were also cataloged to account for temporal fluctuations in biomarker levels.

## ctDNA Profiling Pipeline

Circulating tumor DNA (ctDNA) was isolated from peripheral blood samples through a standardized dual-centrifugation protocol. Plasma separation was performed by initial centrifugation at 1600×g for 10 minutes at 4°C. This was followed by high-speed clarification at 16,000×g for 10 minutes at 4°C to obtain platelet-free plasma (PFP) supernatant. Cell-free DNA (cfDNA) was subsequently extracted from PFP using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications, with elution in 40 μL AE buffer. Libraries were prepared from 10 ng of purified cfDNA by using the Kapa HyperPrep Kit (Roche Sequencing Solutions, Wilmington, MA, USA) with enzymatic fragmentation (37°C for 30 minutes) and adapter ligation (20°C for 60 minutes). Final libraries underwent paired-end sequencing (2 × 150 bp) on either the Illumina HiSeq X Ten or NovaSeq 6000 platforms.

The raw data were mapped to the human genome using BWA-MEM software (University of California Santa Cruz genome browser hg19). Low-quality reads, N reads, and the 9 bp trimmed from the 5' end of the read were removed by Cutadapt software version 2.10. Base quality score recalibration was performed via GATK software version 4. Picard software version 2.18 was used to count alignment quality metrics. After removing low-quality reads (mapping quality score < 30) and PCR duplicates, the clean sequencing reads were equally divided into 100-kb bins. Then, QDNAseq software version 1.22.0 was used to analyze CNA detection in these cfDNA samples based on the mapping results. To reduce noise, low-quality bins (mappability < 60%, GC content < 0.3 or > 0.6, N ratio > 0.5) and regions in the Duke blacklist were removed, and the final CNA results were quantified by calculating the *Z* score of each 1-Mb bin according to the following formula: $Zj = \frac{RDj - \overline{RDj}}{SDj}$. Zj: The *Z* score of each 1-Mb region j, : the mean value of reads adjusted by cfDNA samples from healthy cells, SDj: the standard value of reads adjusted by cfDNA samples from healthy cells, RDj: the adjusted number of region j. The CNA status of a bin was determined by whether the *Z* score was < −3 or > 3.

As previously established, cell-free DNA (cfDNA) derived from non-malignant cellular turnover typically exhibits fragment lengths exceeding 167 base pairs (bp), whereas circulating tumor DNA (ctDNA) originating from apoptotic or necrotic tumor cells predominantly demonstrates shorter fragments (<150 bp) due to chromatin fragmentation patterns in malignancy [18]. After paired-end sequencing of ctDNA-enriched samples, bioinformatic processing included quality filtering (Phred score ≥ 30) and removal of PCR duplicates with unique molecular identifiers. Insert size of paired-end reads was used to enumerate fragment length distribution (150 bp read length, average sequencing insert size of 250 bp). The ctDNA burden in each sample was then calculated as the proportion of sub-150 bp fragments per sample, normalized to total cfDNA content. This size-based stratification was highly correlated with ctDNA burden assessed by orthogonal digital PCR analysis of tumor-specific mutations ($R^2 = 0.89$, $P < 0.001$).

After integration of copy number aberration (CNA) profiles and fragment size (FS) distributions from low-pass whole-genome sequencing (lpWGS), a composite ctDNA score was computationally derived from the remaining fraction of the sample's genome using a machine learning algorithm (Random Forest, 500 trees). This score was weighted by chromosomal instability indices and sub-150 bp fragment proportions. This metric reflects global ctDNA aberrational load and is scored on a continuous scale, with higher scores representing greater amounts of tumor-derived molecular heterogeneity. This metric was validated against ctDNA burden quantified by orthogonal digital PCR analysis of driver mutations (Spearman's $\rho = 0.82$, $P < 0.001$).

## Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY), with a two-tailed significance threshold set at $P < 0.05$. The optimal ctDNA score cutoff (0.61199) was established through receiver operating characteristic (ROC) curve analysis using the Youden index, maximizing sensitivity (88.2%) and specificity (66.1%) for recurrence prediction. Intergroup comparisons of baseline clinical and demographic variables between ctDNA score strata were conducted via Pearson's chi-square test, with continuity correction applied for categorical data. Kaplan-Meier survival curves were generated to evaluate disease-free survival (DFS) and overall survival (OS) probabilities, with differences in survival curves between groups assessed by Log rank testing. Multivariable Cox

proportional hazards regression, incorporating backward stepwise selection (retention criteria: $P < 0.10$), identified independent prognostic factors. The Kaplan-Meier curves now include risk tables showing the number of patients at risk at each time point. Significant variables from the univariate analysis ($P < 0.05$) were subsequently included in a multivariable Cox proportional hazards regression model with backward stepwise selection (retention criteria: $P < 0.10$) to identify independent prognostic factors. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to quantify the strength of associations between prognostic factors and survival outcomes. Model assumptions were verified through Schoenfeld residual analysis and proportional hazards testing to confirm the validity of the Cox regression models.

