






Impact of Metabolic Activity in Hepatocellular Carcinoma: Association With Immune Status and Vascular Formation

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We evaluated the prognostic value of fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography/computed tomography (PET/CT) in hepatocellular carcinoma (HCC). Their association with programmed death ligand 1 (PD-L1) expression and vascular formation was further investigated. In this retrospective study, using a database of 418 patients who had undergone ¹⁸F-FDG PET/CT before hepatic resection for HCC, immunohistochemical staining of PD-L1, clusters of differentiation (CD) 8, CD68, and CD34 was performed. Patients with a high maximum standardized uptake value (SUVmax) on ¹⁸F-FDG PET/CT showed a significantly worse recurrence-free survival (RFS) (hazard ratio [HR]: 1.500; 95% confidence interval [CI]: 1.088-2.069; $P = 0.0133$) and overall survival (OS) (HR: 2.259; 95% CI: 1.276-4.000; $P = 0.0052$) than patients with a low SUVmax. Logistic regression analysis showed that a high SUVmax in HCC was significantly associated with PD-L1-positive expression (odds ratio: 4.407; 95% CI: 2.265-8.575; $P < 0.0001$). SUVmax values of HCC were associated with intratumoral CD8-positive T-cell counts ($P = 0.0044$) and CD68-positive macrophage counts ($P = 0.0061$). Stratification based on SUVmax, PD-L1 expression, and the vessels that encapsulate tumor clusters (VETC) status was also significantly associated with RFS and OS. SUVmax, VETC, and PDL1 expression were independently predictive of survival on multivariable analysis. **Conclusion:** Our large cohort study showed that a high SUVmax on ¹⁸F-FDG PET/CT is associated with a poor clinical outcome and PD-L1 expression in patients with HCC. Additionally, stratification of patients based on the combination of SUVmax, PD-L1 expression, and the VETC status predicts poor clinical outcome. (*Hepatology Communications* 2021;5:1278-1289).

Hepatocellular carcinoma (HCC) is a common malignancy worldwide and is considered one of the most common causes of cancer-related death.⁽¹⁾ Although hepatic resection has been established as a safe and effective treatment for patients with HCC, the long-term survival rate remains unsatisfactory. The number of patients who develop recurrence remains high, even after curative

Abbreviations: ¹⁸F-FDG, fluorine-18 fluorodeoxyglucose; AFP, alpha-fetoprotein; ANG2, angiopoietin 2; BCLC, Barcelona Clinic Liver Cancer; CD, clusters of differentiation; CI, confidence interval; DCP, des-gamma-carboxyprothrombin; FDG, fluorodeoxyglucose; HCC, hepatocellular carcinoma; HCV-Ab, hepatitis C virus antibody; HR, hazard ratio; IQR, interquartile range; OS, overall survival; PD-1, programmed cell death-1; PD-L1, programmed death ligand 1; PET/CT, positron emission tomography/computed tomography; RFS, recurrence-free survival; ROC, receiver operating characteristic; SUVmax, maximum standardized uptake value; VEGF, vascular endothelial growth factor; VETC, vessels that encapsulate tumor clusters.

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hepatic resection.^(2,3) Therefore, the risk assessment of HCC recurrence is useful for treatment-method selection.

Positron emission tomography/computed tomography (PET/CT) using fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) for metabolic assessment is valuable for cancer staging and detecting the recurrence of many malignant tumors. We have previously reported that the maximum standardized uptake value (SUVmax) on ¹⁸F-FDG PET/CT is significantly correlated with microvascular invasion, poor differentiation, and immunohistochemical expression of glucose transporter 1 in HCC.^(4,5)

Immune checkpoint inhibitor targeting the programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway has become a new innovative treatment for various types of cancer.^(6,7) Cancer immunotherapy uses the patient's immune system to defeat cancer by suppressing the immune checkpoint pathway. Especially, combination therapy with immune checkpoint inhibitors for HCC is expected to be an effective treatment option and is currently attracting much attention.⁽⁸⁾ Moreover, the expression of PD-L1 protein in cancer cells is expected to be a prognostic and predictive biomarker for responses to antiPD-1/PD-L1 antibodies in HCC. We recently revealed that PD-L1 expression in cancer cells is associated with a poor clinical outcome and vascular formation in 387 patients with HCC.⁽⁹⁾ High PD-L1 expression was associated with the uptake of ¹⁸F-FDG PET/CT in several cancers.^(10,11) However, the relationship between glucose metabolism and PD-L1 protein expression in HCC is unclear.

In this translational study, we examined the prognostic impact of glucose metabolism on patients with HCC, and investigated PD-L1 expression, immune status, and the vascular formation status by

immunohistochemistry. Additionally, we evaluated the association of SUVmax on ¹⁸F-FDG PET/CT with PD-L1 expression and vascular formation.

Materials and Methods

PATIENTS

Four hundred eighteen patients with HCC who had undergone hepatic resection at the Department of Surgery and Science, Kyushu University Hospital, between January 2010 and December 2019 were enrolled in this retrospective study. The details of the surgical techniques and patient-selection criteria for hepatic resection in HCC have been previously reported.⁽¹²⁾ Fifty five patients had undergone preoperative treatments for HCC. Transcatheter arterial chemoembolization had been done in 37 of 55 patients, hepatic arterial infusion chemotherapy in 5, local ablation therapy in 10, and molecular targeted agents in 3. No patient underwent immune checkpoint inhibitor treatment for preoperative treatment. The patients were followed up as outpatients every 1 to 3 months after discharge. Dynamic CT was performed by radiologists every 3 months, and magnetic resonance imaging was performed if recurrence was suspected. The clinical information and follow-up data were obtained from medical records. This study was approved by the Ethics Committee of Kyushu University (approval codes 30-33 and 30-454).

