Impact of vascular endothelial growth factor (VEGF) on predictor of poor response and survival in advanced HCC patients with HAIC

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ABSTRACT

Background: Advanced hepatocellular carcinoma (HCC) with macroscopic vascular invasion (MVI) still has a poor prognosis. The aim of this study was to investigate the usefulness of hepatic arterial infusion chemotherapy (HAIC) by determining the predictors for response and survival in HCC patients undergoing HAIC.

Methods: Seventy-one patients with advanced HCC underwent HAIC through a subcutaneously implanted infusion port. One chemotherapy course consisted of low-dose cisplatin (10 mg/body on days 1-5) and 5-fluorouracil (250 mg/body on days 1-5) (FP) and 1 treatment cycle consisted of 2 to 3 courses of chemotherapy. Serum vascular endothelial growth factor (VEGF) levels were measured by the Bio-Plex Suspension Array System.

Results: The median survival time (MST) of all patients was 10.2 months, and the 1-, 2-, 3-, and 5-year survival rates were 46.5%, 21.9%, 12.8%, and 3.7%, respectively. Of the 71 patients, 3 and 22 patients achieved a complete response (CR) or a partial response (PR), respectively (response rate [CR+PR/71] = 35%). The serum VEGF level (≥ 100 pg/mL, $P = 0.026$) was an independent predictor of therapeutic effect. Serum VEGF levels were positively correlated with platelet count ($r = 0.569$, $P < 0.001$) and tumor size ($r = 0.543$, $P < 0.001$). Child-Pugh class ($P = 0.046$), serum VEGF level ($P = 0.004$), and therapeutic effect ($P = 0.005$) were identified by multivariate analysis as independent predictors of survival.

Conclusions: HAIC is a useful and safe therapeutic option in advanced HCC patients with especially low serum VEGF level and Child-Pugh class A.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Recent advanced imaging procedures have led to increased detection of early-stage of HCC and improved survival, because curative therapies, such as hepatic resection, liver transplantation, and radiofrequency ablation, are possible in early-stage patients.[1] However, long-term survival is still unsatisfactory because of high recurrence rates, even after these curative therapies.[2] The development of advanced HCC with macroscopic vascular invasion (MVI) especially hinders the use of additional curative therapies, and therefore contributes to poor survival. MVI, including presence of a tumor thrombus in the major portal vein, is known to be the most important negative risk factor impacting survival after resection or liver transplantation for patients with HCC.[3, 4] The median survival time of HCC patients with MVI has been reported to be 2 to 3 months.[5, 6]

Angiogenesis plays an important role in HCC proliferation, invasion, and metastasis. Tumor aggressiveness is mediated by angiogenic factors such as vascular endothelial growth factor (VEGF), one of the most important factors.[7] Recently, sorafenib, an oral multikinase inhibitor, has become available as a new molecular-targeted therapy for advanced HCC. Sorafenib has been shown to suppress tumor growth and angiogenesis by inhibiting the Raf/MEK/ERK signaling pathway and by inhibiting receptor tyrosine kinases such as vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3, and platelet-derived growth factor receptor-beta (PDGFR-beta).[8] In 2 randomized phase III placebo-controlled trials, overall survival was significantly longer in the

sorafenib-treated groups than in the placebo-treated groups.[9, 10] Consequently, sorafenib has become the standard treatment for advanced HCC in the United States, Europe, and many other countries.[11] However, patients with Child-Pugh class B or low platelet counts (less than 60 $\times 10^9$/L) were not included in these studies; therefore, the survival benefit of sorafenib is still not known for these patients.[10-12]

Before sorafenib regulatory approval, hepatic arterial infusion chemotherapy (HAIC) via an implanted port system had been reported to be a useful therapeutic modality for patients with advanced HCC, especially for those with a major portal vein tumor thrombus.[13-17] Various chemotherapeutic regimens were used for HAIC, although the combination of cisplatin and 5-fluorouracil (5-FU) is one of the most common regimens.[14-17] Ando et al first reported that repeated HAIC using low-dose cisplatin and 5-fluorouracil (FP) was useful for patients with advanced hepatocellular carcinoma and tumor thrombus in the portal vein.[13, 14] Several other studies have also demonstrated the usefulness of HAIC using low-dose FP.[18-20] However, these studies did not clearly determine the prognostic indicators of therapeutic effect and had a negative impact on patient quality of life (QOL), because of the long duration of therapy and hospitalization (about 4 consecutive weeks). In this study, we determined the predictors of therapeutic effect and survival in patients with advanced HCC and MVI who underwent short-course HAIC (2 to 3 consecutive weeks) using low-dose FP. In addition, we also examined the usefulness of HAIC in advanced HCC patients with Child-Pugh class B and low platelet counts.

