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Role of Plasma methylated SEPT9 for Predicting Microvascular Invasion and Tumor Proliferation in Hepatocellular Carcinoma

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Abstract

Background: Methylated SEPT9 (mSEPT9) has a role in the occurrence and development of hepatocellular carcinoma (HCC). Here, we studied the significance of plasma mSEPT9 for predicting prognosis-associated pathological parameters in patients with HCC. **Methods:** We retrospectively analyzed data from 205 subjects, including 111 HCC patients, 53 patients with at-risk liver disease (ARD) and 41 healthy donors (HDs). Analysis of plasma mSEPT9 was performed using methylation-specific polymerase chain reaction. Levels of mSEPT9 among different groups were compared using a nonparametric Mann-Whitney *U* test or a one-way ANOVA test. Correlations between pretreatment plasma mSEPT9 and clinicopathological characteristics were analyzed using the Chi-square. Univariate and multivariate analyses were used to identify factors related to microvascular invasion (MVI). Performance of variables for MVI prediction was evaluated by receiver operating characteristics curve. **Results:** A specific increase of plasma mSEPT9 in HCC was found when compared with ARD and HDs (HCC vs ARD, $P = 1.1 \times 10^{-5}$ and HCC vs HDs, $P = 3.7 \times 10^{-10}$). Pretreatment plasma mSEPT9 was significantly correlated tumor number ($P = .004$), tumor size ($P = 4.6 \times 10^{-5}$), MVI ($P = .002$) and Barcelona Clinic Liver Cancer stage ($P = .012$). Levels of plasma mSEPT9 correlated significantly with Ki67 expression in tumor ($r = 0.356$, $P = 1.3 \times 10^{-4}$). Univariate and multivariate analyses showed that plasma mSEPT9 and serum protein induced by vitamin K absence or antagonist-II (PIVKA-II) were independent predictors for MVI. A combination of these 2 markers exhibited a larger areas under the curve (areas under the curve [AUC] = 0.72) than mSEPT9 or PIVKA alone (AUC = 0.67 and 0.65), especially in early-stage HCC. **Conclusions:** Plasma mSEPT9 is a promising noninvasive biomarker for predicting MVI and tumor proliferation in HCC. Integration plasma mSEPT9 detection into clinical settings might facilitate the patient management.

Keywords

methylated SEPT9, circulating tumor DNA, hepatocellular carcinoma, microvascular invasion, tumor proliferation

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Abbreviations

ACTB, β-actin; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; ARD, at-risk liver disease; AUC, areas under the curve; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HDs, healthy donors; HR, hazard ratio; mSEPT9, methylated SEPT9; MVI, microvascular invasion; NLR, neutrophil-to-lymphocyte ratio; PIVKA-II, protein induced by vitamin K absence or antagonist-II; ROC, receiver operating characteristics; TCGA, The Cancer Genome Atlas.

Introduction

Hepatocellular carcinoma (HCC), the most prevalent form of liver cancer, is a leading cause of cancer-related mortality both globally and in China.[1] The outcome remains poor, with a five-year survival rate of less than 30%.[2] Pathological parameters such as microvascular invasion (MVI) is one of the major hazardous factors for early recurrence.[3,4] However, MVI can be only detected based on microscopic examination of tumor tissues, which limits its clinical applicability. Therefore, discovery of a noninvasive biomarker to assist preoperative MVI prediction would play an important role in making treatment decision and improving the prognosis of patients with HCC.

Epigenetic modification plays a crucial role in early carcinogenesis and metastasis, mainly regulating the chromatin structure and gene expression.[5,6] Among them is DNA methylation, which induces the repression of gene expression through hypermethylation of the CpG island in gene promoters. It is reported that 9.34% of patients occurred genetic variations of 20 DNA methylation regulators in HCC from the Cancer Genome Atlas (TCGA) cohort.[7] Previous studies have demonstrated that DNA methylation in HCC correlates with poor clinical outcomes, such as TERT, p16, RASSF1A, GSTP1, CDH1, APC, RUNX3, SOCS1, MGMT, SFRP1, WIF1, PRDM2, DAPK1, p53, SPINT2, OPCML, and WT1.[8,9] DNA methylation, released by dying cancer cells into the blood stream, can be detected in circulating tumor DNA within plasma.[10] This approach has a potential value in preinterventional stratification for HCC.