¹⁸F-FDG PET/CT

In each patient, 185 MBq of fluorodeoxyglucose (FDG) was intravenously administered after fasting

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for at least 4 hours. Scans were conducted from the middle of the thigh to the top of the skull 60 minutes after FDG administration. FDG-PET/CT images were obtained using an integrated PET/CT scanner (Discovery STE; GE Medical Systems, Milwaukee, WI) or Biograph mCT (Siemens Medical Solutions, Erlangen, Germany). All emission scans were performed in the three-dimensional mode, and the acquisition time per bed position was 3 minutes for Discovery STE and 2 minutes for Biograph mCT. We reconstructed PET images using the ordered-subset expectation-maximization method (VUE Point Plus) with two full iterations of 28 subsets for the Discovery STE, and iterative True-X algorithm and TOF (Ultra HD-PET) with two full iterations of 21 subsets. The True-X algorithm incorporates an additional specific correction for the point-spread function. The full-width at half-maximum values of the Discovery STE and Biograph mCT were 5.2 mm and 4.4 mm, respectively. A low-dose 16-slice CT (tube voltage 120 kV; effective tube current 30-250 mA; Discovery STE) and a low-dose 32-slice CT (tube voltage 120 kV; use of angular and longitudinal dose modulation; CAREdose4D; Biograph mCT) from the vertex to the proximal thigh were performed for attenuation correction and to determine the precise anatomic location of the lesions before the acquisition of PET images. CT scans were reconstructed by filtered back projection into 512 × 512-pixel images with a slice thickness of 5 mm, to match the PET scan. FDG uptake in lesions was evaluated using SUV_{max}, calculated using a dedicated workstation for each scanner. The best cutoff values of these markers were determined by the receiver operating characteristic (ROC) curve.

IMMUNOHISTOCHEMICAL EXAMINATION

Immunohistochemistry was performed in 418 cases of surgically resected HCC using formalin-fixed tissue sections according to our PD-L1 immunohistochemistry protocol, as described previously.^(3,9) The primary antibody used was an anti-human PD-L1 rabbit monoclonal antibody (clone 28-8; dilution 1:450; Abcam, Cambridge, United Kingdom). Carcinoma cells showing membranous staining for PD-L1 were evaluated as positive cells. The proportion of PD-L1-positive cells was independently estimated as the

percentage of total carcinoma cells in whole sections by three investigators (S.I., K.Y., and K.K.). If the independent assessments did not agree, the slides were reviewed by all three investigators together, to achieve a consensus. The consensus judgments were adopted as the final results. Cases with <1% tumor membrane staining were considered negative in this study. Sections from human placentas were used as positive controls. Immunohistochemical staining was performed using anti-CD8 (C8/144B, 1:50; Dako, Glostrup, Denmark), anti-CD68 (PG-M1, 1:100; Dako), and anti-CD34 (QBEnd10, 1:50, Dako) antibodies. The numbers of central intratumoral clusters of differentiation (CD) 8-positive lymphocytes and CD68-positive macrophages with cytoplasmic or membrane staining in three high-power fields were counted, as described previously.^(9,13) The index of vessels that encapsulated tumor clusters (VETC) was calculated as reported previously.^(9,14) Briefly, five representative fields were recorded for the total number of individual tumor clusters that were surrounded by endothelium, and the average number of endothelium-coated tumor clusters per field is presented as the index of VETC. Cases with a VETC pattern in all or part of the HCC section were defined as VETC-positive, and those without a VETC pattern in a whole HCC section were defined as VETC-negative.

STATISTICAL ANALYSIS

Standard statistical analyses were used to evaluate descriptive statistics such as medians, frequencies, and percentages. Continuous variables without a normal distribution were compared using the Mann-Whitney U test. Categorical variables were compared using χ^2 test or Fisher's exact test. Survival data were used to establish a univariate Cox proportional hazards model. Covariates that were significant at $P < 0.05$ were included in the multivariate Cox proportional hazards model. Cumulative overall survival (OS) and recurrence-free survival (RFS) rates were calculated using the Kaplan-Meier method, and differences between the curves were evaluated using the log-rank test. Logistic regression analysis was performed to identify variables for SUV_{max}. Differences were considered to be significant at $P < 0.05$. All statistical analyses were performed using JMP14 software (SAS Institute Inc., Cary, NC).

Results

SUV_{max} AND CLINICOPATHOLOGICAL FACTORS

In this study comprised of 418 patients with HCC, 301 patients (72.0%) were males and 159 patients (38.0%) had diabetes mellitus. Seventy-six (18.2%) and 193 (46.1%) patients had positive hepatitis B surface antigen and hepatitis C antibody expression, respectively. The median observation period was 3.3 years (range, 0.1-10.3 years). The best cutoff values of SUV_{max} for the postoperative prognosis were determined using ROC curves (Supporting Fig. S1). The best cutoff points for the operative prognosis of SUV_{max} and area under the ROC curve of SUV_{max} were 4.0 and 0.736, respectively.

Table 1 summarizes the clinicopathological characteristics of the patients with a high or low SUV_{max}. The high SUV_{max} was higher in patients with a high alpha-fetoprotein (AFP) concentration ($P < 0.0001$), a high *des*-gamma-carboxyprothrombin (DCP) concentration ($P < 0.0001$), a large tumor size ($P < 0.0001$), a high rate of Barcelona Clinic Liver Cancer (BCLC) staging B or C ($P < 0.0001$),

poorly differentiated HCC ($P < 0.0001$), microscopic vascular invasion ($P < 0.0001$), microscopic intrahepatic metastasis ($P < 0.0001$), and a low rate of single nodular type according to the gross classification ($P < 0.0001$), and liver fibrosis ($P = 0.0004$). Patients with a high SUV_{max} showed a higher incidence of postoperative recurrence than those with a low SUV_{max} (102 of 171; 59.7% vs. 101 of 247; 40.9%; $P = 0.0002$).