# Results

## Patient Characteristics and ctDNA Score Stratification

The retrospective cohort initially enrolled 100 consecutive HCC patients. After excluding one patient with non-HCC malignancy and six patients lost to follow-up, 93 analyzable cases were retained (Table 1). The cohort was predominantly male (86%, 80/93), with a median age of 54 years (range 20–88 years). Surgical resection constituted the primary intervention (74.2%, 69/93), while 7.5% (7/93) received treatments such as transarterial

**Table 1** Comparison of Clinical Characteristics Between HCC Patients with Different CtDNA Score Levels

| Category | Subject | ctDNA | | *P* value |
|---|---|---|---|---|
| | | ≤0.61199 | >0.61199 | |
| Gender | Male<br>Female | 36<br>7 | 44<br>6 | 0.553 |
| Age | ≤50<br>>50 | 14<br>29 | 26<br>24 | 0.059 |
| HBsAg | Positive<br>Negative | 36<br>7 | 43<br>7 | 0.759 |
| Child-Pugh stage | A<br>B<br>C | 41<br>2<br>0 | 45<br>3<br>2 | 0.393 |
| Preoperative tumor therapy | Yes<br>No | 2<br>33 | 5<br>25 | 0.156 |
| AFP (ng/mL) | ≤400<br>>400 | 37<br>6 | 26<br>24 | <0.001 |
| PIVKA-II (mAU/mL) | ≤2454<br>>2454 | 39<br>4 | 22<br>26 | <0.001 |
| The largest tumor size (cm) | <5<br>5 to8<br>≥8 | 37<br>4<br>2 | 10<br>12<br>28 | <0.001 |
| Macro-vascular invasion | Yes<br>No | 43<br>1 | 41<br>14 | 0.001 |
| TNM stage | I<br>II<br>III<br>IV | 28<br>9<br>5<br>1 | 13<br>12<br>16<br>9 | 0.001 |

(*Continued*)

**Table 1** (Continued).

| Category | Subject | ctDNA | | *P* value |
|---|---|---|---|---|
| | | ≤0.61199 | >0.61199 | |
| Surgery | Yes<br>No | 35<br>8 | 30<br>20 | 0.019 |
| Tumor number | ≤3<br>>3 | 42<br>1 | 43<br>7 | 0.045 |

**Abbreviations**: HBsAg, Hepatitis B surface Antigen; AFP, Alpha-fetoprotein; PIVKA-II, Protein Induced by Vitamin K Absence or Antagonist-II.

chemoembolization (TACE) and systemic therapies, including targeted therapy and immunotherapy. Liver function stratification showed preserved hepatic function (Child-Pugh A: 92.5%, 86/93) and decompensated disease (Child-Pugh B/C: 7.5%, 7/93). Chronic hepatitis B infection was highly prevalent (84.9%, 79/93). Tumor staging distribution comprised early-stage (I–II: 69.9%, 65/93) and advanced disease (III–IV: 29.1%, 27/93). Over a median follow-up of 637 days (IQR: 415–892), disease recurrence occurred in 44.1% (41/93) of patients, and HCC-specific mortality, attributable to tumor progression, was observed in 36.6% (34/93) of patients. These data were derived from follow-up of the study cohort.

ROC analysis identified a ctDNA score threshold of 0.61199 as the optimal prognostic classifier (Youden index). The model demonstrated robust discriminatory capacity with an AUC of 0.806 (95% CI: 0.711–0.900), achieving 88.2% sensitivity and 66.1% specificity for recurrence prediction (Figure 1). Subsequent stratification partitioned the cohort into low (≤0.61199) and high (>0.61199) ctDNA subgroups for comparative survival analysis.