MATERIALS AND METHODS

Patients

HCC and vascular invasion were diagnosed using a combination of contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US), and digital subtraction angiography (DSA), in addition to determination of alpha-feto-protein (AFP) and des-gamma-carboxy prothrombin (DCP) serum levels within 1 month before treatment. Hepatic functional reserve was evaluated before treatment using the Child-Pugh scoring system. From June 1996 to May 2003, 124 consecutive HCC patients with tumor thrombus in the portal vein were referred to Kurume University School of Medicine. Of these 124 patients, 92 patients underwent HAIC; 14 patients received other treatments, including hepatic resection, transcatheter arterial infusion chemoembolization (TACE), and systemic chemotherapy; and 18 patients received best supportive care. Eligibility criteria for this study were as follows: 1) Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, 2) Child-Pugh class A or B, 3) serum bilirubin level < 3.0 mg/dL, 4) serum creatinine level ＜ 1.5 mg/dL, 5) no extrahepatic metastasis, and 6) patients not previously treated with low-dose FP. Among the 92 patients undergoing HAIC, 71 patients met these criteria and were enrolled in the study. The study protocol was approved by the institutional review board, and informed consent for participation in the study was obtained from each subject and conformed to the guidelines of the 1975 Declaration of Helsinki.

Response Assessment

To determine therapeutic effect, baseline tumor measurements were determined within 1 month before treatment by combining the largest diameters of selected target lesions in each patient as measured by CT or MRI. Four to 6 weeks after the initial treatment cycle and every 2 to 3 months thereafter, CT or MRI was performed. Therapeutic effect was determined according to the best overall response, which was defined by the Response Evaluation Criteria in Solid Tumors (RECIST) as follows: complete response (CR), all measurable lesions disappeared for more than 4 weeks; partial response (PR), the sum of the largest target lesion diameters decreased more than 30% and there was no development of a new lesion for more than 4 weeks; progressive disease (PD), the sum of the largest diameters increased more than 25% or a new lesion appeared; and stable disease (SD), neither PR nor PD was seen for more than 8 weeks.[21]

Implantation of Arterial Catheter

An indwelling intra-arterial catheter (Anthron P-U Catheter, Toray Medical, Tokyo, JAPAN) was inserted through the femoral or brachial artery, with the distal end of the catheter extended into the hepatic artery or gastroduodenal artery, and the proximal end connected to the port system (P-U Celsite Port, Toray Medical), which was implanted subcutaneously. The right gastric, gastroduodenal and posterior superior pancreaticoduodenal arteries were embolized to prevent gastroduodenal ulcers caused by anticancer drugs.

Therapeutic HAIC Regimen

Chemotherapeutic agents were administered via a mechanical portable infusion pump. One course of

chemotherapy consisted of daily administration of cisplatin (Nippon Kayaku, Tokyo, Japan) (10 mg/body, for 30 minutes, on days 1-5) followed by 5-FU (Kyowa Hakko Kogyo, Tokyo, Japan) (250 mg/body, for 3 hours, on days 1-5). Days 6 and 7 were rest days. In principle, one cycle of treatment consisted of 2 to 3 weekly courses of HAIC (completion of protocol) and was performed on admission. This was followed by continuous HAIC chemotherapy with cisplatin (20 mg/body) and 5-FU (250 mg/body), repeated every 2 weeks at the outpatient clinic. Treatments were discontinued for the occurrence of grade 3 or higher adverse effects according to ECOG classification[22] with the exception of total bilirubin, platelet counts, and leukocyte counts of >3.0 mg/dL, < 25 $\times 10^9$/L, and < 1500/mm^3, respectively. Patients received 1.0–5.0 (median, 3.0) chemotherapy courses. More than 2 consecutive HAIC courses were delivered in 66 patients (completion of protocol). In 5 patients (including 4 patients with Child-Pugh class B), HAIC was stopped after less than 2 consecutive courses (protocol not completed).