SUV_{max} AND PATIENT SURVIVAL

We performed survival analysis based on SUV_{max} using the Kaplan-Meier method, which revealed that patients with a high SUV_{max} had significantly shorter RFS (log-rank $P < 0.0005$) and OS (log-rank $P < 0.0001$) than those with a low SUV_{max} (Fig. 1A,B).

We grouped the patients with HCC according to liver fibrosis. A high SUV_{max} was significantly associated with poor RFS (log-rank $P < 0.0001$) and OS (log-rank $P < 0.0001$) for patients with liver fibrosis. Regarding patients without liver fibrosis, there was a significant difference for RFS (log-rank $P < 0.0001$) and OS (log-rank $P < 0.0001$) between patients with a high and low SUV_{max} (Supporting Fig. S2).

TABLE 1. ASSOCIATION BETWEEN SUV_{max} AND PATIENT CLINICOPATHOLOGICAL FACTORS

Variable	SUV _{max} ≤ 4.0 (n = 247)	SUV _{max} > 4.0 (n = 171)	PValue
Age (years)	70 (64-76)	72 (65-78)	0.0640
Sex, male/female	174/73	127/44	0.3919
BMI (kg/m ²)	23.14 (21.18-25.64)	23.21 (21.27-25.44)	0.8711
Diabetes mellitus	86 (34.8%)	73 (42.6%)	0.1031
HBs-Ag positive	49 (19.9%)	27 (15.7%)	0.2827
HCV-Ab positive	116 (46.9%)	77 (45.0%)	0.6965
Albumin (g/dL)	4.1 (3.8-4.3)	4.1 (3.7-4.3)	0.2959
Child-Pugh classification, grade B	10 (4.1%)	5 (2.9%)	0.5433
AFP (ng/mL)	5.3 (3.2-17.7)	12.6 (4.4-51.3)	<0.0001
DCP (mAU/mL)	33 (21-101)	295 (52-1984)	<0.0001
Tumor size (cm)	2.2 (1.5-3.2)	4.0 (2.7-6.5)	<0.0001
Solitary/multiple	197/50	125/46	0.1116
BCLC staging, B or C	17 (6.9%)	49 (28.7%)	<0.0001
Gross classification, single nodular type	196 (79.4%)	96 (56.1%)	<0.0001
Poorly differentiation	31 (16.6%)	80 (46.7%)	<0.0001
Microscopic vascular invasion	24 (9.7%)	70 (40.9%)	<0.0001
Microscopic intrahepatic metastasis	24 (9.7%)	46 (26.9%)	<0.0001
F3 or F4	104 (42.1%)	43 (25.1%)	0.0004

Note: The data are presented as n (%) or median (IQR).

Abbreviations: BMI, body mass index; HBs-Ag, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody.

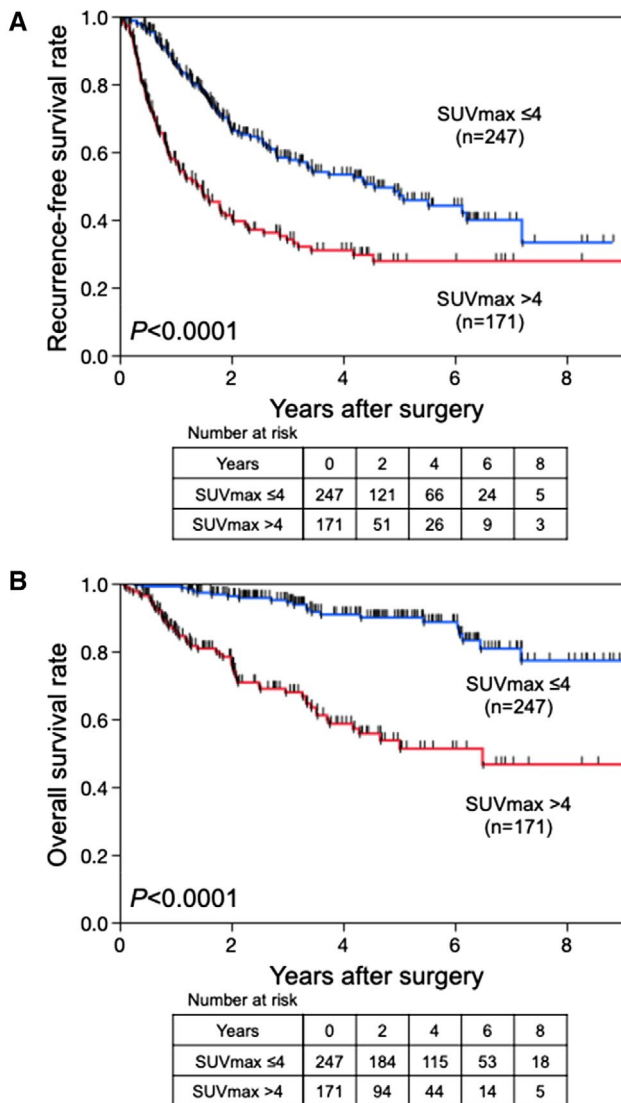


FIG. 1. Kaplan-Meier curves showing the survival of patients with HCC according to the SUVmax on ^{18}F -FDG PET/CT. (A,B) RFS (A) and OS (B) in all patients according to SUVmax between ≤ 4 and > 4 .

