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Preoperative plasma cell-free DNA chromosomal instability predicts microvascular invasion in hepatocellular carcinoma: a prospective study

Zheyue Shu[1,2,4†], Ting Ye[4†], Wei Wu[5], Menghan Su[4], Jingcheng Wang[1,2,3], Min Zhang[1,2,3], Ziliang Qian[6], Haifen Huang[6], Shusen Zheng[1,2,3*] and Qi Xia[4,5,7*]

Abstract

Background Microvascular invasion (MVI) has been recognized as a risk factor for early recurrence after hepatectomy in patients with hepatocellular carcinoma (HCC). This study aimed to estimate the performance of an ultrasensitive chromosomal aneuploidy detector (UCAD) model for preoperative MVI prediction in operable HCC patients based on plasma cell-free DNA (cfDNA).

Methods A prospective study included HCC patients who underwent surgery in 2021. Preoperative peripheral plasma samples of eligible patients were collected to extract cfDNA, which was then subject to next generation sequencing. Low-coverage whole-genome sequencing data were analyzed for chromosomal instability using different parameters, including Z-score, chromosomal instability score (CIN score), tumor fraction (TFx) and a UCAD model (UCAD = CIN score + TFx + Z-score of all chromosomes). Receiver operating characteristic (ROC) curve was used to evaluate the performance of these parameters in preoperative MVI prediction.

Results Finally, a total of 74 patients with HCC who undergone hepatectomy were prospectively enrolled. Chromosomal instability was evaluated by copy number alterations and oncogenes *MCL1* (located at 1q), *MYC* (located at 8q), *TERT* (located at 5p), *EGFR* (located at 7p), and *VEGFA* (located at 6p) were identified in plasma cfDNA. The UCAD model was a superior parameter in predicting preoperative MVI, with an area under curve (AUC) value 0.749 with a sensitivity of 0.938 specificity of 0.466. Univariate analysis revealed that tumor size (≥ 5 cm) and UCAD (> 0.199) significantly increased the risk of MVI, which were further demonstrated by multivariate analysis, with odd ratio of 1.338 (95%CI, 1.060–1.689) and 2.028 (95%CI, 1.053–3.908) ($P < 0.05$).

†Zheyue Shu and Ting Ye contributed equally to this work.

*Correspondence:
Shusen Zheng
shusenzheng@zju.edu.cn
Qi Xia
xiaqi@zju.edu.cn

Full list of author information is available at the end of the article

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Conclusions Our cfDNA-based UCAD model has shown a promising performance for preoperative MVI prediction in operable HCC patients.

Trial registration This study was registered at https://clinicaltrials.gov/ on 16 May 2022, retrospectively registered, Registration number: NCT05371873.

Keywords Hepatocellular carcinoma, Microvascular invasion, Cell-free DNA, Low-coverage whole-genome sequencing, Ultrasensitive chromosomal aneuploidy detector model

Introduction

Primary liver cancer is the fifth most common type of cancer and the second most common cancer-related mortality across the world [1, 2]. Hepatocellular carcinoma (HCC) is the predominant pathological type of primary liver cancer, accounting for approximately 90% of cases, thus being a huge health issue and socioeconomic burden [2]. Liver resection remains the first-line potential treatment to obtain long-term survival for patients with HCC, especially for those with early-stage disease. However, the five-year recurrence rate after hepatectomy is up to 50-70%, which may be due to preoperative microscopic foci or multicenter occurrence [2].

Recently, microvascular invasion (MVI) has been recognized as a risk factor for early tumor recurrence after hepatectomy in HCC patients and preoperative MVI prediction will guide the choice of surgery and prognosis management strategies in operable HCC patients [3–7]. Currently, confirmation of MVI still relies on pathological examination of surgical tissue [8, 9]. Thus, other non-invasive approaches that can predict MVI presence are urgently needed. Actually, many non-invasive indicators have been explored to investigate their performance in preoperative MVI prediction, such as clinicopathological characteristics, radiographic images, and laboratory test indicators (e.g. alpha fetoprotein [AFP]) [10–18]. However, no satisfactory preoperative MVI predictor has been widely recognized and recommended so far.

Genomic instability is a hallmark of human cancers, including gene mutation and copy number variation [19]. Previous studies have shown that more than 90% of HCC have genomic alterations at the early stages of tumor progression [20]. Genes frequently mutated in HCC patients include *TERT*, *TP53*, *CDKN2A*, *CTNNB1*, *AXIN1*, and *ARID1A* [21, 22]. Loss of chromosomes 1p, 4q, 8p, 9p, 9q, 10q,13p, 16p and 16q, and gain of 1q, 5p, 6p, 7p, 7q, 8q, 13q and 17p were frequently detected in HCC patients [23–26]. However, tumor heterogeneity is a thorny issue for the genomics research in HCC using tumor tissue samples [27].