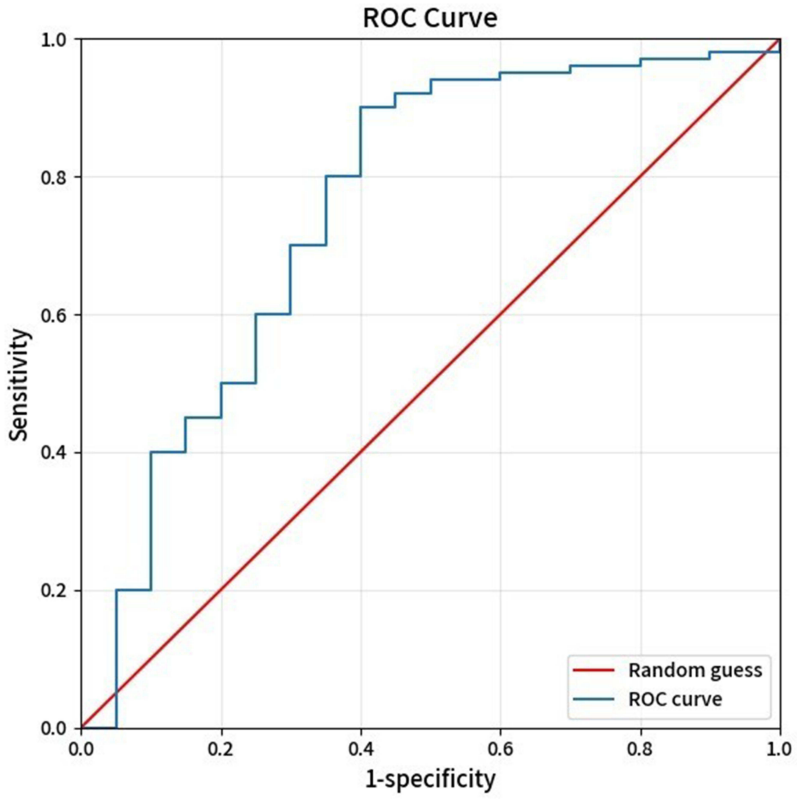


**Figure 1** The optimal cutoff value of ctDNA score was 0.61199 by using receiver operating characteristic (ROC) curves.


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## Association Between ctDNA Score and Clinicopathological Features

Comparative analysis of baseline characteristics between ctDNA score-stratified groups revealed no significant inter-group disparities in sex distribution (P = 0.553), median age (P = 0.059), HBsAg seropositivity (P = 0.759), Child-Pugh class (P = 0.393), or preoperative neoadjuvant therapy (P = 0.156). However, elevated ctDNA scores (>0.61199) demonstrated strong associations with aggressive tumor biology. Specifically, these patients exhibited significantly higher median AFP levels above 400 ng/mL versus 400 ng/mL or below (P < 0.001), elevated PIVKA-II concentrations (>2454 vs ≤2454 mAU/mL; P < 0.001), increased prevalence of the macrovascular invasion (48.0% vs 2.3%; P = 0.001), advanced AJCC TNM stage (III/IV: 50.0% vs 14.0%; P = 0.001), and larger tumor diameters (≥8 cm: 93.3% vs 4.7%; P < 0.001). Importantly, survival outcomes diverged markedly between cohorts, with patients with low ctDNA scores exhibiting superior 3-year overall survival (83.4% vs 29.6%; P < 0.001) and prolonged disease-free survival (51.1% vs 8.4%; P < 0.001), underscoring the prognostic relevance of ctDNA quantification in HCC progression.

Given its association with aggressive tumor biology, we next evaluated the impact of the ctDNA score on survival. Kaplan-Meier analysis of the entire cohort (n = 93) revealed striking survival disparities between ctDNA score-stratified groups. Patients with low ctDNA scores (≤0.61199) demonstrated superior 3-year overall survival (OS: 83.4% vs 29.6%; HR = 6.99, P < 0.001), with consistent survival rates observed at Year 1 (96.6%) and Year 2 (83.4%). Surgical subgroup analysis (n = 65) further validated this pattern: patients with favorable ctDNA profiles (subgroup 1a, n = 35) achieved 100% 1-year and 84.8% 3-year OS, versus 67.6% and 40.7% in high-score counterparts (subgroup 1b, n = 30; log-rank P = 0.001). Disease-free survival (DFS) analysis showed similar trends, with 3-year DFS rates of 51.1% vs 8.4% (P < 0.001). Notably, non-surgical patients (n = 28) exhibited analogous prognostic stratification; low ctDNA scores correlated with 75.0% 3-year OS versus 11.9% in high-score counterparts (P = 0.018), confirming ctDNA's prognostic value across therapeutic modalities. These stratified outcomes persist across all follow-up time points, establishing elevated ctDNA scores as robust biomarkers of aggressive HCC biology and poor clinical trajectories (Table 2 and Figure 2).

**Table 2** (A) OS for All Participators Who with a Different Level of ctDNA Score. (B) DFS for Participators Who Received Surgical Treatment with Different Levels of ctDNA Score. (C) OS for Participators Who Received Surgical Treatment with Different Levels of ctDNA Score. (D) OS for Participators Who Have Not Received Surgical Treatment with Different Levels of ctDNA Score

| A | | | | | |
|---|---|---|---|---|---|
| Group | Cases | Overall survival | | | *P* value |
| | | 1 year | 2 year | 3 year | |
| ≤0.61199 | 43 | 0.966 | 0.834 | 0.834 | <0.001 |
| >0.61199 | 50 | 0.475 | 0.296 | 0.296 | |
| B | | | | | |
| Group | Cases | Disease-free survival | | | *P* value |
| | | 1 year | 2 year | 3 year | |
| ≤0.61199 | 35 | 0.749 | 0.532 | 0.532 | <0.001 |
| >0.61199 | 30 | 0.177 | 0.089 | 0.089 | |