UNIVARIATE AND MULTIVARIATE ANALYSES OF PROGNOSTIC FACTORS FOR RFS AND OS

Table 2 lists the results of the univariate and multivariate analyses that were used to identify the factors that were significantly associated with RFS and OS after hepatic resection in patients with HCC. The multivariate analysis showed that a high SUVmax was associated with a significantly worse RFS (hazard ratio [HR]: 1.500; 95% confidence

interval [CI]: 1.088-2.069; $P = 0.0133$). Similar results were observed for OS (HR: 2.259; 95% CI: 1.276-4.000; $P = 0.0052$).

ASSOCIATION OF METABOLIC CHANGES WITH IMMUNE STATUS AND VASCULAR FORMATION

We assessed the association between immunohistochemical staining for PD-L1 and SUVmax in 418 patients with HCC. Figure 2A-D shows immunohistochemical staining for PD-L1 in HCC tissues. Among the 418 HCC tissues examined, 20.8% were from PD-L1-positive patients (87 of 418). PD-L1-positive expression in cancer cells was associated with a significantly higher SUVmax compared with PD-L1-negative expression (PD-L1-positive median, 5.1; range, 2.23-20.1; PD-L1-negative median, 3.5; range, 1.69-17.0; $P < 0.0001$; Fig. 2E). In addition, immunohistochemical staining for CD8 and CD68 was performed in our clinical population (Supporting Fig. S3A,B). The patients with HCC with a high SUVmax had lower intratumoral CD8-positive T-cell counts than those with a low SUVmax (high SUVmax median, 8; interquartile range [IQR], 5-17.7; low SUVmax median, 11.7; IQR, 7-19; $P = 0.0044$; Supporting Fig. S3C). Higher CD68-positive macrophage counts was associated with a significantly high SUVmax compared with low CD68 positive macrophage counts (high SUVmax median, 127.5; range, 71.5-154.5; low SUVmax median, 100.3; range, 66.8-136.1; $P = 0.0061$; Supporting Fig. S3D).

We next performed immunohistochemical staining for CD34 in human HCC tissue and observed two distinct vascular patterns: vessels with discrete lumens (the reported classical capillary vessels) and sinusoid-like vessels that form VETCs (Fig. 2F-I). Among the 418 HCC tissues examined, 16.9% were from VETC-positive patients (71 of 418). The presence of VETC was associated with a significantly high SUVmax compared with the absence of VETC (VETC positive median, 4.6; range, 2.45-20.1; VETC negative median, 3.58; range, 1.69-19.5; $P < 0.0001$; Fig. 2J).

We examined the association between SUVmax and characteristics, especially immune status and the VETC status. Multivariate analysis showed that a high SUVmax in HCC is significantly associated with PD-L1 expression (Table 3).

TABLE 2. UNIVARIATE AND MULTIVARIATE ANALYSES OF FACTORS RELATED TO RFS AND OS IN PATIENTS WITH HCC WHO HAD UNDERGONE HEPATIC RESECTION (COX PROPORTIONAL HAZARDS ANALYSIS)

Factors		RFS		OS	
		Univariate Analysis	Multivariate Analysis	Univariate Analysis	Multivariate Analysis
		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
		PValue	PValue	PValue	PValue
Age (years)		1.005 (0.991-1.020)		1.035 (1.010-1.061)	1.041 (1.013-1.070)
		0.4231		0.0069	0.0037
Sex	Male	1.069 (0.785-1.454)		0.827 (0.515-1.327)	
	Female	0.6704		0.4323	
Diabetes mellitus	Positive	0.866 (0.649-1.155)		0.633 (0.387-1.035)	
	Negative	0.3284		0.0687	
HBsAg	Positive	1.006 (0.708-1.428)		0.868 (0.487-1.546)	
	Negative	0.9730		0.6319	
HCV-Ab	Positive	0.981 (0.744-1.293)		1.261 (0.813-1.956)	
	Negative	0.8951		0.2997	
Albumin		0.616 (0.457-0.841)	0.726 (0.535-0.995)	0.336 (0.224-0.518)	0.566 (0.335-0.953)
		0.0019	0.0400	<0.0001	0.0326
Child-Pugh classification	B	1.549 (0.762-3.150)		4.705 (2.244-9.867)	3.653 (1.357-9.829)
	A	0.2264		<0.0001	0.0103
AFP		1.000 (1.000-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)	0.999 (0.999-1.000)
		<0.0001	0.0022	<0.0001	0.1248
DCP		1.000 (1.000-1.000)	0.999 (0.999-1.000)	1.000 (1.000-1.000)	0.999 (0.999-1.000)
		0.0034	0.7942	0.0004	0.3280
Tumor size		1.081 (1.052-1.106)	1.042 (1.000-1.087)	1.102 (1.070-1.129)	1.060 (1.008-1.115)
		<0.0001	0.0499	<0.0001	0.0209
Macroscopic tumor number	Multiple	2.161 (1.598-2.921)	1.338 (0.861-2.079)	1.817 (1.123-2.941)	0.618 (0.290-1.317)
	Single	<0.0001	0.1950	0.0149	0.2127
BCLC staging	B or C	1.732 (1.294-2.318)	1.679 (0.993-2.841)	5.574 (3.533-8.793)	2.842 (1.270-6.356)
	0 or A	0.0002	0.0531	<0.0001	0.0110
Poor differentiation	Present	1.732 (1.294-2.318)	0.990 (0.706-1.390)	3.294 (2.122-5.115)	1.279 (0.761-2.151)
	Absent	0.0002	0.9578	<0.0001	0.3523
Microscopic vascular invasion	Present	2.460 (1.816-3.331)	1.550 (1.083-2.218)	3.720 (2.386-5.797)	1.861 (1.072-3.232)
	Absent	<0.0001	0.0165	<0.0001	0.0273
Microscopic intrahepatic metastasis	Present	3.473 (2.502-4.821)	1.694 (1.085-2.644)	4.540 (2.850-7.230)	2.211 (1.186-4.122)
	Absent	<0.0001	0.0203	<0.0001	0.0125
Microscopic liver fibrosis	F3 or F4	1.127 (0.848-1.496)		1.106 (0.705-1.736)	
	F0 or F1 or F2	0.4086		0.6587	
SUVmax	>4	2.181 (1.654-2.876)	1.500 (1.088-2.069)	5.132 (3.150-8.363)	2.259 (1.276-4.000)
	≤4	<0.0001	0.0133	<0.0001	0.0052