Cell-free DNA (cfDNA) has provided a new type of biological analyte for liquid biopsy, because it may contain tumor-derived DNA, namely, circulating tumor DNA (ctDNA), which currently can be detected by polymerase chain reaction or next generation sequencing techniques [28–31]. Previous studies have reported that plasma cfDNA can serve as an alternative to tumor tissue for studying the genetic alterations of operable patients with HCC [32, 33]. In addition, several studies have shown that cfDNA has high sensitivity and specificity in the diagnosis and prognosis prediction of urothelial carcinoma [34], breast cancer [35] and liver cancer [36]. In liver cancer, several studies have reported the association between cfDNA variant allele frequency and MVI status [37–39]; however, proofs are still not sufficient.

In this study, we attempted to analyze chromosomal instability based on cfDNA-derived low-coverage whole-genome sequencing data, and evaluate the performance of preoperative MVI prediction using different parameters, including an ultrasensitive chromosomal aneuploidy detector (UCAD) model, with the goal to provide a new cfDNA-based approach for preoperative MVI prediction in operable HCC patients.

Materials and methods

Study design and population enrollment

This was a prospective study conducted at the First Affiliated Hospital of Zhejiang University during 2021. Patients were included in this study if they met the following key criteria: (1) 18–80 years old; (2) pathologically diagnosed with HCC; (3) intending to receive liver resection. Exclusion criteria were as follows: (1) concurrent serious complications, including other malignancy, unstable coronary heart disease or congestive heart failure (grade 3–4), chronic kidney disease (stage 4–5), cirrhosis (Child-Pugh grade C), immunodeficiency; (2) pregnancy. This study was conducted in accordance with Declarations of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital (approval number: IIT20220097B). All participants provided written informed consent. This study was registered at clinicaltrials.gov with number of NCT05371873.

Collection of blood samples and clinicopathological data

A total of 8–10 mL peripheral blood was collected from each patient prior to liver resection, and centrifuged to yield plasma.

The demographics of the participants were collected, as well as the laboratory test indicators, such as AFP, alanine aminotransferase (ALT), aspartate aminotransferase

(AST), Hepatitis B Virus (HBV). The pathological characteristics of each participant was also determined by postoperative pathological examination, including tumor size, tumor count, tumor encapsulation, tumor differentiation and MVI. HCC staging was determined according to Barcelona Clinic Liver Cancer (BCLC) staging system, which divides HCC patients into very early stage (BCLC stage 0), early stage (BCLC stage A), intermediate stage (BCLC stage B), advanced stage (BCLC stage C) and end-stage stage (BCLC stage D) [40]. MVI is defined as the nesting mass of cancer cells lining the vascular cavities of endothelial cells or portal and hepatic venous systems, which is graded as M0 (no MVI), M1 (invaded vessels were no more than 5 and located at the peritumoral region adjacent to the tumor surface within 1 cm), and M2 (invaded vessels of more than 5 or at more than 1 cm away from the tumor surface) as described previously [8]. A small number of cancer cells or small clusters of cancer cells, not covered by endothelium but floating freely within isolated blood vessels, were not considered MVI.

Next generation sequencing and chromosomal instability evaluation

Plasma cfDNA was isolated using the QIAseq cfDNA Extraction Kit (Qiangen). Then 10 ng cfDNA was used to prepare the sequencing libraries with the NEBnext Ultra II FS DNA Library Prep Kit following the manufacturer's instructions. DNA fragments were ligated with 8 bp-barcoded sequencing adaptors and amplified by PCR. Purified sequencing libraries were massively sequenced on Illumina HiSeq X10 platform as previously described [20, 41].

For each sample, at least 10 M paired reads (about 4 G data) were collected and filtered. The reads were mapped to human reference genome hg19. Then, average genomic coverage was calculated using samtools mpileup for each 200 k bin [42], and Z-scores which refer to a score that indicates how many standard deviations a value is above or below the mean for each bin was then normalized using the formula below:

$$coverage_{normalized} = \frac{coverege_{raw} - mean\left(coverage_{controls,\,raw}\right)}{stdev\left(coverage_{controls,\,raw}\right)}$$

R package DNACopy (Version 3.4.3) based on circular binary segmentation (CBS) algorithm was used to identify significant genomic breakpoints and genomic segments with copy number variation [43]. A P value of <0.05 was considered as statistically significant binary segmentation. Absolute segment value was used for further analysis. Chromosomal instability score (CIN score) was calculated using the formula below,

$$CIN_{Score} = \sum_{seg \in\, all\ segment} L_{seg} \times V_{seg}$$

The cfDNA fraction derived from tumor cells (TFx) was evaluated by ichorCNA software based on copy number alterations detected by NGS as previously described [41].

Lastly, chromosomal instability was evaluated by a proprietary bioinformatics model UCAD, which has integrated chromosome Z-scores, CIN score, and TFx (formula is that: UCAD = CIN score + TFx + Z-score of all chromosomes).