Serum VEGF measurements

Fasting morning blood samples were obtained from all subjects and stored at -20˚C until analysis. Blood samples were collected before HCC therapy was initiated. Serum VEGF levels were measured using the Bio-Plex Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical Analysis

Mean values ± standard deviations were determined for measured variables. Comparisons between 2 groups of patients, those achieving CR or PR and those with SD or PD, were performed using the

Mann-Whitney *U* test for continuous variables, and the chi-square test for discrete variables. Multiple logistic regression analysis was used to identify factors associated with therapeutic effect and serum VEGF levels. Pearson correlation coefficients were calculated to examine the association of serum VEGF levels with platelet count and tumor size. Kaplan-Meier survival curves were compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were used to identify any independent variables that were related to survival. Data are reported as hazard ratios (HRs) and 95% confidence intervals (95% CIs). *P*-values < 0.05 were considered to be statistically significant. Statistical analysis was performed by SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient Characteristics

Table 1 summarizes the clinical profiles of the 71 HCC patients enrolled in this study. There were 56 male and 15 female patients, with a mean age of 64 years (range, 44-85). With respect to viral markers, 42 patients were positive for hepatitis C virus (HCV) antibody, and 19 patients were positive for hepatitis B surface antigen. There were 43 and 26 patients with Child-Pugh class A and B, respectively. The mean maximum tumor size was 95 mm (range, 24-217). There were 22 patients with portal vein tumor thrombus of the main trunk, and 15 patients with hepatic vein tumor thrombus.

Therapeutic Effect and Predictors of Response

Of the 71 patients, 3 (4%) patients achieved CR, 22 (31%) patients achieved PR, 25 (35%) patients had SD, and 21 (30%) patients had PD; the response rate (CR+PR/71) was 35%. Characteristics of the 2 patient groups are shown in Table 2. The mean VEGF level in patients with response to therapy (97.2 ± 198.8 pg/ml) was significantly lower compared to the mean level in patients without response to therapy (143.7 ± 149.5 pg/ml) ($P = 0.014$). There were no significant differences between other patient and tumor characteristics. In addition, multiple logistic regression analysis identified the serum VEGF level (≥ 100 pg/mL, HR: 4.77, 95% CI: 1.21-18.90, $P = 0.026$) as an independent predictor of therapeutic effect.

Serum VEGF Levels, Platelet Count, and Tumor Size

Multiple logistic regression analysis was performed to identify which variables were independently associated with serum VEGF levels (Table 3). The platelet count (≥ 120 × 10^9/L, HR: 9.85, 95% CI: 2.38-40.77, $P = 0.002$), tumor size (≥ 100mm, HR: 4.60, 95% CI: 1.13-19.01, $P = 0.034$), and tumor location (bilobular, HR: 8.01, 95% CI: 1.28-50.30, $P = 0.026$) were independent predictors of serum VEGF levels. Serum VEGF levels were positively correlated with platelet count ($r = 0.569$, $P < 0.001$ [Figure. 1A]) and tumor size ($r = 0.543$, $P < 0.001$ [Figure. 1B]) by Pearson correlation calculations.

Survival and Predictors of Outcome

The cumulative survival curve of 71 patients is shown in Figure 2. The median survival time (MST) of these patients was 10.2 months. The 1-, 2-, 3-, and 5-year survival rates were 46.5%, 21.9%, 12.8%, and 3.7%, respectively. Cox proportional hazards regression analysis was performed to identify independent predictors of survival (Table 4). The results of univariate analysis showed that Child-Pugh class B ($P = 0.002$), platelet count (≥ 120 × 10^9/L, $P = 0.006$), serum VEGF levels (≥ 100 pg/mL, $P < 0.001$), tumor size (> 100 mm, $P = 0.002$), tumor location (bilobular, $P = 0.023$), portal vein invasion (main trunk, $P < 0.001$) and therapeutic effect (SD + PD, $P < 0.001$) were found to be significant risk factors adversely affecting survival. By multivariate analysis, Child-Pugh class B (HR: 1.81, 95% CI: 1.01-3.25, $P = 0.046$), serum VEGF level (≥ 100 pg/mL; HR: 2.42, 95% CI: 1.33-4.38, $P = 0.004$), and therapeutic effect (SD + PD, HR: 2.46, 95% CI: 1.31-4.62, $P = 0.005$) were identified as independent predictors of survival. Cumulative survival curves plotted for

therapeutic effects shown in Figure 3. Cumulative survival curves plotted for serum VEGF level, Child-Pugh class, platelet counts, and tumor size are shown in Figure 4.