Plasma methylated SEPT9 (mSEPT9) assay has received approval from Chinese Food and Drug Administration as a plasma-based early screening for colorectal cancers. Emerging evidence suggests that mSEPT9 has a role in hepatocarcinogenesis.[11] SEPT9 expression was frequently lower in HCC due to aberrant promoter hypermethylation of SEPT9 gene.[12] Previous studies have demonstrated that patients with mSEPT9 exhibited significantly poorer survival rates.[13,14] Hypermethylation of SEPT9 was reported to lead to loss of apoptotic cellular function and activation of hepatic stellate cells.[15] The activation of hepatic stellate cells was proved to promote angiogenesis in HCC.[16]

Based on these evidences, we recruited 205 subjects including HCC, those at risk of developing HCC and healthy individuals to investigate whether the plasma mSEPT9 can be a potential noninvasive biomarker in HCC. Further, we explored correlations between plasma mSEPT9 and clinicopathological characteristics of HCC patients. Importantly, we evaluated the value of pretreatment mSEPT9 for predicting MVI.

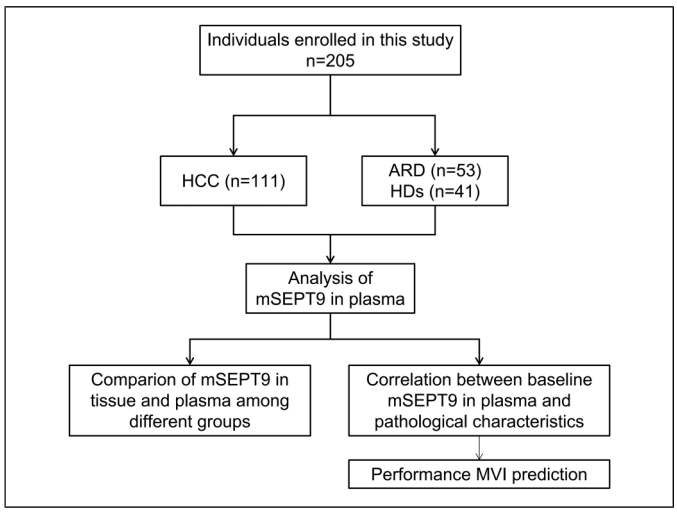

Figure 1. Workflow of present study. Abbreviations: ARD, at-risk liver disease; HCC, hepatocellular carcinoma; HDs, healthy donors; mSEPT9, methylated SEPT9; MVI, microvascular invasion.

Materials and Methods

UALCAN Database

DNA methylation data in tumors and related clinical information of HCC were obtained from UALCAN database (available at http://ualcan.path.uab.edu), an online analysis of gene methylation and clinical data of cancers from TCGA.[17] We obtained levels of the promoter methylation of SEPT9 in HCC tumor and normal tissues from UALCAN database. The screening conditions were: "Gene: SEPT9"; "TCGA dataset: Liver hepatocellular carcinoma"; "Links for analysis: Methylation"; "Profile based on: Sample types, Individual cancer stages and Tumor grade".

Patient Collection

We consecutively enrolled 111 patients with HCC between July 2021 and June 2022. This retrospective study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of Zhongshan Hospital (B2021-539). Informed consent was obtained from every individual and all detailed information of patients was de-identified in this study. Enrollment criteria were as follows: (1) definite HCC

diagnosis based on histopathological examinations; (2) age >18 years; and (3) having no history of the prior treatment. Exclusion criteria were as follows: (1) Child-Pugh C or severe liver dysfunction; (2) having history of any malignancy other than HCC; (3) pregnant woman; and (4) no active inflammation. In addition, a total of 41 healthy donors (HDs) and 53 patients with at-risk liver disease (ARD), including cirrhosis, hepatitis, and focal nodular hyperplasia, were enrolled (Figure 1). The reporting of this study conformed to the Strengthening the Reporting of Observational Studies in Epidemiology guideline.[18]

Data Collection

Preoperative results of hepatitis B surface antigen (HBsAg), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II (PIVKA-II) and neutrophil-to-lymphocyte ratio (NLR) were collected in all individuals enrolled. However, no remaining samples from HDs were available for serum PIVKA-II detection. The threshold of PIVKA-II and NLR was defined as 90 mAU/mL and 2.4, according to previous studies.[19,20] Clinicopathologic data from pathology reports were also included.

Sample Collection and Storage

A total of 10 mL of venous blood was collected from each subject before surgery using EDTA-K_2 anticoagulant tubes. Plasma samples were isolated within 4 hours after sampling by repeated centrifugation at 1500 g for 12 min at 4 °C, and subsequently stored at −80 °C.