Abbreviation: HBsAg, hepatitis B surface antigen.

COMBINATION OF SUV_{max}, PD-L1 EXPRESSION, AND VETC

Consistent with previous findings, PD-L1-positive expression influenced the outcomes for patients with HCC for both RFS (log-rank $P = 0.0013$; Supporting

Fig. S4A) and OS (log-rank $P < 0.0001$; Supporting Fig. S4B). Moreover, patients with VETC positivity showed a significantly worse prognosis than those with VETC negativity for both RFS (log-rank $P < 0.0001$; Supporting Fig. S4C) and OS (log-rank $P < 0.0001$; Supporting Fig. S4D). We evaluated the significance

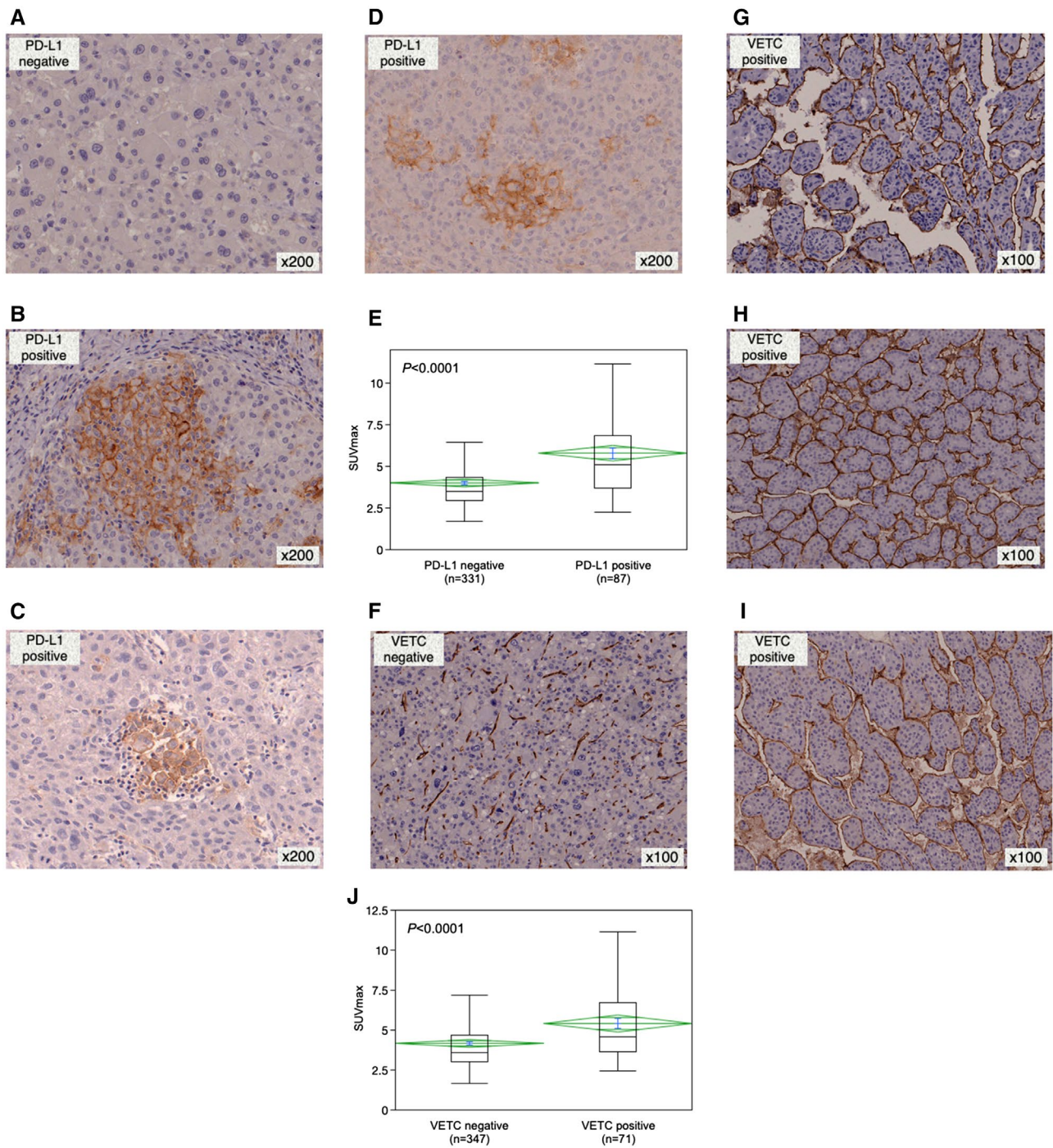


FIG. 2. Immunohistochemical staining of PD-L1 and CD34 in patients with HCC. (A) Negative staining for PD-L1. Positive membrane staining for PD-L1 in case 1 (B), case 2 (C), and case 3 (D). (E) The median SUVmax values on ^{18}F -FDG PET/CT with PD-L1-negative and PD-L1-positive expression were 3.5 (range 1.69–17.0) and 5.1 (range 2.23–20.1), respectively ($P < 0.0001$). Negative case (F) and positive cases (G–I) with VETC by CD34 staining. (J) The median SUVmax values with VETC negativity and positivity were 3.58 (range 1.69–19.5) and 4.6 (range 2.45–20.1), respectively ($P < 0.0001$).