Adverse Reactions and Complications

Twenty-two of 71 patients (31%) developed more than 1 adverse reaction or complication. The most common adverse reaction was mild liver dysfunction and gastrointestinal symptoms, which were primarily controlled by medical treatment and/or suspension of HAIC. Two patients with Child-Pugh class B developed liver dysfunction thought to be HCC progression or treatment-related toxicity, and HAIC was stopped. Brief and reversible grade 3 or 4 leukocytopenia developed in 9 (8%) patients. The development of thrombocytopenia or anemia was infrequent, and if it did occur, was mild. None of the patients with platelet counts less than 60 $\times 10^9$/L platelet needed to have HAIC discontinued because of thrombocytopenia.

The complications that occurred were associated mainly with implantation of the infusion catheter. Infection of the port system occurred in 5 patients. These infections were controlled by antibiotics and conservative care, but there were 2 patients with Child-Pugh class B who discontinued HAIC. Occlusion of the hepatic artery, which interfered with HAIC and caused its termination, occurred in 1 patient.

DISCUSSION

In the current study, the response rate and MST of patients undergoing HAIC with low-dose FP were 35% and 10.2 months, respectively. Ando et al and Lai et al reported similar response rates of 48% and 33%, respectively, and MSTs of 10.2 months and 9.5 months, respectively, from their HAIC with low-dose FP studies.[14, 20] In addition, our data has shown that short-course HAIC with low-dose FP provided improvement in patient QOL. Results from the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) study, also demonstrated a MST of 10.7 months in advanced HCC patients.[9] Moreover, in sub-analysis of SHARP study, MST of advanced HCC patients with MVI was 8.2 months in the sorafenib group. Thus, our data also indicated that efficacy of HAIC may be equal or better compared to sorafenib in advanced HCC patients with MVI.

We determined the predictive factors associated with the therapeutic effect of HAIC. By multiple logistic regression analysis, the serum VEGF level was found to be an only independent predictor of therapeutic effect. VEGF is known to be the most important factor in tumor angiogenesis.[7] Recently, a high VEGF level in patients with various cancers, including hepatocellular carcinoma, was reported to be an independent marker for predicting poor response to chemotherapy.[23-26] Regarding the mechanism for the association of serum VEGF levels to chemotherapy response, several studies have suggested that tumor vasculature provides a relatively inefficient blood supply that differs from normal vascular networks and is secondary to VEGF.[27, 28] Therefore, increases in this abnormal tumor vascular network may lead to chemotherapy resistance.

Jain reported that VEGF-targeted therapy can normalize tumor vasculature.[27] Normalization of the tumor network can theoretically lead to increased delivery of chemotherapy. Therefore, our study results suggest that the response to HAIC may be improved by combining it with anti-VEGF therapy using sorafenib treatment of advanced HCC patient subgroup with high serum VEGF level.

By multiple logistic regression analysis, platelet count and tumor size were found to be independent predictors of serum VEGF levels. In addition, we demonstrated that serum VEGF levels were positively correlated with platelet count and tumor size (Fig. 1). Several previous studies have shown that the degree of serum VEGF elevation was positively correlated with tumor size and HCC tumor stage.[29, 30] Poon et al demonstrated that there was significantly higher VEGF mRNA expression in tumor than in normal liver tissue. There was a significant correlation of VEGF mRNA expression with VEGF protein expression in tumor. Both tumor cytosolic VEGF protein and VEGF mRNA increased significantly with advancing tumor stage.[31] Platelets contain several angiogenic growth factors affecting such processes as wound healing and tumor growth, which are released on activation. VEGF is stored in large quantities in platelet α-granules.[32] Previous studies have reported that serum VEGF levels were significantly elevated and correlated with platelet counts in HCC patients.[26, 31] Poon et al demonstrated that when corrected for platelet count, the amount of serum VEGF per platelet indicated platelet release of VEGF, and was significantly correlated with tumor VEGF protein level. Increased serum VEGF level per platelet and serum VEGF levels were also associated with advancing tumor stage.[31] These results suggest that VEGF released from tumor cells

is stored and transported by platelets in the blood stream, and that this VEGF reservoir may have a role in tumor angiogenesis and progression. Moreover, these reports support our results that indicate that platelet count and tumor size are simple and useful markers for predicting patient subgroup with high serum VEGF level.