Measurement of mSEPT9 in Plasma

Analysis of plasma mSEPT9 was performed in all subjects enrolled before treatment. Measurement of mSEPT9 was performed using the Septin9 assay kit (BioChain Science and Technology), according to the manufacturer's instructions. Cell-free DNA was extracted from plasma and treated with bisulfite conversion, followed by a subsequent real-time polymerase chain reaction (PCR) for mSEPT9 detection. β-actin (ACTB) was applied to ensure the quality and quantity of cfDNA. If a Ct value of ACTB was over 32.1, DNA sample was retreated with bisulfite conversion and PCR. Positive and negative controls were used to determine the validity of each assay. The results of mSEPT9 were regarded as positive when the Ct of mSEPT was below 41.0 according to the manufacturer's instructions.[21] To obtain relative quantification of plasma mSEPT9, the $-\Delta\Delta$Ct value for each sample was calculated as follows:

$$-\Delta\Delta Ct_{Sample} = -(\Delta Ct_{Sample} - \Delta Ct_{positive})$$
$$\Delta Ct_{Sample} = Ct_{SEPT9\ of\ sample} - Ct_{\beta\text{-actin of sample}}$$
$$\Delta Ct_{positive} = Ct_{SEPT9\ of\ positive} - Ct_{\beta\text{-actin of positive}}$$

Statistical Analysis

Statistical analyses were performed using SPSS version 23 (IBM, USA). Categorical variables were described by frequency counts and percentages, and continuous variables were featured by means and standard deviation. Correlations between clinicopathological characteristics and plasma SEPT9 status were analyzed using the Chi-square test. If variances within groups were not homogeneous, a Mann-Whitney U test was used to compare the differences between 2 independent groups and a one-way ANOVA test was used among 3 groups. Nonparametric spearman's rank correlation was employed to evaluate the relationship between plasma mSEPT9 and other biomarkers in HCC patients. Univariate and multivariate logistic regression analyses were conducted to identify factors predictive of MVI. The receiver operating characteristics (ROC) curve analysis was applied to evaluate the value of variables for MVI prediction. A nomogram was built on the predictive model as a graphical presentation. A P value <.050 was considered statistically significant.

Results

Patient Characteristics

The clinical characteristics of the 205 patients were summarized in Supplemental Table 1. Mean age was 58.4 ± 10.6 years for HCC group, 51.7 ± 15.0 years for ARD group and 61.4 ± 12.0 years for HDs, respectively. The majority of the HCC patients were male (n = 97, 87.4%). According to Barcelona Clinic Liver Cancer (BCLC) staging criteria, 94 patients were classified as early-stage HCC (stage 0, n = 28; stage A, n = 66), and the other 17 patients were classified as intermediate-stage HCC (stage B, n = 10) or advanced HCC (stage C, n = 7).

Specific Elevation of mSEPT9 Level in Tissue and Plasma From HCC Patients

DNA methylation levels of SEPT9 were significantly higher in tumors compared with normal tissues from UALCAN database ($P = 4.8 \times 10^{-8}$, Figure 2A), which demonstrated that mSEPT9 had a pivotal role in molecular pathogenesis and development of HCC. Plasma samples from 205 individuals, including HCC (n = 111), ARD (n = 53) and HDs (n = 41), were obtained for further study (Figure 1). The positive rate of plasma mSEPT9 was significant higher in HCC than in ARD ($P = 1.1 \times 10^{-5}$, Figure 2B) and HDs ($P = 3.7 \times 10^{-10}$, Figure 2B). Meanwhile, the mean $-\Delta\Delta$Ct value of mSEPT9 was −3.7 ± 4.3, −7.0 ± 3.0, and −7.4 ± 1.3 in HCC, ARD, and HDs, with significant differences among the 3 groups (HCC vs ARD, $P = 5.2 \times 10^{-7}$; HCC vs HDs, $P = 9.0 \times 10^{-6}$, Figure 2C). In addition, no significant difference was found in the mSEPT9 levels between ARD and HDs (P = .623, Figure 2C). Male HCC patients had higher mSEPT9 levels (male HCC vs female HCC, P = .055; male HCC vs ARD + HDs, $P = 1.1 \times 10^{-4}$, Supplemental Figure 1B), similar to data obtained from UALCAN database