Statistically analysis

Continuous variables were expressed as mean and standard deviation, median and interquartile ranges; categorical variables were compared by chi-square test. Logistic regression analysis was used to investigate the risk factors of MVI using hazard ratios or odds ratios with 95% confidence intervals, as appropriate. Missed data was removed from the analyses. Receiver operating characteristic (ROC) curve was used to evaluate the performance of preoperative MVI prediction of the UCAD model, and the optimal cutoff value was identified by Youden's index. Correlation coefficient was calculated by Pearson correlation analysis. Statistical analyses were performed using R software.

Results

Patient characteristics

Among 102 patients recruited in this study, 24 patients were excluded due to deficiency of preoperative plasma samples, or failure of quality control; one patient with history of other malignancy; and 3 patients due to tumor tissue necrosis. Finally, a total of 74 participants were eligible for this study. As shown in Table 1, 71.6% of the participants were ≥55 years old, 79.73% were male, 64.86% were detected with pre-operative AFP less than 20 ng/mL, 32.43% were HBV-positive, and 79.73% were negative for MVI. Most of the participants were at early-stage HCC (BCLC stage 0-A, 86.5%). Forty-two patients were detected with tumor size of less than 3 cm and 19 patients had tumors measuring ≥5 cm. Sixty-two patients were observed to have only one single tumor. Tumor encapsulation was presented in 44 patients. Only 15 of the 74 patients had positive MVI and 16 cases had hepatic trans-arterial chemoembolization (TACE) treatment before liver surgery.

Performance of Z-score, CIN score, TFx and UCAD model for MVI prediction in HCC

The analysis of chromosome copy number gains of well-studied oncogenes *MCL1* (located at 1q), *MYC* (located at 8q), *TERT* (located at 5p), *EGFR* (located at 7p), and *VEGFA* (located at 6p) were identified in plasma cfDNA.

Table 1 Patients' demographic and clinical characteristics

Parameters		Patients (*n*) *N* = 74	
		N	%
Age, years	≥ 55	53	71.6%
	< 55	21	28.4%
Gender	Male	59	79.73%
	Female	15	20.27%
AFP (pre)	≥ 20 ng/mL	26	35.14%
	< 20 ng/mL	48	64.86%
ALT (pre)	≥ 40 U/L	14	18.92%
	< 40 U/L	59	79.73%
	no detection	1	1.35%
AST (pre)	≥ 40 U/L	13	17.57%
	< 40 U/L	60	81.08%
	no detection	1	1.35%
HBV	positive	24	32.43%
	negative	49	66.22%
	no detection	1	1.35%
Tumor size	< 3 cm	42	56.76%
	≥ 3 and < 5 cm	13	17.57%
	≥ 5 cm	19	25.68%
Tumor count	multiple	12	16.22%
	single	62	83.78%
Tumor encapsulation	yes	44	59.46%
	no	30	40.54%
Tumor differentiation	well	5	6.76%
	medium	60	81.08%
	poor	7	9.46%
	unknown	2	2.70%
MVI	0	59	79.73%
	1	13	17.57%
	2	2	2.70%
BCLC stage	0	17	22.97%
	A	47	63.51%
	B	5	6.76%
	C	5	6.76%
Child-Pugh score	A	73	98.65%
	unknown	1	1.35%
TACE treatment	yes	16	21.62%
	no	58	78.38%
Primary	yes	52	70.27%
	no	22	29.73%
Drinking history	yes	25	33.78%
	no	49	66.22%
Smoking history	yes	28	37.84%
	no	46	62.16%

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; MVI, microvascular invasion; TACE, trans-arterial chemoembolization. Unknown means the tumor was destroyed and not to be analyzed in terms of tumor differentiation; Child-Pugh score was missed due to the absence of certain indicators

ROC curve for MVI prediction in HCC was plotted using Z-score, CIN score, TFx and UCAD model individually (Fig. 1). The AUC obtained by using Z-score of each chromosome arm loss or gain ranged from 0.31 to 0.70 (Table 2). Among them, chr1q, chr5p, chr8q, and chr16p had the largest AUC values of 0.70, 0.68, 0.66 and 0.66, respectively ($P \leq 0.05$), showing a high diagnostic potential for MVI prediction. Next, we used Z-score of all chromosome to predict MVI, and the AUC value was 0.637 (95% CI, 0.503–0.77), with a sensitivity of 68.8% and a specificity of 58.6% at the optimal cutoff value of 2.26 (Table 3), showing a better performance than any single chromosome alteration. CIN score had an AUC of 0.702 (95% CI, 0.612–0.79) with a sensitivity of 93.8% and a specificity of 46.6% at the optimal cutoff value of 693.19, which showed a promising potential in MVI prediction. The AUC value of TFx was 0.64 (95% CI, 0.503–0.777) with 62.5% sensitivity and 65.6% specificity, respectively. Among these parameters, UCAD model had the largest AUC value of 0.749 (95% CI, 0.635–0.863) (Table 3; Fig. 1), achieving a sensitivity of 0.938 and a specificity of 0.466 at the optimal cutoff value of 0.199. However, AFP only demonstrated an AUC value of 0.565 (95% CI, 0.438–0.691). It was worth noting that the AUC value of UCAD was significantly higher than that of AFP ($P<0.05$).