In the current study, multivariate analysis demonstrated that the therapeutic effect of HAIC was the independent prognostic factor. Several studies have also reported that therapeutic effect was the significant prognostic factor in patients with advanced HCC who were treated with HAIC.[14, 20] As the reason, these results demonstrated that the short-term reduction or disappearance of intrahepatic tumor, including MVI, and/or continuation of this state, is the main factor related to prolonged survival of patients treated with HAIC. Moreover, the VEGF level was also independent prognostic factor on multivariate analysis in the current study. This result suggests that high serum VEGF level lead to poor response to HAIC, active angiogenesis, and rapidly progressive disease, resulting in poor survival for these patients. Therefore, our data suggests that the combination of HAIC and sorafenib may be also useful in improving the survival of advanced HCC patient subgroup with high serum VEGF level.

In previous studies, no safety data have been reported for advanced HCC patients treated with sorafenib who have platelet counts of less than $60 \times 10^9/L$[9, 10]. The advantage of HAIC over systemic chemotherapy is that in HAIC, chemotherapeutic agents can be delivered to the liver at high concentrations with lower systemic toxicity.[33] Indeed, in our current study there were no patients

with platelet counts of less than 60 × 10^9/L who needed discontinuation of HAIC because of thrombocytopenia. Moreover, in our current study, patients with low platelet counts achieved significantly longer survival times than patients with high platelet counts, with a MST of 19.6 months for patients with platelet counts of less than 60 × 10^9/L. Therefore, HAIC may be a useful therapeutic modality, especially in advanced HCC patients with low platelet counts who cannot receive sorafenib and low serum VEGF level is predicted.

In the SHARP study, the survival benefit of sorafenib was restricted to patients with Child-Pugh class A.[11] Pinter et al showed that sorafenib treatment of patients with unresectable HCC resulted in significantly shorter survival in patients with Child-Pugh class B than in patients with Child-Pugh class A.[12] Similarly, in this current study of advanced HCC patients treated with HAIC, the Child-Pugh classification was an independent prognostic factor. Hepatic reserve is important for hepatic extraction and metabolism of HAIC agents. In this study, liver dysfunction necessitating suspension or discontinuation of HAIC more frequently occurred in patients with Child-Pugh class B than in patients with Child-Pugh class A; therefore we presume that liver function is an important predictor of survival.

In conclusion, HAIC with low-dose FP is a useful and safe therapeutic option in advanced HCC patients with especially low serum VEGF level and Child-Pugh class A. Moreover, our results that indicate that platelet count and tumor size are simple and useful markers for predicting serum VEGF level. Based on these results, we hope that a randomized controlled trial will further evaluate the

benefits of treatments using HAIC alone or the combination of HAIC with sorafenib.

Figure Legends

Figure 1. Correlation between serum VEGF level and platelet count ($r = 0.569$, $P < 0.001$) (A). Correlation between serum VEGF level and tumor size ($r = 0.543$, $P < 0.001$) (B).

Figure 2. Cumulative survival of 71 hepatocellular carcinoma patients with macroscopic vascular invasion treated with hepatic arterial infusion chemotherapy. The median survival time (MST) of these patients was 10.2 months. The 1-, 2-, 3-, and 5-year survival rates were 46.5%, 21.9%, 12.8%, and 3.7%, respectively.

Figure 3. Cumulative survival of patients by therapeutic effect. The median survival times (MSTs) of responders (complete response [CR]) + partial response [PR]) and nonresponders (stable disease [SD] + progressive disease [PD]) were 21.7 and 7.2 months, respectively. (P = 0.0001) (A). Cumulative survival of patients by serum VEGF level. The MSTs of patients with serum VEGF level less than 100 pg/mL and equal to or greater than 100 pg/mL were 16.8 months and 5.8 months, respectively. (P = 0.0002) (B). Cumulative survival of patients by Child-Pugh class. The MSTs of patients with Child-Pugh class A and B cirrhosis were 16.4 months and 7.4 months, respectively. (P = 0.0014) (C). Cumulative survival of patients by platelet counts. The MST of groups 1, 2, and 3 were 19.6 months, 15.9 months, and 6.9 months, respectively. The cumulative survival of group 3 was significantly shorter than the survival of group 1 (P = 0.027) and 2 (P = 0.023) (D).