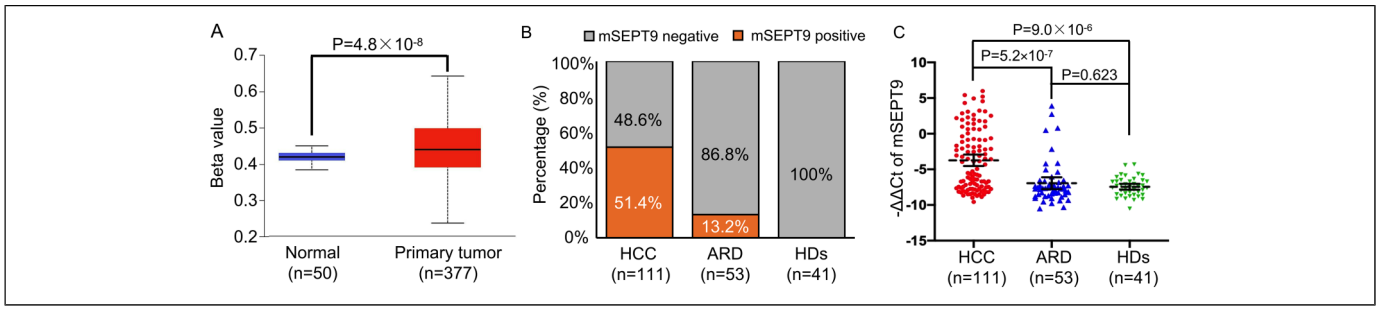

Figure 2. Analysis of mSEPT9 in tumor and plasma using methylation-specific fluorescence PCR. (A) Promoter methylation levels of SEPT9 in HCC obtained from UALCAN database. (B) Positive rates of plasma mSEPT9 in each of the study groups. (C) Distributions of plasma mSEPT9 in each of the study groups. Bars represent the mean and 95% CI. *P* values were determined by using the one-way ANOVA test. Abbreviations: ARD, at-risk liver disease; HCC, hepatocellular carcinoma; HDs, healthy donors; mSEPT9, methylated SEPT9; PCR, polymerase chain reaction; mSEPT9, methylated SEPT9.

(Supplemental Figure 1A). Plasma mSEPT9 showed a better ability in identifying male HCC from HDs (male AUC = 0.77, 95% confidence interval [CI] 0.71-0.84; female AUC = 0.60, 95% CI 0.43-0.77, Supplemental Figure 1C).

Among 111 HCC patients enrolled in this study, −ΔΔCt value of mSEPT9 showed a weak correlation with AFP (r = 0.190, P = .046, Supplemental Figure 2A) and PIVKA-II (r = 0.289, P = .002, Supplemental Figure 2B) in serum. Although the correlation of mSEPT9 level and NLR was not significant (r = 0.174, P = .068, Supplemental Figure 2C), the positive rate of plasma mSEPT9 significantly increased in NLR group (P = .007, Table 1).

Correlations Between Plasma mSEPT9 and Clinicopathologic Characteristics of HCC

Correlations between pretreatment plasma mSEPT9 and clinicopathologic characteristics are shown in Table 1. Plasma mSEPT9 was significantly correlated with tumor number (P = .004), tumor size ($P = 4.6 \times 10^{-5}$), MVI (P = .002) and BCLC stage (P = .012). Further investigation indicated that levels of plasma mSEPT9 were significantly higher in HCC patients with multiple tumor lesions (P = .024, Figure 3A) and large tumor sizes ($P = 1.4 \times 10^{-4}$, Figure 3B). In addition, −ΔΔCt value of mSEPT9 increased with high BCLC stage (BCLC 0 vs BCLC A, $P = 3.27 \times 10^{-3}$; BCLC A vs BCLC B + C, P = .040, Figure 3C). Our observation demonstrated that baseline plasma mSEPT9 might indicate the severity of HCC. Meanwhile, we found that patients with multiple tumors have higher Ki67 expression (P = .015) and plasma mSEPT9 level correlated significantly with Ki67 expression (r = 0.356, $P = 1.3 \times 10^{-4}$, Figure 3E). This indicated that patients with high mSEPT9 level likely have a more aggressive tumor phenotype.

Pretreatment mSEPT9 as an Independent Predictor of MVI

MVI was found in 26.1% of patients with surgery on histologic evaluation. Occurrence rates of MVI in the BCLC stage 0, A, and B + C subgroups were 10.7%, 30.3%, and 35.3%, respectively. The positive rate of mSEPT9 was higher in patients with MVI (75.9% vs 42.7%). In addition, −ΔΔCt value of mSEPT9 was significantly increased in patients with MVI (P = .008, Figure 3D). Similar results were found in patients with high PIVKA-II (75.9% vs 45.1%, P = .004, Supplemental Figure 3A; P = .003, Supplemental Figure 3B). In univariate analysis, pretreatment plasma mSEPT9 and serum PIVKA-II were significantly associated with MVI (Table 2). Further, multivariate analysis showed mSEPT9 (hazard ratio [HR] = 3.24, 95% CI 1.20–8.76, P = .020) and PIVKA-II (HR = 2.83, 95% CI 1.04–7.69, P = .041) were independent predictors of MVI (Table 2).