TABLE 3. UNIVARIATE AND MULTIVARIATE ANALYSES OF HIGH SUV_{max} AND CLINICOPATHOLOGICAL FACTORS IN PATIENTS WITH HCC WHO HAD UNDERGONE HEPATIC RESECTION

Factors		Univariate Analysis	Multivariate Analysis
		Odds Ratio (95% CI)	Odds Ratio (95% CI)
		<i>P</i> Value	<i>P</i> Value
Age (years)		1.015 (0.995-1.036)	
		0.1346	
Sex	Male	1.210 (0.781-1.877)	
	Female	0.3922	
BMI		0.994 (0.941-1.051)	
		0.8554	
Diabetes mellitus	Positivity	1.394 (0.480-1.070)	
	Negativity	0.1036	
HBs-Ag	Positivity	0.753 (0.449-1.263)	
	Negativity	0.2836	
HCV-Ab	Positivity	0.925 (0.625-1.368)	
	Negativity	0.6965	
Albumin		0.738 (0.467-1.165)	
		0.1924	
AFP		1.000 (1.000-1.000)	0.999 (0.999-1.000)
		0.0021	0.8926
DCP		1.000 (1.000-1.000)	0.999 (0.999-1.000)
		0.0003	0.1639
Tumor size		1.401 (1.269-1.546)	1.294 (1.141-1.468)
		<0.0001	<0.0001
Macroscopic tumor number	Multiple	1.449 (0.916-2.294)	
	Single	0.1126	
BCLC staging	B or C	5.433 (3.000-9.839)	2.020 (0.829-4.921)
	O or A	<0.0001	0.1214
Poor differentiation	Present	4.417 (2.816- 6.927)	2.586 (1.473-4.539)
	Absent	<0.0001	0.0009
Microscopic vascular invasion	Present	6.439 (3.829-10.82)	3.007 (1.544-5.858)
	Absent	<0.0001	0.0012
Microscopic intrahepatic metastasis	Present	3.419 (1.992-5.867)	1.416 (0.618-3.219)
	Absent	<0.0001	0.4102
Microscopic liver fibrosis	F3 or F4	0.461 (0.301-0.708)	0.535 (0.310-0.924)
	F0 or F1 or F2	0.0004	0.0249
PD-L1 expression	Positivity	5.420 (3.211-9.146)	4.407 (2.265-8.575)
	Negativity	<0.0001	<0.0001
Intratumoral CD8-positive T-cell counts		0.997 (0.985-1.009)	
		<0.0001	
CD68-positive macrophage counts		1.006 (1.002-1.010)	0.996 (0.990-1.0002)
		0.0037	0.2502
VETC	Positivity	3.267 (1.915-5.574)	1.391 (0.694-2.785)
	negativity	<0.0001	0.3514

Abbreviations: BMI, body mass index; HBs-Ag, hepatitis B surface antigen.

of SUVmax stratified by PD-L1 expression and the VETC status in HCC samples. The patients were divided into the following four groups: group 1, low SUVmax group (n = 247); group 2, high SUVmax/PD-L1-negative/VETC negative (n = 86); group 3, high SUVmax/PD-L1-positive or VETC positive (n = 69); and group 4, high SUVmax/PD-L1-positive/VETC-positive (n = 24). We found that RFS and OS were significantly different among the four groups, excluding analysis between groups 3 and 4 in terms of RFS

(Fig. 3A,B). Moreover, multivariate analysis for RFS and OS using the combination of SUVmax, PD-L1 expression and the VETC status, and significant factors of univariate analysis indicated a significant association between the number of these described predictors and poor outcome for both RFS (high SUVmax/PD-L1-negative/VETC-negative HR, 1.306; 95% CI, 0.884-1.915; *P* = 0.1812; high SUVmax/PD-L1-positive or VETC-positive HR, 1.655; 95% CI, 1.098-2.493; *P* = 0.0159; high SUVmax/PD-L1-positive/