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Fig. 1

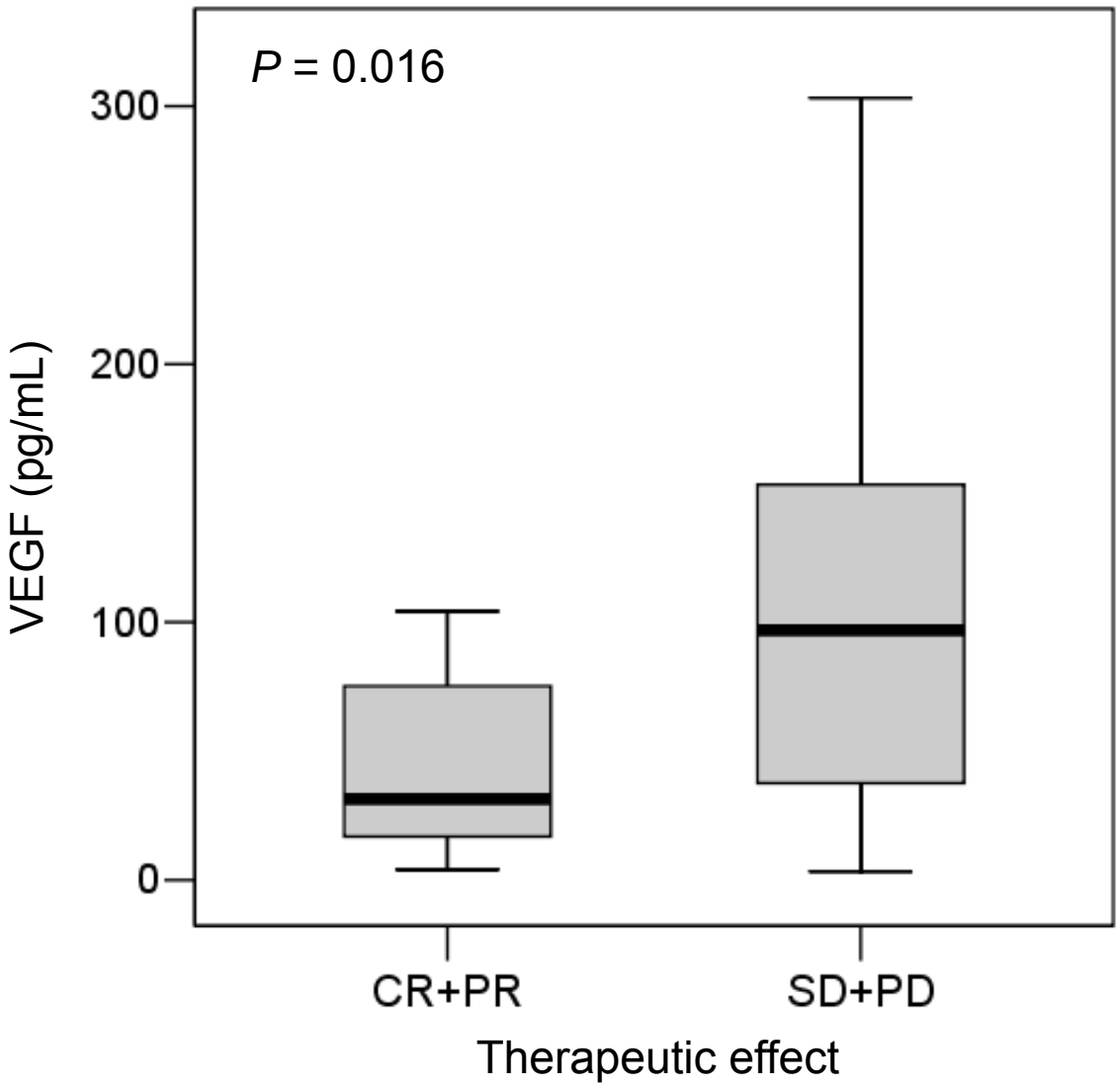
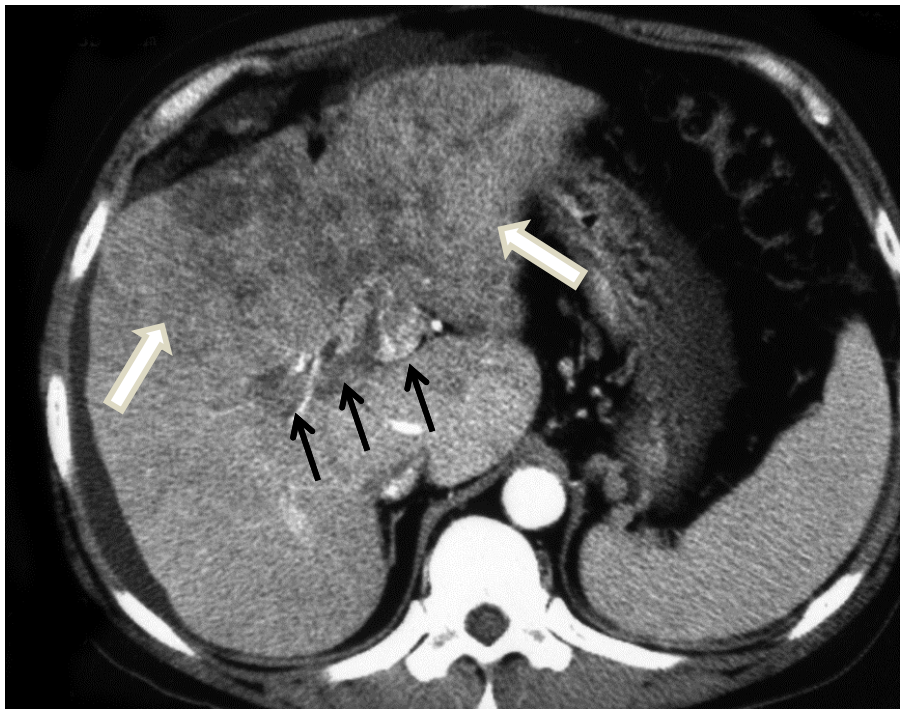