Performance of Combining Pretreatment Plasma mSEPT9 and serum PIVKA-II for MVI Prediction in HCC Patients

Areas under the curve (AUC) of plasma mSEPT9 and serum PIVKA-II in identifying MVI among HCC participants were 0.67 (95% CI 0.55-0.78) and 0.65 (95% CI 0.54-0.77), respectively (Figure 4A). However, other involved variables including HBVDNA, NLR, ALT, HBsAg, AFP, BCLC, tumor number, tumor size and cirrhosis showed unsatisfactory performance (Supplemental Figure 4A-1). Further, combination of plasma mSEPT9 and serum PIVKA-II exhibited a better performance of MVI prediction in HCC (AUC = 0.72, 95% CI 0.61-0.82) with a sensitivity and specificity of 62.1% and 74.4% than one single marker. A nomogram based on the combined model for MVI prediction was presented in Figure 4B. We further explored the performance of combined model for prediction of MVI in patients with early stage (BCLC 0 + A), of which 43.6% carried single tumour less than 3 cm. The combined model also provided a better predictive performance (AUC = 0.72, 95% CI 0.60-0.84) with a sensitivity and specificity of 62.1% and 74.4% (Supplemental Figure 5).

Discussion

HCC is one of the most prevalent malignancies worldwide, with higher morbidity and mortality rates worldwide.

Table 1. Correlations Between Plasma mSEPT9 Status and Clinicopathological Characteristics of HCC Patients.

Variables		HCC patients			*P*
		All n = 111	mSEPT9 negative n = 54	mSEPT9 positive n = 57	
Age	<50	20 (18.0%)	12 (22.2%)	8 (14.0%)	.262
	≥50	91 (82.0%)	42 (77.8%)	49 (86.0%)	
Gender	Female	14 (12.6%)	10 (18.5%)	4 (7.0%)	.124
	Male	97 (87.4%)	44 (81.5%)	53 (93.0%)	
HBsAg	Negative	26 (23.4%)	12 (22.2%)	14 (24.6%)	.771
	Positive	85 (76.6%)	42 (77.8%)	43 (75.4%)	
ALT, U/L	<50	99 (89.2%)	49 (90.7%)	50 (87.7%)	.608
	≥50	12 (10.8%)	5 (9.3%)	7 (12.3%)	
AFP, ng/mL	<20	55(49.5%)	33 (61.1%)	22 (38.6%)	***.018***
	≥20	56 (50.5%)	21 (38.9%)	35 (61.4%)	
PIVKA-II, mAU/mL	<90	52 (46.8%)	34 (63.0%)	18 (31.6%)	***.001***
	≥90	59 (53.2%)	20 (7.0%)	39 (68.4%)	
NLR	≥2.4	89 (80.2%)	49 (90.7%)	40 (70.2%)	***.007***
	<2.4	22 (19.8%)	5 (9.3%)	17 (29.8%)	
Cirrhosis	No	56 (50.5%)	26 (48.1%)	30 (52.6%)	.637
	Yes	55 (49.5%)	28 (51.9%)	27 (47.4%)	
No. of tumor	Single	88 (79.3%)	49 (90.7%)	39 (68.4%)	***.004***
	Multiple	23 (20.7%)	5 (9.3%)	18 (31.6%)	
Tumor size, cm	<3	52 (46.8%)	36 (66.7%)	16 (28.1%)	$\boldsymbol{4.6 \times 10^{-5}}$
	≥3	59 (53.2%)	18 (33.3%)	41 (71.9%)	
Satellite lesion	No	108 (97.3%)	54 (100.0%)	54 (94.7%)	/
	Yes	3 (2.7%)	0 (0.0%)	3 (5.3%)	
MVI	Absent	82 (73.9%)	47 (87.0%)	35 (61.4%)	***.002***
	Present	29 (26.1%)	7 (13.0%)	22 (38.6%)	
Macrovascular invasion	Absent	108 (97.3%)	53 (98.1%)	55 (96.5%)	/
	Present	3 (2.7%)	1 (1.9%)	2 (3.5%)	
Differentiation	I-II	85 (76.6%)	45 (83.3%)	40 (70.2%)	.102
	III-IV	26 (23.4%)	9 (16.7%)	17 (29.8%)	
BCLC stage	0 + A	94 (84.7%)	51 (94.4%)	43 (75.4%)	***.012***
	B + C	17 (15.3%)	3 (5.6%)	14 (24.6%)	

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; HBsAg: hepatitis B surface antigen; HCC, hepatocellular carcinoma; mSEPT9, methylated SEPT9; MVI, microvascular invasion; NLR, neutrophil-to-lymphocyte ratio; PIVKA-II, protein induced by vitamin K absence or antagonist-II.