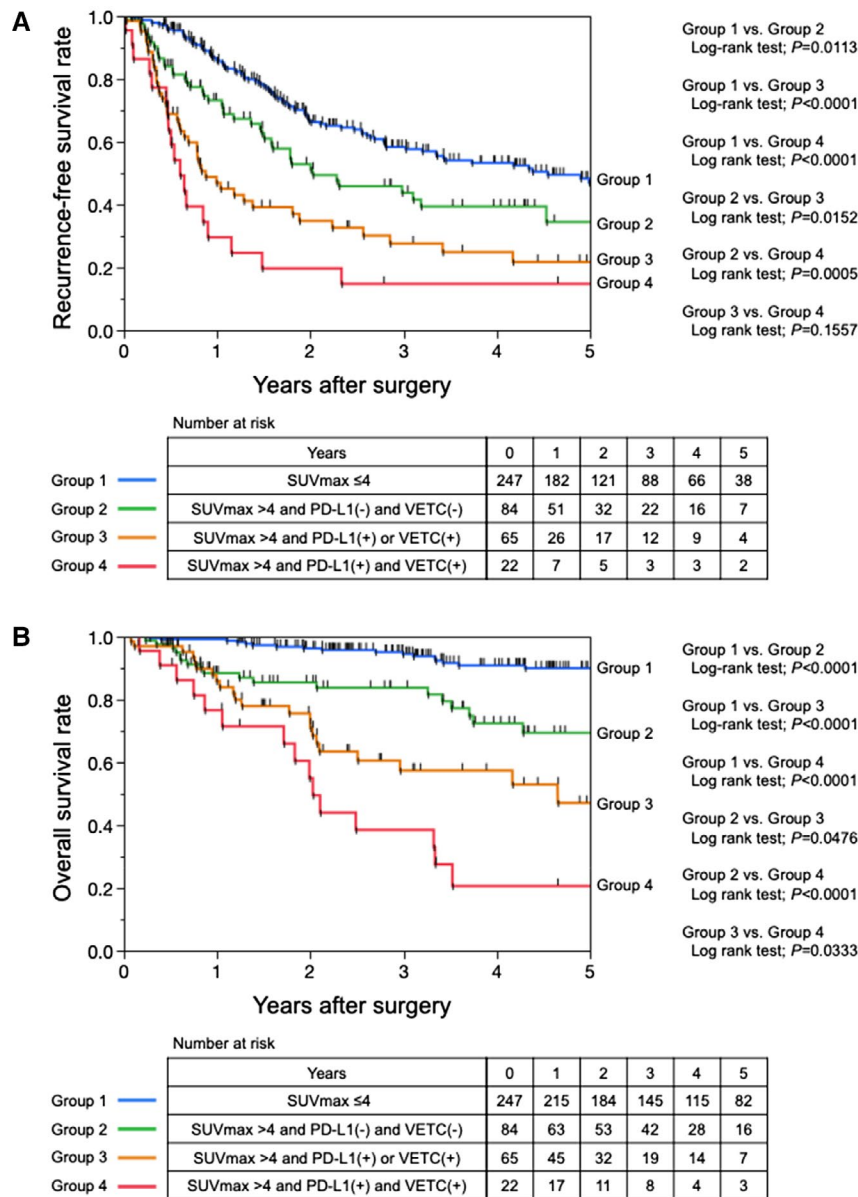


FIG. 3. Kaplan-Meier curves for RFS (A) and OS (B) in patients with HCC according to the SUVmax on ¹⁸F-FDG PET/CT, PD-L1 expression, and VETC status.

TABLE 4. MULTIVARIATE ANALYSES OF FACTORS RELATED TO RFS IN PATIENTS WITH HCC WHO HAD UNDERGONE HEPATIC RESECTION (COX PROPORTIONAL HAZARDS ANALYSIS)

Variable	HR	95% CI	PValue
Albumin	0.718	0.529-0.975	0.0338
AFP	1.000	1.000-1.000	0.0009
DCP	0.999	0.999-1.000	0.7621
Tumor size	1.043	1.000-1.087	0.0472
Multiple tumors	1.355	0.873-2.105	0.1752
BCLC staging, B or C	1.566	0.919-2.670	0.0990
Poor differentiation	0.939	0.665-1.328	0.7252
Microscopic vascular invasion	1.476	1.021-2.133	0.0384
Microscopic intrahepatic metastasis	1.691	1.080-2.648	0.0215
SUVmax ≤ 4/PD-L1-negative/ VETC-negative	Ref	Ref	Ref
SUVmax > 4/PD-L1-negative/ VETC-negative	1.306	0.884-1.915	0.1812
SUVmax > 4/PD-L1-positive or VETC-positive	1.655	1.098-2.493	0.0159
SUVmax > 4/PD-L1-positive and VETC-positive	2.578	1.399-4.751	0.0024

TABLE 5. MULTIVARIATE ANALYSES OF FACTORS RELATED TO OS IN PATIENTS WITH HCC WHO HAD UNDERGONE HEPATIC RESECTION (COX PROPORTIONAL HAZARDS ANALYSIS)

Variable	HR	95% CI	PValue
Age	1.046	1.017-1.076	0.0016
Albumin	0.602	0.346-1.044	0.0712
Child Pugh classification, grade B	4.433	1.572-12.49	0.0049
AFP	1.000	1.000-1.000	0.0312
DCP	0.999	0.999-1.000	0.3328
Tumor size	1.058	1.003-1.116	0.0367
Multiple tumors	0.696	0.330-1.467	0.3416
BCLC staging, B or C	2.438	1.072-5.546	0.0335
Poor differentiation	1.166	0.647-1.925	0.6916
Microscopic vascular invasion	1.750	0.988-3.102	0.0550
Microscopic intrahepatic metastasis	2.299	1.214-4.354	0.0106
SUVmax ≤ 4/PD-L1-negative/ VETC-negative	Ref	Ref	Ref
SUVmax > 4/PD-L1-negative/ VETC-negative	2.000	1.045-3.830	0.0365
SUVmax > 4/PD-L1-positive or VETC-positive	2.215	1.089-4.513	0.0280
SUVmax > 4/PD-L1-positive and VETC-positive	5.841	2.517-13.55	<0.0001

VETC-positive HR, 2.578; 95% CI, 1.399-4.751; $P = 0.0024$) and OS (high SUVmax/PD-L1-negative/VETC-negative HR, 2.000; 95% CI, 1.045-3.830; $P = 0.0365$; high SUVmax/PD-L1-positive or

VETC-positive HR, 2.215; 95% CI, 1.089-4.513; $P = 0.0280$; high SUVmax/PD-L1-positive/VETC-positive HR, 5.841; 95% CI, 2.517-13.55; $P < 0.0001$) after hepatic resection (Tables 4 and 5).