Performance of the UCAD model in predicting MVI grades in different subgroups of HCC patients

The performance for MVI prediction between UCAD model and AFP was compared in different subgroups according to the clinical and pathological characteristics of patients. For patients with tumor size less than 3 cm ($N = 42$), patients who had received TACE treatment before surgery ($N = 15$), patients with HCC recurrence ($N = 18$), and patients at late BCLC stage (B or C stage) ($N = 10$), the UCAD model had a larger AUC value than that of the AFP in each subgroup, although with no significant difference (Fig. 2). For HCC patients with tumor count ≥ 2 ($N = 12$), UCAD model showed a significantly better predictive performance of MVI than AFP ($P = 0.01$) (Fig. 2).

Factors associated with MVI in HCC

We also performed univariate and multivariate logistic regression to evaluate factors associated with MVI in HCC. Univariate analysis revealed that tumor size (≥ 5 cm) and UCAD (> 0.199) significantly increased the risk of MVI, which were further demonstrated by multivariate analysis, with odd ratio of 1.338 (95%CI, 1.06–1.689) and 2.028 (95%CI, 1.053–3.908) ($P < 0.05$) (Table 4).

According to the logistic regression results, we could predict the probability of MVI for each subject with the

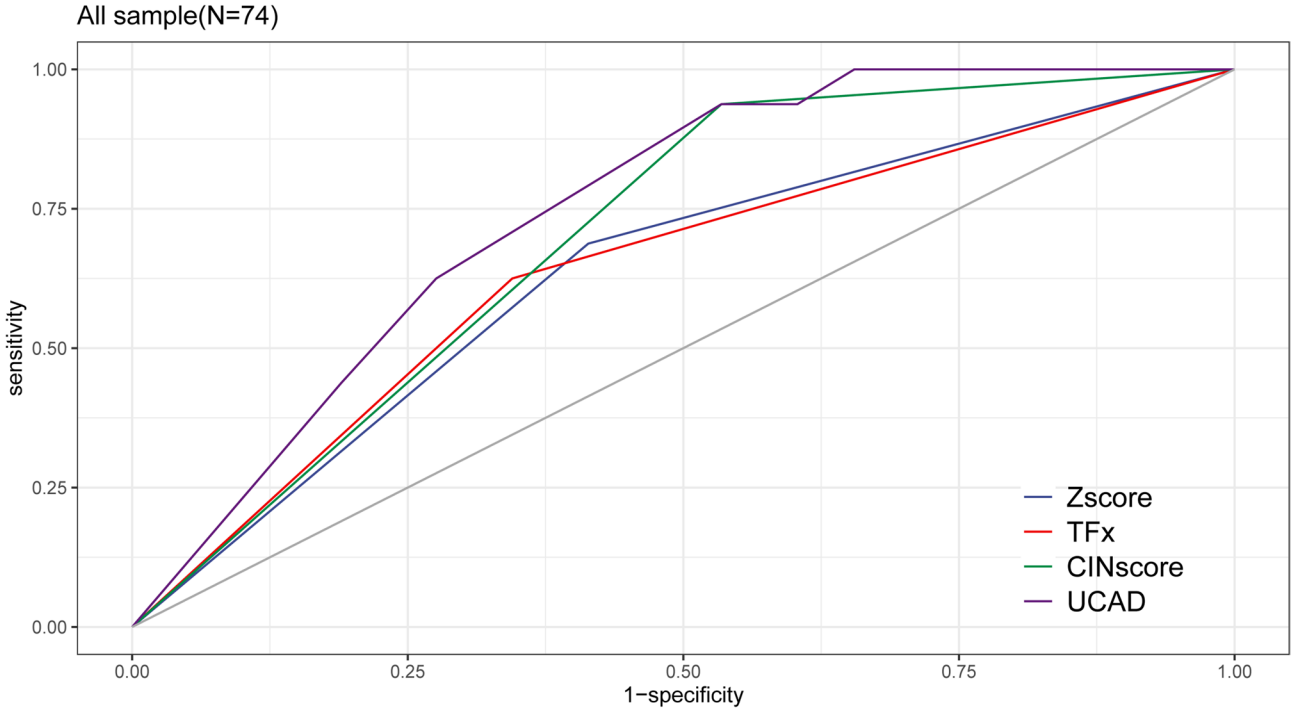

Fig. 1 ROC curve of all detectable chromosome instability and combined index. ROC, receiver operating characteristic

following formula: Logit(P)= -5.06 + 0.49 * Zscore + 0.18 * TFx + 2.11 * CINscore + 1.58 * AFP– 0. * BCLC + 1.56 * TumorCount + 1.65 * TumorSize. The formula of predicting probability for each subject was P = eLogit(P)/(1 + eLogit(P)). The AUC value of the logistic regression model was 0.838 (95% CI: 0.727–0.949), with optimal sensitivity and specificity of 75% and 83% (Fig. 3).