Hypermethylated SEPT9 was demonstrated to be closely related to tumorigenesis and progression in HCC. However, the significance of plasma mSEPT9 for predicting MVI and tumor proliferation in HCC is rarely reported. Here, we studied the clinical value of plasma mSEPT9 as a noninvasive biomarker in HCC.

In this study, we analyzed the data of promoter methylation of SEPT9 in HCC cancer from UALCAN database and found that levels of mSEPT9 in HCC tumors was significantly higher. Further, we found a specific increase of plasma mSEPT9 in HCC, suggesting it could be a potential marker of HCC. In addition, levels of mSEPT9 showed a weak correlation with AFP, PIVKA-II, and NLR.

We further studied the relationship between plasma mSEPT9 and clinicopathologic characteristics of HCC. Notably, mSEPT9 was significantly correlated with multiple tumor number, larger tumor size and advanced BCLC stage. These findings demonstrated plasma mSEPT9 as a noninvasive marker to reflect the severity of the progression of hepatic lesions, in accordance with previous studies on other malignancies.[22,23] We found that patients with multiple tumors have higher Ki67 expression. Also, plasma mSEPT9 level was significantly correlated with Ki67 expression. This study provides direct clinical evidence of the correlation between plasma mSEPT9 and cell proliferation. In addition, mSEPT9 level increased with the BCLC stage, indicating it acted outstandingly as an auxiliary stratification for therapeutic management of HCC patients. Also we found that plasma mSEPT9 level correlated significantly with Ki67 expression. Taken together, our findings indicate that positive mSEPT9 detection in plasma, especially in high level, may indicate a more aggressive tumor phenotype and will therefore significantly improve the evaluation of tumor prognosis.

Multiple retrospective studies determined the presence of MVI as a critical predictor of worse clinical outcomes in HCC.[24] In clinical settings, identification of MVI can help to guide postoperative adjuvant therapy.[25] However, MVI can be detected on histological examination of tumor tissues. Thus, noninvasive approach is urgently needed to predict the presence of MVI before operation. The occurrence rates of MVI in our study is consistent with the previous study.[26] Notably, we found that patients with plasma mSEPT9 had a higher possibility of harboring MVI. Previous studies were