(*Continued*)

**Table 2** (Continued).

| C | | | | | |
|---|---|---|---|---|---|
| Group | Cases | Overall survival | | | *P* value |
| | | 1 year | 2 year | 3 year | |
| ≤0.61199 | 35 | 1 | 0.848 | 0.848 | 0.001 |
| >0.61199 | 30 | 0.676 | 0.407 | 0.407 | |
| D | | | | | |
| Group | Cases | Overall survival | | | *P* value |
| | | 1 year | 2 year | 3 year | |
| ≤0.61199 | 8 | 0.750 | 0.750 | 0.750 | 0.018 |
| >0.61199 | 20 | 0.298 | 0.119 | 0.119 | |

## Univariate and Multivariate Analyses for Overall Survival

Univariate Cox regression analysis identified multiple potential prognostic determinants in HCC patients (Table 3). Elevated ctDNA scores (>0.61199) emerged as the strongest predictor, alongside clinical parameters including elevated BMI (>21.79 kg/m$^2$), leukocytosis (WBC >6.38×10$^9$/L), and systemic inflammatory markers (NLR >2.33, neutrophil count >3.77×10$^9$/L). Hepatic dysfunction indicators demonstrated significant prognostic value: ALT >34.0 U/L (HR=1.84), hyperbilirubinemia (TBIL >20.15 μmol/L, DBIL >3.65 μmol/L, IBIL >10.65 μmol/L), and hypoalbuminemia (ALB <35.6 g/L). Coagulation abnormalities (APTT >37.6 s, fibrinogen >2.865 g/L) and established tumor biomarkers (PIVKA-II >2454 mAU/mL, AFP >40 ng/mL) further correlated with poor outcomes. Notably, advanced tumor characteristics, including maximal diameter exceeding 8 cm (HR=3.12) and multifocal lesions (>3 nodules, HR=4.05), were identified as independent prognostic factors in multivariate analysis, highlighting the complex determinants of HCC progression.

Prior to multivariate modeling, tumors were stratified by maximal diameter into clinically relevant categories (≤5 cm, 5–8 cm, >8 cm). To build a robust prognostic model, significant univariate variables were entered into a multivariate Cox regression with backward selection. Different AFP thresholds were used: >40 ng/mL in univariate analysis for sensitivity, and ≥400 ng/mL in multivariate analysis for clinical specificity in advanced disease. Elevated ctDNA scores (>0.61199) demonstrated the strongest mortality hazard (HR = 6.99, 95% CI = 2.34–20.85; P < 0.001). Hypoalbuminemia (<35.6 g/L) was associated with a 3.3-fold increased risk (HR = 3.29, 1.34–8.06; P = 0.009). Tumor multifocality (>3 lesions) conferred a 3.9-fold mortality hazard (HR = 3.85, 1.58–9.40; P = 0.003). Paradoxically, elevated AFP (>40 ng/mL) exhibited an inverse prognostic relationship (HR = 0.28, 0.13–0.61; P = 0.001). The final multivariate model demonstrated strong explanatory capacity, accounting for 78% of the variance in survival outcomes (Nagelkerke $R^2$ = 0.78), with ctDNA scores contributing the majority of predictive power (partial $R^2$ = 0.52) (Table 4). These findings underscore the dominant prognostic role of ctDNA quantification alongside traditional clinicopathological parameters in HCC risk stratification.

# Discussion

Detailed investigations have confirmed the clinical significance of circulating tumor DNA (ctDNA) across various malignancies, such as pancreatic cancer, breast cancer, and lung cancer, and shown great promise for early detection and prognosis stratification. Meanwhile, there is still large variability in the methods used to analyze ctDNA aberrations in hepatocellular carcinoma (HCC). In early studies, researchers quantified the concentration of serum cell-free DNA (cfDNA). Wong et al[27,28] demonstrated that in a cohort consisting of 24 HCC patients and 62 hepatitis B virus-induced liver fibrosis patients, cfDNA concentrations (not alpha-fetoprotein [AFP] concentrations) were