Discussion

Previous studies have reported that cfDNA is a potential biomarker for MVI prediction and may predict tumor recurrence after hepatectomy in HCC patients [37, 38]. But proofs are still not sufficient. In this study, we analyzed chromosomal instability based on cfDNA-derived low-coverage whole-genome sequencing data, and evaluated the performance of preoperative MVI prediction using different parameters, including Z score, CIN score, TFx and a proprietary UCAD model which integrated the parameters above. And the results showed that our proprietary cfDNA-based UCAD model outperformed well for preoperative MVI prediction in operable HCC patients.

Compared to tumor sampling, cfDNA is less invasive. In addition, cfDNA has overcome the disadvantages of tumor tissue samples, namely, heterogeneity and subclonal evolution when used for genomics research. In this study, we used low-coverage whole-genome sequencing of cfDNA to evaluate chromosomal instability. And we identified substantial genomic copy number alterations, including *MCL1* (located at 1q), *MYC* (located at 8q), *TERT* (located at 5p), *EGFR* (located at 7p), and *VEGFA* (located at 6p), which have been reported previously [23–25].

Previous studies have proved the association between ctDNA variant allele fraction (VAF) and MVI in HCC [37, 38], and two of them have reported that ctDNA VAF can predict preoperative MVI status [37, 38]. Wang et al. divided 73 participants into training cohort ($N = 49$) and validation cohort ($N = 24$) and found ctDNA VAF was the only independent risk factor for MVI prediction; ROC analysis using the training cohort obtained an AUC of 0.92, with a sensitivity of 89.7% and a specificity of 80.0% when the cut-off value of ctDNA VAF was set at 0.83% [37, 38]. Using a smaller size of patients ($N = 41$), Xin et al. found that ctDNA maximal VAF could predict the presence of MVI with an AUC of 0.85, a sensitivity of 64.71% and a specificity of 100% when ctDNA VAF of 0.018 was set as the cutoff value [37, 38]. However, both studies employed DNA targeted sequencing with deep sequencing depth. Unlike targeted panels, low-coverage whole-genome sequencing we used here captures CIN across all chromosomes, including unexpected alterations beyond known HCC-associated genes. While our AUC (0.749) is lower than deep sequencing, low-coverage whole-genome sequencing requires < 10% of the sequencing depth, making it feasible for clinical screening and more cost-effective. Based on the sequencing data, we compared the performance of different parameters in

Table 2 The AUC values, 95% CI and P-values of each chromosome arm losses and gains for MVI prediction in terms of Z-scores

Chromosome	AUC	95% CI	*P* value
chr1q	0.70	(0.551,0.856)	0.01
chr5p	0.68	(0.527,0.83)	0.02
chr8q	0.66	(0.505,0.822)	0.05
chr16p	0.66	(0.478,0.835)	0.06
chr4p	0.64	(0.462,0.815)	0.10
chr20p	0.63	(0.468,0.797)	0.11
chr7p	0.59	(0.436,0.737)	0.30
chr18p	0.59	(0.414,0.76)	0.30
chr21p	0.58	(0.421,0.736)	0.35
chr7q	0.57	(0.385,0.748)	0.43
chr20q	0.56	(0.386,0.737)	0.46
chr5q	0.55	(0.389,0.712)	0.55
chr17p	0.54	(0.373,0.7)	0.66
chr6p	0.53	(0.35,0.7)	0.76
chr11q	0.52	(0.346,0.683)	0.86
chr18q	0.51	(0.338,0.681)	0.91
chr15q	0.50	(0.327,0.678)	0.98
chr19p	0.50	(0.336,0.664)	1.00
chr19q	0.50	(0.336,0.664)	1.00
chr11p	0.49	(0.325,0.658)	0.92
chr17q	0.49	(0.316,0.663)	0.90
chr10p	0.49	(0.317,0.656)	0.87
chr9q	0.47	(0.316,0.627)	0.73
chr22q	0.47	(0.32,0.609)	0.67
chr3q	0.46	(0.286,0.636)	0.64
chr6q	0.46	(0.287,0.636)	0.64
chr16q	0.46	(0.278,0.639)	0.62
chr13q	0.44	(0.286,0.601)	0.50
chr3p	0.43	(0.252,0.616)	0.43
chr2p	0.43	(0.253,0.604)	0.39
chr1p	0.40	(0.244,0.546)	0.21
chr10q	0.39	(0.215,0.562)	0.18
chr9p	0.38	(0.218,0.541)	0.15
chr21q	0.38	(0.228,0.533)	0.15
chr12q	0.38	(0.195,0.561)	0.14
chr12p	0.38	(0.199,0.553)	0.14
chr8p	0.38	(0.205,0.546)	0.14
chr2q	0.37	(0.199,0.537)	0.12
chr4q	0.36	(0.215,0.506)	0.10
chr14q	0.31	(0.165,0.448)	0.02