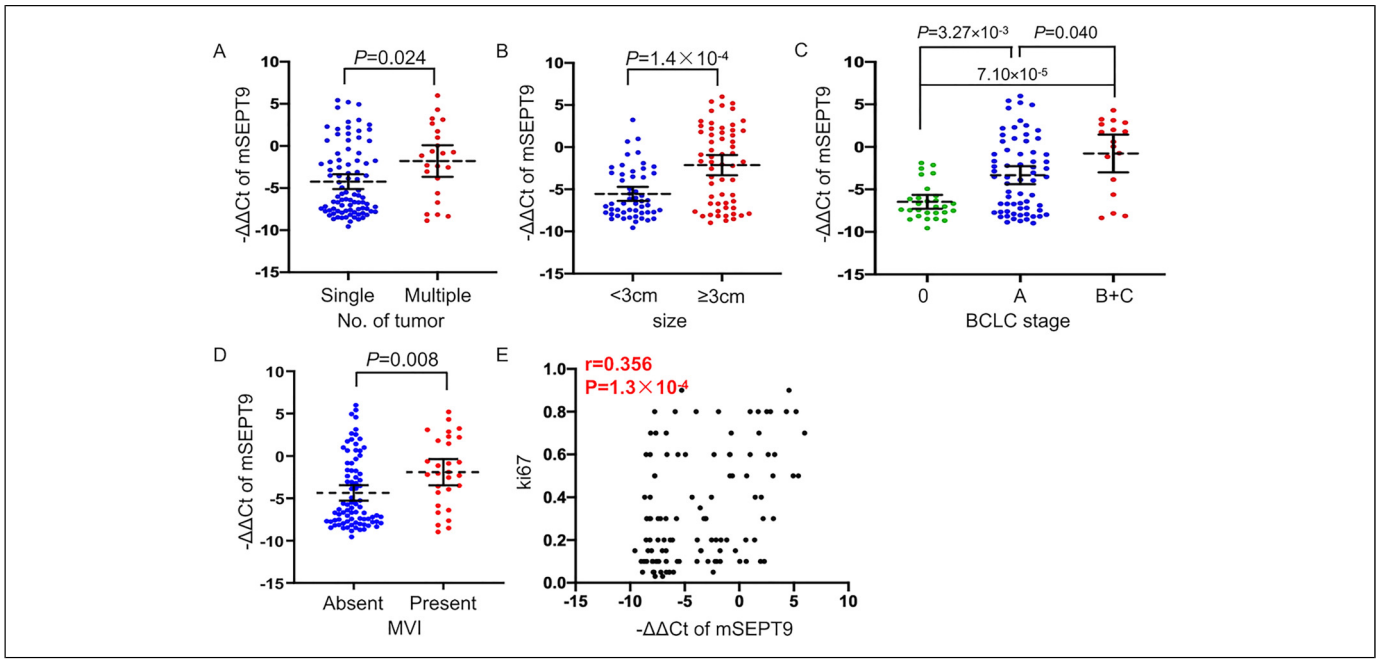

Figure 3. Correlations between plasma mSEPT9 and clinicopathologic characteristics of HCC. Levels of plasma mSEPT9 from HCC patients with different tumor number (A), distinct tumor size (B), BCLC stage (C), and MVI (D). Bars represent the mean and 95% CI. *P* values were determined by using the Mann-Whitney *U* test between 2 independent groups and the one-way ANOVA test among 3 groups. (E) Correlations of plasma mSEPT9 and Ki67 expression in tumor from HCC patients using the nonparametric Spearman's rank correlation analysis. Abbreviations: BCLC, Barcelona Clinic Liver Cancer; mSEPT9, methylated SEPT9; MVI, microvascular invasion; HCC, hepatocellular carcinoma.

Table 2. Univariate and Multivariate Analysis of Predictors of MVI.

	Univariate		Multivariate	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (years) (≥50 vs < 50)	1.075 (0.353-3.275)	.899		
Gender (Male vs Female)	1.343 (0.347-5.197)	.670		
HBsAg (Positive vs Negative)	1.652 (0.559-4.884)	.364		
HBVDNA (IU/mL) (≥50 vs < 50)	1.272 (0.516-3.133)	.601		
ALT (U/L) (≥50 vs < 50)	1.480 (0.410-5.339)	.549		
AFP (ng/mL) (≥20 vs < 20)	1.562 (0.663-3.679)	.308		
PIVKA-II (mAU/mL) (≥90 vs < 90)	3.822 (1.470-9.936)	.006	2.834 (1.044-7.690)	***.041***
NLR (≥2.4 vs < 2.4)	1.421 (0.513-3.934)	.499		
mSEPT9 (≤41.0 vs Ct > 41.0)	4.220 (1.622-10.984)	.003	3.242 (1.200-8.760)	***.020***
Cirrhosis (Yes vs No)	1.357 (0.580-3.177)	.482		
No. of tumor (Multiple vs Single)	1.312 (0.478-3.606)	.598		
Tumor size (cm) (≥3 vs < 3)	2.450 (0.998-6.015)	.051		
Differentiation (III-IV vs I-II)	1.354 (0.515-3.565)	.539		

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; mSEPT9, methylated SEPT9; MVI, microvascular invasion; NLR, neutrophil-to-lymphocyte ratio; PIVKA-II, protein induced by vitamin K absence or antagonist-II.

defined PIVKA-II as an independent predictor of MVI in patients with HCC.[27] Our findings showed that preoperative plasma mSEPT9 and serum PIVKA-II were confirmed as independent predictors for MVI. Combination of mSEPT9 and PIVKA-II could improve the predictive power for MVI compared with mSEPT9 or PIVKA-II alone, especially in early-stage HCC (BCLC 0 + A). The prediction of MVI in early-stage HCC is important for making a personalized therapeutic decision. Adjuvant therapy after radical resection could be considered for early-stage HCC with both mSEPT9 and PIVKA-II positive detection. Therefore, integration plasma mSEPT9 detection into clinical settings might facilitate patient management.

There are several limitations in our study. First, no power calculation was done for the estimation of the sample size. Second, the sample size was small for subgroup analysis and from a single institution, which limits the possibility of data mining. Therefore, more multicentered, prospective studies are needed in the future. Due to