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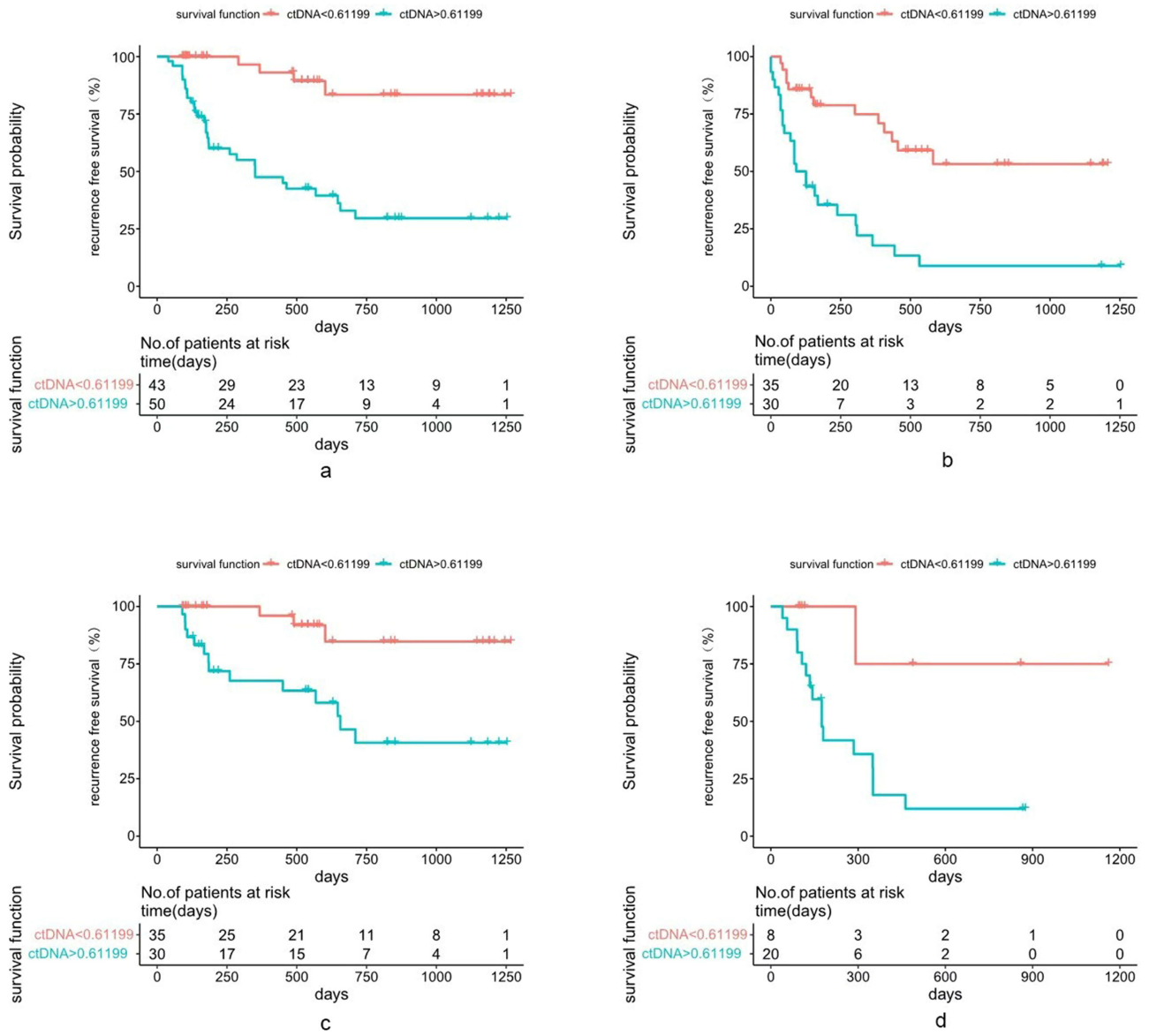


**Figure 2** (**a**) OS for all participants who with a different level of ctDNA score. (**b**) DFS for all participants who with different levels of ctDNA score. (**c**) OS for participants who received surgical treatment with different levels of ctDNA score. (**d**) OS for participants who have not received surgical treatment with different levels of ctDNA score.

independent HCC predictors. This model showed low specificity (AUC = 0.68) because other fibrotic and inflammatory conditions were also associated with cfDNA elevations.[29] Subsequently, tumor-informed mutation analyses were conducted, similar to the deep sequencing of 2924 single-nucleotide variants in 69 genes across 59 HCC patients by Kim et al[30] They identified MLH1 mutations as tissue-plasma concordant biomarkers; however, their data also highlighted the substantial challenges associated with mutation-dependent liquid biopsy strategies (48% of tumor mutations were not detectable in matched plasma).[30] Methylation profiling was then explored as an alternative approach; an eight-marker CpG score developed by Xu et al exhibited strong prognostic ability (training cohort: hazard ratio [HR] = 2.405, 95% confidence interval [CI] = 1.904–3.038, P < 0.001; validation cohort: HR = 1.548, 95% CI = 1.246–1.924, P < 0.001), although it required targeted sequencing, which is laborious and resource-consuming.[26,31] Recently, several innovative methods based on copy number aberration (CNA) profiling using low-pass whole-genome sequencing (lpWGS) have been combined with fragmentomic signatures (FS), providing cost-effective solutions.[32] Our CNA/FS composite algorithm is clinically applicable due to its enhanced specificity (82% specificity vs. 67% for mutation panels) and rapid turnaround (48-hour processing time), thus overcoming the