Abbreviations: AUC, area under curve; CI, confidence interval; MVI, microvascular invasion

preoperative MVI prediction, including Z score, CIN score, TFx and a UCAD model, which integrated the parameters above. Among them, UCAD model showed the best performance in preoperative MVI prediction for the entire patient population, with an AUC value of 0.749, a sensitivity of 0.938 and specificity of 0.466, followed by CIN score (AUC of 0.702), while Z score and TFx had similar performance (AUC of approx. 6.4), which was significantly better than AFP (AUC of 0.565), a frequently-used biomarker for HCC diagnosis. We further investigated the performance of UCAD model in patient subgroups stratified by clinical and pathological characteristics, and found that UCAD remained superior to AFP in each subgroup, especially in those with multiple tumor (tumor count ≥ 2) with significant difference. Next, both univariate and multivariate logistic regression analysis further confirmed UCAD model as an independent risk factor associated with MVI. Compared to previous approaches employing ctDNA VAF [37, 38], our low-coverage whole-genome sequencing-based UCAD model has an outstanding sensitivity but a lower specificity. What's more, we established a formula by taking into account the multiple parameters aforementioned, as well as the clinical and pathological characteristics, which can predict MVI status with a larger AUC value of 0.838 (95% CI: 0.727–0.949), and higher sensitivity (75%) and specificity (83%), thus deserving further follow-up and validation.

Finally, although our low-coverage whole-genome sequencing-based UCAD model has provided a promising approach for preoperative MVI prediction, there are some limitations that can't be neglected. First, the small sample size, especially the low MVI-positive patient proportion, may to some extent affect the solidness of our findings. Secondly, the study was a single center research and the model should be validated in more centers or with more data. Thirdly, the specificity of the UCAD model is not ideal, which should be explored and improved later. Thus, a study enrolling more patients and more centers should be conducted in the future to validate and enhance our findings here.

Conclusions

In conclusion, our low-coverage whole-genome sequencing-based UCAD model has provided a promising novel approach for preoperative MVI prediction in operable HCC patients.

Table 3 Performance of Z-score of all chromosomes, CIN scores, TFx, UCAD and AFP for MVI prediction in HCC patients

	AUC (95%CI)	Cutoff	TN	TP	FN	FP	Sensitivity	Specificity	NPV	PPV	Accuracy
Z-score of all chromosomes	0.637 (0.503–0.77)	|Z|>2.26	34	11	5	24	0.688	0.586	0.872	0.314	0.608
CIN score	0.702 (0.612–0.79)	> 693.19	27	15	1	31	0.938	0.466	0.964	0.326	0.568
TFx	0.64 (0.503–0.777)	> 0.07	38	11	5	20	0.625	0.656	0.863	0.355	0.649
UCAD	0.749 (0.635–0.863)	> 0.199	27	15	1	31	0.938	0.466	0.964	0.326	0.568
AFP	0.565 (0.438–0.691)	< 20	22	12	4	36	0.75	0.379	0.846	0.25	0.459

Abbreviations: UCAD = CIN score + TFx + Z-score of all chromosomes; The predictive performance of the UCAD by combination all chromosomal aberrations; sensitivity = TP/(TP + FN), specificity = TN/(TN + FP), NPV = TN/(TN + FN), PPV = TP/(TP + FP), (TN + TP)/(TN + FN + TP + FP). AFP, alpha fetoprotein; AUC, area under curve; CIN, chromosomal instability; HCC, hepatocellular carcinoma; TFx, fraction derived from tumor cells; FN, false negative; FP, false true positive; MVI, microvascular invasion; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive