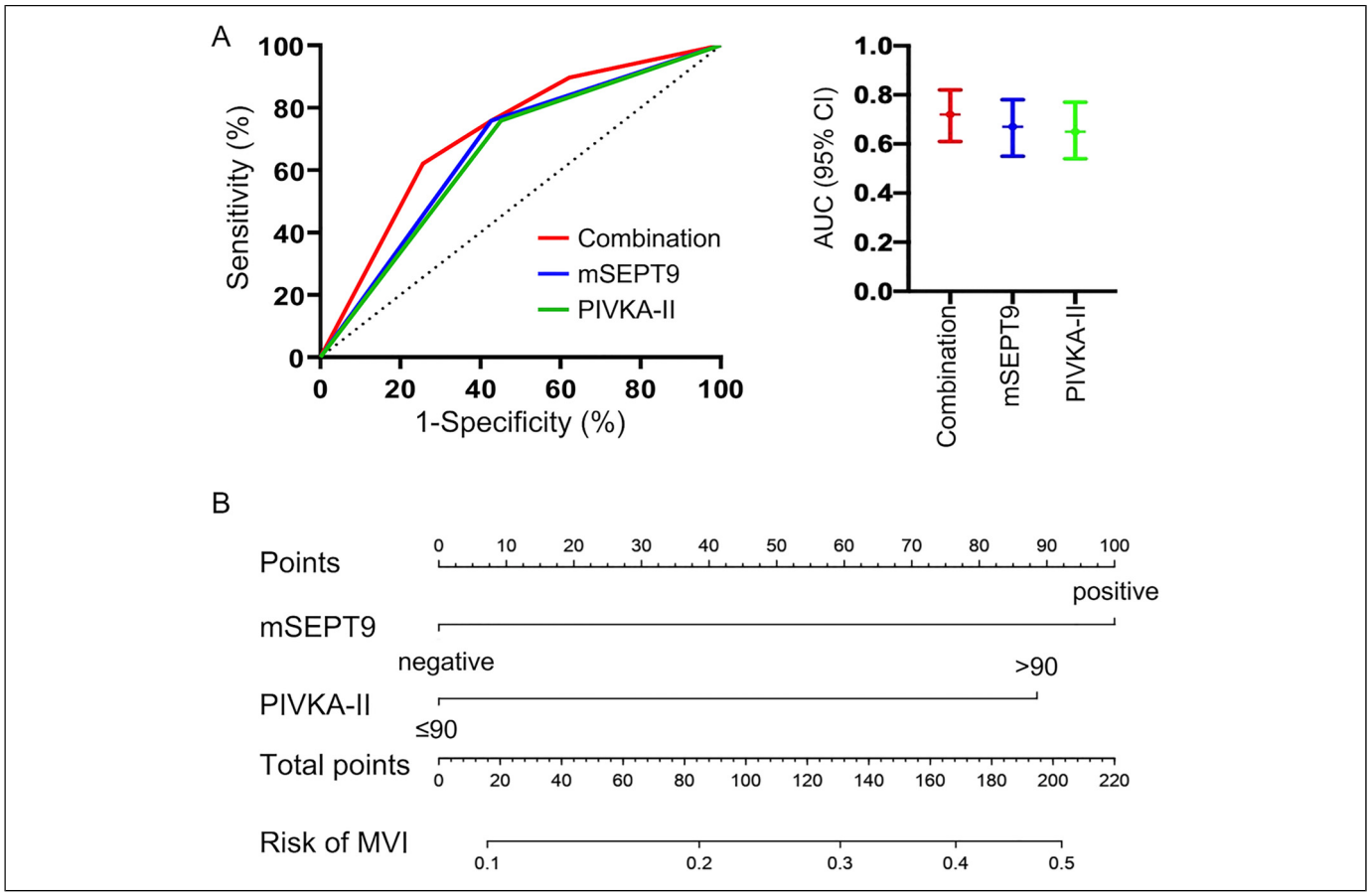

Figure 4. Performance of combination of pretreatment plasma mSEPT9 and serum PIVKA-II for MVI prediction in HCC patients. (A) ROC curves of plasma mSEPT9, PIVKA-II and combined model for predicting MVI in HCC patients. (B) A nomogram was built on the predictive model as a graphical presentation. Abbreviations: mSEPT9, methylated SEPT9; MVI, microvascular invasion; PIVKA-II, protein induced by vitamin K absence or antagonist-II; ROC, receiver operating characteristics curve; HCC, hepatocellular carcinoma.

various etiology backgrounds of HCC patients in real-world, larger sample size is necessary for further confirmation.

Conclusion

In summary, our findings demonstrated plasma mSEPT9 have potential value in predicting MVI and tumor proliferation in HCC. Moreover, combination of preoperative plasma mSEPT9 and serum PIVKA-II has a good predictive efficacy of MVI in patients with HCC.

Author Contribution

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. F.H. and G.Y. created the concept. W.G., B.W., F.H., Y.Yang, H.W. and T.L. designed the experiments. G.Y., H.J. and Q.Y. collected clinical samples. F.H., X.C. and Y.Y. performed experiments, analyzed data, and interpreted the results. F.H. drafted the manuscript. B.P., B.W., W.G., W.Y. and C.Z. edited the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability Statement

The data that support the findings of this study are available in this article.

Ethical Approval

Approval for the use of human subjects was obtained from the Research Ethics Committee of Zhongshan Hospital (B2021-539).

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Informed Consent

Informed consent was obtained from every individual in this study.

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Supplemental Material

Supplemental material for this article is available online.

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