**Table 3** Clinicopathological Variables Affecting OS in HCC Patients

| Variables | Cases | OS | | | P |
|---|---|---|---|---|---|
| | | 1-year | 2-year | 3-year | |
| BMI | | | | | 0.037 |
| ≤21.79 | 33 | 0.549 | 0.372 | 0.372 | |
| >21.79 | 60 | 0.775 | 0.617 | 0.617 | |
| WBC | | | | | 0.002 |
| ≤6.38 | 63 | 0.788 | 0.629 | 0.629 | |
| >6.38 | 30 | 0.459 | 0.310 | 0.310 | |
| NEU | | | | | 0.001 |
| ≤3.77 | 60 | 0.820 | 0.655 | 0.655 | |
| >3.77 | 33 | 0.433 | 0.275 | 0.275 | |
| NLR | | | | | <0.001 |
| ≤2.33 | 58 | 0.835 | 0.642 | 0.642 | |
| >2.33 | 35 | 0.417 | 0.278 | 0.278 | |
| MONO% | | | | | 0.007 |
| ≤0.455 | 35 | 0.837 | 0.746 | 0.746 | |
| >0.455 | 58 | 0.588 | 0.380 | 0.380 | |
| ALT | | | | | 0.019 |
| ≤34.0 | 46 | 0.814 | 0.658 | 0.658 | |
| >34.0 | 47 | 0.564 | 0.383 | 0.383 | |
| TBIL | | | | | <0.001 |
| ≤20.15 | 71 | 0.778 | 0.606 | 0.606 | |
| >20.15 | 22 | 0.413 | 0.258 | 0.258 | |
| DBIL | | | | | <0.001 |
| ≤3.65 | 61 | 0.840 | 0.629 | 0.629 | |
| >3.65 | 32 | 0.388 | 0.302 | 0.302 | |
| IBIL | | | | | 0.005 |
| ≤10.65 | 43 | 0.834 | 0.644 | 0.644 | |
| >10.65 | 50 | 0.550 | 0.396 | 0.396 | |
| ALB | | | | | 0.003 |
| ≤35.6 | 26 | 0.426 | 0.383 | 0.383 | |
| >35.6 | 67 | 0.795 | 0.574 | 0.574 | |
| GLB | | | | | 0.018 |
| ≤29.75 | 46 | 0.753 | 0.652 | 0.652 | |
| >29.75 | 47 | 0.614 | 0.388 | 0.388 | |
| APTT | | | | | 0.005 |
| ≤37.60 | 40 | 0.828 | 0.789 | 0.789 | |
| >37.6 | 53 | 0.588 | 0.348 | 0.348 | |
| Fib | | | | | 0.006 |
| ≤2.865 | 44 | 0.763 | 0.731 | 0.731 | |
| >2.865 | 49 | 0.612 | 0.360 | 0.360 | |
| PIVKA-II (mAU/mL) | | | | | <0.001 |
| ≤2454 | 60 | 0.854 | 0.673 | 0.673 | |
| >2454 | 31 | 0.414 | 0.248 | 0.248 | |

(*Continued*)

**Table 3** (Continued).

| Variables | Cases | OS | | | P |
|---|---|---|---|---|---|
| | | 1-year | 2-year | 3-year | |
| AFP (ng/mL) | | | | | 0.001 |
| ≤40 | 44 | 0.844 | 0.740 | 0.740 | |
| >40 | 49 | 0.562 | 0.363 | 0.363 | |
| Tumor number | | | | | |
| ≤3 | 85 | 0.733 | 0.562 | 0.562 | |
| >3 | 8 | 0.156 | 0 | 0 | <0.001 |
| The largest tumor size (cm) | | | | | |
| <5 | 47 | 0.948 | 0.733 | 0.733 | |
| 5 to8 | 16 | 0.549 | 0.509 | 0.509 | |
| ≥8 | 30 | 0.369 | 0.219 | 0.219 | <0.001 |

**Abbreviations**: BMI, Body Mass Index; WBC, White Blood Cell; NEU, Neutrophil; NLR, Neutrophil-to-Lymphocyte Ratio; MONO%, Monocyte Percentage; ALT, Alanine Aminotransferase; TBIL, Total Bilirubin; DBIL, Direct Bilirubin; IBIL, Indirect Bilirubin; ALB, Albumin; GLB, Globulin; APTT, Activated Partial Thromboplastin Time; Fib, Fibrogen; PIVKA-II, Protein Induced by Vitamin K Absence or Antagonist-II; AFP, Alpha-fetoprotein.