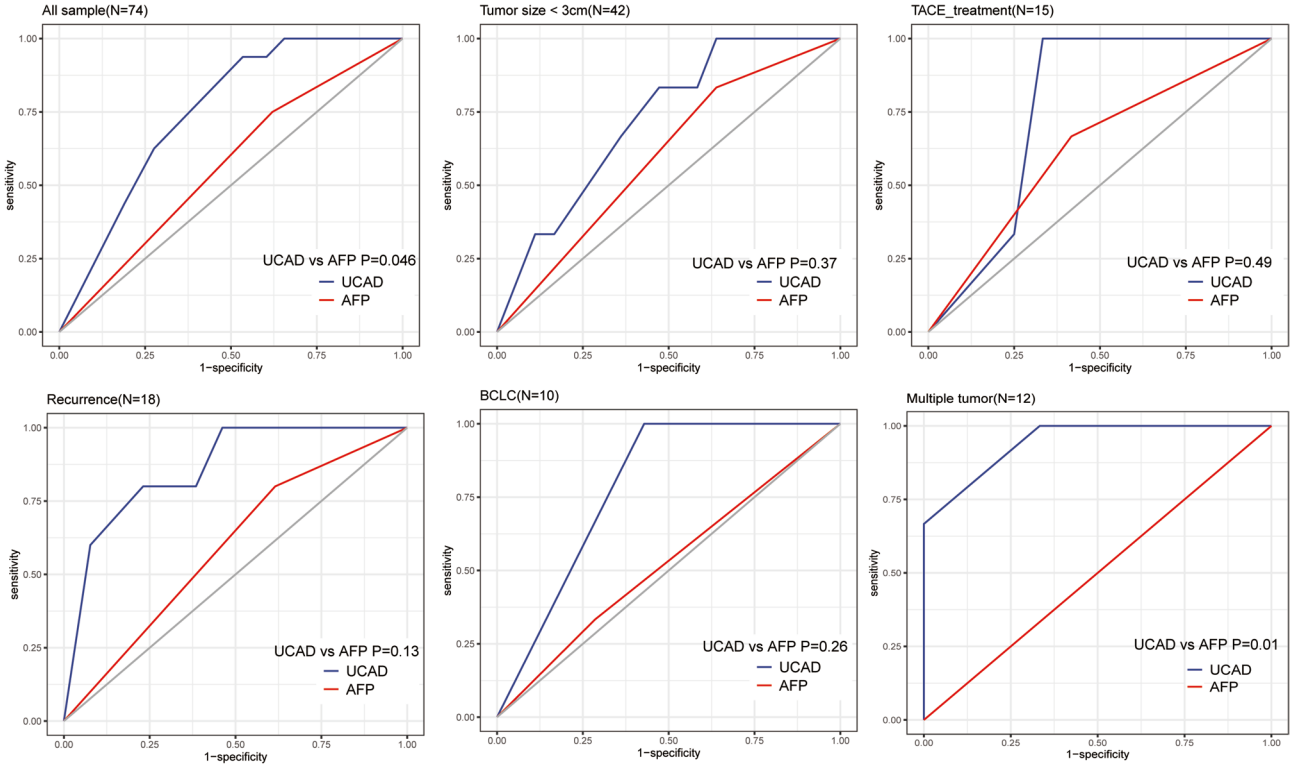

Fig. 2 ROC curve of MVI prediction with the UCAD and AFP models in different subgroups of HCC. (**a**) ROC of MVI prediction with the UCAD and AFP models in whole group patients (*N* = 74). (**b**) ROC of MVI prediction with the UCAD and AFP models in the subgroup of HCC patients with tumor size less than 3 cm (*N* = 42). (**c**) ROC of MVI prediction with the UCAD and AFP models for HCC patients with TACE treatment before liver surgery (*N* = 15). (**d**) ROC of MVI prediction with the UCAD and AFP models for HCC patients with recurrence (*N* = 18). (**e**) ROC of MVI prediction with the UCAD and AFP models for HCC patients at BCLC stage B and C (*N* = 10). (**f**) ROC of MVI prediction with the UCAD and AFP models in HCC patients with total tumor count ≥ 2 (*N* = 12). AFP, alpha fetoprotein; MVI, microvascular invasion. HCC, hepatocellular carcinoma; MVI, microvascular invasion; ROC, receiver operating characteristic; TACE, trans-arterial chemoembolization

Table 4 Univariate and multivariate Stepwise logistic regression analysis of risk factors for MVI in patients with HCC

	Univariate analysis		Multivariate analysis	
	P value	OR (95%CI)	*P* value	OR (95%CI)
Age (≥ 55)	0.341	1.108(0.895,1.371)	0.489	1.072 (0.878,1.348)
Gender	0.118	1.206(0.952, 1.528)	0.285	1.134 (0.898,1.433)
AFP	0.344	1.1(0.9,1.346)	0.257	1.126 (0.916,1.384
Tumor size (≥ 5 cm)	0.001	1.414(1.151,1.737)	0.015	1.338(1.06,1.689)
Tumor count (Multiple)	0.76	1.041 (0.801,1.353)	0.203	1.203 (0.903,1.604)
BCLC (0 stage)	0.369	1.06 (0.932,1.204)	0.565	0.959(0.829,1.109)
UCAD (> 0.199)	0.001	2.718 (1.488,4.966)	0.035	2.028(1.053,3.908)

Abbreviations: AFP, alpha fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; MVI, microvascular invasion; OR, odd ratio