The associations between high SUVmax/PD-L1-positive/VETC-positive and patient clinicopathological characteristics are found in Supporting Table S1. The high SUVmax/PD-L1-positive/VETC-positive patients showed high AFP concentration ($P < 0.0001$), high DCP concentration ($P = 0.0002$), large tumor size ($P = 0.0007$), high rate of BCLC staging B or C ($P = 0.0001$), poorly differentiated HCC ($P = 0.0002$), microscopic vascular invasion ($P < 0.0001$) and microscopic intrahepatic metastasis ($P = 0.0113$), and low rate of single nodular type ($P = 0.0104$).

Discussion

In the present study, we investigated the prognostic impact of SUVmax, PD-L1 expression, VETC, and their combined effect in a large group of patients with HCC who had undergone hepatic resection. We showed that a high SUVmax in HCC is associated with a poor prognosis and PD-L1 expression, and we stratified the patient prognosis based on SUVmax, PD-L1 expression, and VETC status.

Several studies have investigated the effect of ^{18}F -FDG PET/CT on long-term outcome after hepatic resection in HCC. Han et al. reported that an SUVmax of ^{18}F -FDG PET/CT > 3.5 is a predictive factor for the histological grade of the tumor, recurrence, and survival in 217 patients with HCC.⁽¹⁵⁾ Yoh et al. showed that FDG uptake is a prognostic factor for survival in 207 patients with solitary HCC.⁽¹⁶⁾ As reported recently, ^{18}F -FDG PET/CT predicts microvascular invasion and early recurrence after liver resection for 78 patients with HCC under a prospective observational study.⁽¹⁷⁾ In the present study, we examine the clinical impact of assessing ^{18}F -FDG PET/CT on the outcome for patients with HCC using large sample sizes of more than 400 cases.

Our data revealed that PD-L1-positive expression in cancer cells is associated with a high SUVmax. Glucose metabolism of cancer cells measured on ^{18}F -FDG PET/CT is a significant biomarker for the metabolic characteristics of cancer cells and correlates

with important features such as proliferation, histologic type, tumor differentiation, and hypoxia.^(4,5,17) Recently, an association was found between the metabolic information of FDG-PET and tissue expression of PD-L1 in patients with several cancers.^(10,11) We hypothesize that a positive correlation between the uptake of FDG and PD-L1 expression might be found in patients with HCC. The present study shows a statistically significant association between the metabolic imaging parameter SUVmax and PD-L1 expression in cancer cells for HCC following hepatic resection. PD-L1-positive HCC demonstrated a high SUVmax, suggesting that PD-L1 expression is related to malignant features with high glucose metabolism.

Although ¹⁸F-FDG PET/CT may be useful to evaluate PD-L1 expression in patients with HCC, the mechanism of the association between FDG uptake and PD-L1 protein expression remains unclear. High glucose metabolism causes the accumulation of lactate in the tumor microenvironment. Fisher et al. revealed that lactate in the tumor environment suppresses the function of cytotoxic T lymphocytes.⁽¹⁸⁾ Feng et al. demonstrated that tumor cell-derived lactate induces TAZ-dependent up-regulation of PD-L1 through GPR81 in lung cancer cells.⁽¹⁹⁾ However, this phenomenon and its mechanism should be investigated and validated in future studies using HCC cells.

In the current study, we observed that HCC with a high SUVmax is significantly correlated with VETC positivity. Fan et al. demonstrated that the VETC pattern has a profound effect on HCC metastasis.⁽¹⁴⁾ Moreover, angiopoietin 2 (ANG2) is a fundamental molecule for VETC formation, and its levels were significantly increased in HCC cells in that study. ANG2 knockdown in HCC cells suppressed VETC formation and reduced the metastasis of HCC xenografts. Vascular endothelial growth factor (VEGF) is a master switch for the angiogenic cascade, whereas ANG2 controls the later events of angiogenesis such as vessel assembly, maturation, and quiescence. Regarding metabolic activity, an inverse correlation was found between glucose metabolism and angiogenesis, as evaluated by the protein expression level of VEGF and tumor microvessel density.⁽²⁰⁾ VETC was associated with ANG2, not VEGF, suggesting that the metabolic activity might contribute to VETC formation in HCC tissues. Therefore, the role of metabolic change in vascular formation requires further investigation.

The scoring systems using the uptake of FDG to predict recurrence and survival for HCC treatments have been assessed. Han et al. established a scoring system to predict the prognosis after surgery using AFP, the SUVmax of ¹⁸F-FDG PET/CT, and the enhanced ratio from magnetic resonance imaging.⁽¹⁵⁾ Rhee et al. showed a risk stratification for locally advanced HCC using AFP and the SUVmax of ¹⁸F-FDG PET/CT.⁽²¹⁾ Our study revealed that four groups using the SUVmax of ¹⁸F-FDG PET/CT, PD-L1 expression, and the VETC status expressed different prognostic features. Therefore, our model may have implications in terms of recurrence and future treatment planning in postoperative adjuvant therapy.

The current study has several limitations. First, it was a single-center retrospective study. Second, certain biases in patient selection could not be ruled out. A prospective validated study is required to confirm the results of the current study.

In summary, our large cohort study showed that the SUVmax of ¹⁸F-FDG PET/CT is correlated with clinical outcome and PD-L1 expression in patients with HCC. Additionally, we found that the four subgroups that were defined based on the SUVmax of ¹⁸F-FDG PET/CT, PD-L1 expression, and VETC status had diverse prognostic features.

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Supporting Information

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