**Table 4** Independent Risk Factors for OS in HCC Patients

| Variables | OS | | |
|---|---|---|---|
| | HR | 95% CI | P |
| DNA score | 6.990 | 2.344–20.845 | <0.001 |
| ALB | 0.284 | 0.132–0.613 | 0.001 |
| AFP | 3.286 | 1.341–8.056 | 0.009 |
| Tumor number | 3.851 | 1.577–9.404 | 0.003 |

**Abbreviations**: ALB, Albumin; AFP, Alpha-fetoprotein.

limitations of our previous work and providing scalable approaches for HCC monitoring in different clinical scenarios.[33–35] Notably, while previous ctDNA studies in HCC have focused on single modalities (mutations, methylation, or fragmentomics), our composite score integrates both global genomic instability (CNA) and fragmentomic features, offering a more comprehensive representation of tumor biology and potentially better prognostic performance.

Recently studies have verified the prognostic value of ctDNA monitoring in HCC patients with different therapies. Sefrioui et al (2023) longitudinally studied 38 HCC patients with transarterial chemoembolization (TACE).[36] They found that both increases in ctDNA and cell-free DNA (cfDNA) levels (pre-TACE, post-TACE, 1-month post-TACE) were significantly associated with treatment failure (AUC=0.82, P<0.01).[36] Consistent with their results, Ye et al (2022) performed postoperative surveillance on 96 resected HCC cases with next-generation sequencing and found tumor-derived mutations (AXIN1, CTNNB1, LRP1B, PDGFRA, TP53) were present in the circulation within 7 days after surgery.[37] They found that ctDNA positivity is an independent predictor of poor overall survival (HR=3.45, 95% CI=1.98–5.99) and disease-free survival (HR=4.12, 95% CI=2.31–7.35).[37] Notably, when combining ctDNA status with α-fetoprotein (AFP), more stratification of prognosis could be achieved, and AFP-high/ctDNA(+) patients presented the worst prognosis (3-year DFS: 12% vs 68% in AFP-low/ctDNA(−), P<0.001)[36–38]. All of these results indicated

that ctDNA is gradually emerging as a dynamic biomarker for therapy response and recurrence risk stratification in HCC treatment.[38–40]

This retrospective cohort study demonstrates that high ctDNA scores (integrated copy number aberration signature [CNA] and fragmentomic signature [FS])) is an independent predictor of poor OS in HCC patients (HR=6.99, 95% CI=2.34–20.85, P<0.001). Although our analysis revealed associations between ctDNA burden and aggressive tumor biology, all retrospective studies are subject to potential selection bias and unmeasured confounding. The modest cohort size (n=93) was statistically powered for the primary endpoint (power=0.85, α=0.05), and thus, the present study should be further validated by multicenter prospective trials to evaluate generalization.[40] To improve the robustness of future studies, we recommend that investigations focus on a dynamic longitudinal sampling across perioperative phases to elucidate ctDNA kinetics and improve predictive algorithms. Then, our study lacks comprehensive data on HBV-DNA viral load and nucleotide analogue treatment history, which may influence HCC prognosis. Furthermore, the degree of liver fibrosis was not systematically assessed. Future prospective studies should incorporate these important viral and liver background factors to validate and refine the prognostic model based on the ctDNA score. Additionally, if this methodology were extended to other therapeutic scenarios, including transarterial chemoembolization (TACE), systemic chemotherapy, and molecular-targeted regimens, the clinical applications of ctDNA could be extended to treatment response and acquired resistance monitoring. These efforts would improve the clinical utility of our findings, thereby filling existing gaps in the literature for the sensitivity and specificity of liquid biopsy applications for HCC. Accumulating evidence has demonstrated that molecular MRD exerts a significant impact on postoperative relapse and mortality in solid malignancies. In recent years, with the aid of novel advances in circulating tumor DNA (ctDNA) analysis, MRD monitoring has become increasingly recognized as a game-changing approach. Longitudinal studies have demonstrated that ctDNA analysis exhibits superior prognostic performance compared with conventional imaging and serum biomarkers. In a meta-analysis of 1532 patients from colorectal, breast, and non-small cell lung cancers, ctDNA positivity within 30 days post-resection was associated with a 7.2-fold higher recurrence risk (95% CI: 5.1–10.1; P<0.001) and 4.8-fold mortality risk (95% CI: 3.4–6.7; P<0.001) relative to ctDNA-negative patients.[40] HCC-specific studies have also demonstrated that postoperative ctDNA clearance is associated with improved disease-free survival (HR=0.32, 95% CI: 0.18–0.57; P<0.001).[41] These results highlight the urgent need for investigation into ctDNA-guided MRD monitoring frameworks, especially for the fine-tuning of adjuvant therapy strategies and early recurrence interception through multimodal integration of fragmentomic, methylation, and copy-number aberration signatures. Overall, these results suggest that quantification of ctDNA is clinically actionable biomarker for prognosis prediction and therapeutic response in HCC. Mortality risk is increased 6.9 times (95% CI: 2.34–20.85; P<0.001) and recurrence risk is increased 5.1 times (95% CI: 2.98–8.72; P<0.001) in patients stratified according to high ctDNA scores (>0.61199) compared with patients with low scores.[41,42] Therefore, this observation strongly suggests the emergence of a risk-stratified therapeutic strategy combining ctDNA variation with clinicopathological parameters. Based on the current evidences, ctDNA-positive resected cases should receive more intense adjuvant therapy, ie. more stringent surveillance strategy during systemic therapy, and early salvage measures to detect subclinical recurrence.[43] These recommendations were also reflected in the recently published National Comprehensive Cancer Network (NCCN) and European Association for the Study of the Liver (EASL) guidelines.[44] The guidelines proposed a precision oncology strategy guided by liquid biopsy to improve HCC management through molecularly profiled therapy continuum.