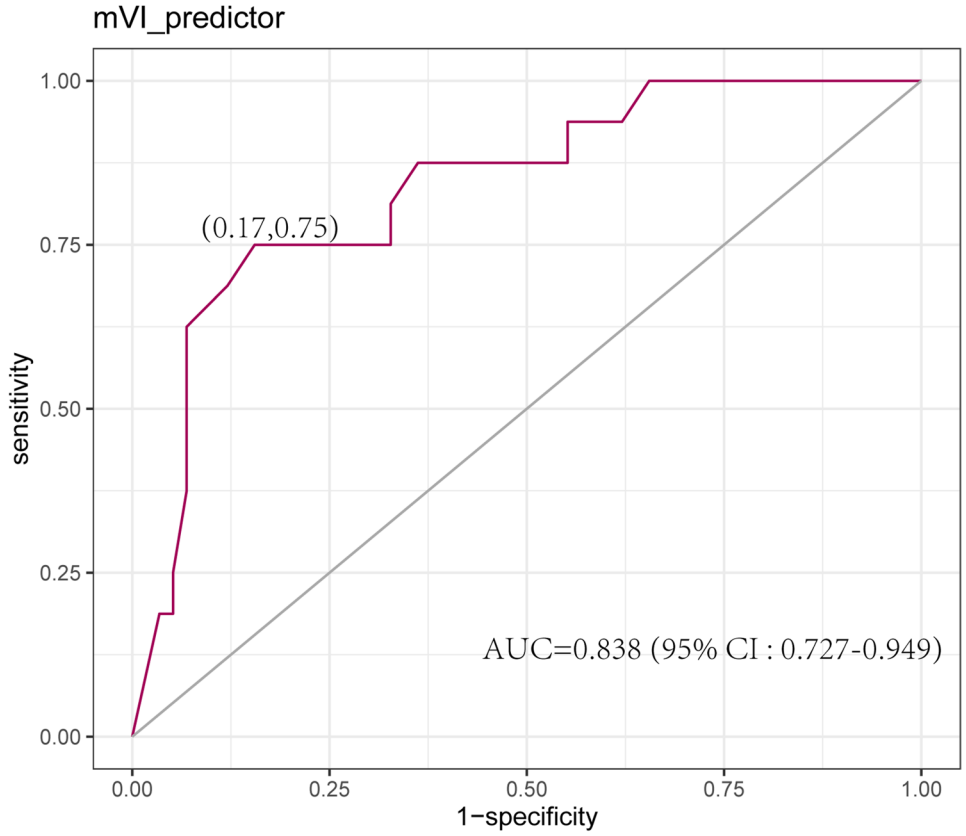

Fig. 3 ROC curve of MVI prediction on the basis of the logistic regression model for HCC patients. HCC, hepatocellular carcinoma; MVI, microvascular invasion; ROC, receiver operating characteristic

Abbreviations

AFP	alpha fetoprotein
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under curve
BCLC	Barcelona Clinic Liver Cancer
CBS	circular binary segmentation
cfDNA	cell-free DNA
CIN score	chromosomal instability score
ctDNA	circulating tumor DNA
HBV	Hepatitis B Virus
HCC	hepatocellular carcinoma
MVI	microvascular invasion
ROC	receiver operating characteristic
TACE	transcatheter artery chemoembolization
TFx	tumor fraction
UCAD	ultrasensitive chromosomal aneuploidy detector
VAF	variant allele fraction

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Author contributions
Conceptualization and Methodology: Qi Xia, Shusen Zheng, Zheyue Shu and Ting Ye; Investigation: Wei Wu, Menghan Su, Jingcheng Wang and Min Zhang; Formal Analysis: Zheyue Shu, Ting Ye, Ziliang Qian and Haifen Huang; Writing–Original Draft: Zheyue Shu and Ting Ye; Supervision: Shusen Zheng and Qi Xia. Writing– Review & Editing: all authors. All authors read and approved the final manuscript.

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Data availability
The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2024), China National Center for Bioinformation/ Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA009795) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Declarations

Ethics approval and consent to participate
All research was conducted in accordance with both the Declarations of Helsinki and Istanbul. All research was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang (Approval No.: IIT20220097B). This study was registered at https://clinicaltrials.gov/ on 16 May 2022, retrospectively registered, Registration number: NCT05371873. Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, No. 79 Qingchun Road, Hangzhou 310003, China
[2]NHC Key Laboratory of Combined Multi-organ Transplantation, Hangzhou 310003, China
[3]Key Laboratory of the Diagnosis and Treatment of Organ Transplantation, Research Unit of Collaborative Diagnosis and Treatment for Hepatobiliary and Pancreatic Cancer, Chinese Academy of Medical Sciences, Hangzhou 310003, China
[4]Zhejiang University School of Medicine, Hangzhou 310058, China
[5]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, No. 79 Qingchun Road, Hangzhou 310003, China
[6]The First People's Hospital of Chenzhou, Chenzhou 424512, China
[7]Key Laboratory for Biomedical Engineering of Ministry of Education, Zhejiang University, Hangzhou 310003, China

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