Our results demonstrate that an composite ctDNA score is a strong independent prognostic factor in HCC. The prognostic ability of our CNA- and FS-based model concurs with the recent tendency towards using more complex genomic/fragmentomic features for cancer management. For example, in mNSCLC, a 2024 paper concluding that a machine learning model using a combination of longitudinal ctDNA metrics, including variant allele frequency dynamics and molecular response, significantly better predicted overall survival than single time-point measures of ctDNA or radiographic imaging in mNSCLC.[40] This reinforces our approach of using a composite, algorithmically derived score rather than a single analyte.

In addition, our results support the notion that ctDNA is essential for detecting MRD and guiding adjuvant therapy, a paradigm that is poised to gain widespread acceptance in solid tumours.[45] The CIRCULATE trials in CRC are seminal.

The CIRCULATE-Japan colorectal cancer study, represented by the GALAXY trial, found post-operative ctDNA positivity to be the strongest independent prognostic factor of recurrence in patients with resected CRC.[46] More importantly, the ongoing ALTAIR trial is prospectively randomising ctDNA-positive patients after resection of colorectal liver metastases to different adjuvant regimens, a step towards implementing ctDNA-guided interventional strategies. Our result that patients with high pre-operative ctDNA scores achieved poor outcomes despite surgery suggests a similar potential for ctDNA to identify HCC patients who harbour MRD and may benefit most from intensified adjuvant therapy or closer surveillance.

For the clinical application of ctDNA analysis to HCC, standardisation and validation of assays such as ours will be needed. In the future, our study should be followed by multi-center, prospective validation of our ctDNA score cutoff. Moreover, it will be important to extend our static pre-operative assessment to a dynamic one, in which ctDNA levels are measured during and after locoregional or systemic therapies, to capture the dynamic tumour biology and evaluate treatment response. It may also be important to integrate our relatively cost-effective lpWGS assay with other analytical dimensions, such as methylation signatures or whole-exome sequencing for tumour-agnostic MRD detection, to increase the analytic sensitivity and provide biological clues to the mechanisms of relapse. In summary, by validating a practical and accessible ctDNA prognostic score, our study provides one of the pieces of evidence needed to facilitate the implementation of a subsequent, ctDNA-guided era of care in HCC.

## Conclusion

In summary, our results suggest that ctDNA quantification represents a more efficacious biomarker system when compared to traditional markers, incorporating molecular tumor biology with patterns of clinical progression to improve hepatocellular carcinoma risk stratification. Elevated ctDNA scores are strongly associated with adverse clinical outcomes in HCC, underscoring their potential as a noninvasive prognostic biomarker. Integration of ctDNA-based assessments into clinical workflows may enhance risk stratification and therapeutic decision-making. Further prospective validation is warranted to confirm these findings and establish standardized protocols for ctDNA monitoring in HCC management.

## Institutional Review Board Statement

The retrospective study was approved by the medical ethic committee of Peking University Shenzhen Hospital followed by Declaration of Helsinki. The ethic approval number was 2020(1). Due to the retrospective design, the written informed consent was waived. Patient data were anonymized and handled in compliance with the Declaration of Helsinki. All patient data were kept confidential.

## Abbreviations

CtDNA, circulating tumor DNA; CNA, copy number aberrations; HCC, hepatocellular carcinoma; FS, fragment size; AFP, alpha-fetoprotein; DFS, disease-free survival; OS, overall survival; cfDNA, Cell-free DNA; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; HBsAg, serum hepatitis B virus antigen.

## Data Sharing Statement

Data are available upon reasonable request from the corresponding authors: Qingqi Ren (renqq@alumni.sysu.edu.cn) and Zewei Lin (szlinzw@126.com).

## Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, data acquisition, analysis, interpretation, or all of these areas. They took part in drafting, revising, or critically reviewing the article; gave final approval of the version for publication; have agreed to the journal to which the article has been submitted; and each author agrees to be accountable for all aspects of the work.

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## Disclosure

Xiaofei Ma and Wenhao Yuan are co-first authors for this study. The authors report no conflicts of interest in this work.

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