Fig. 2

(a)



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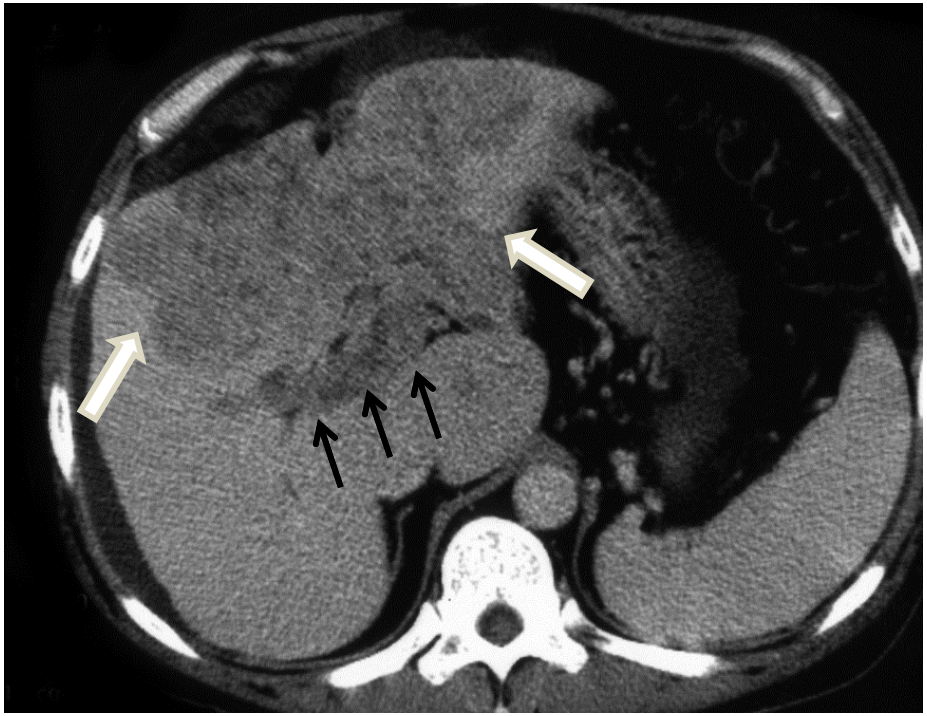
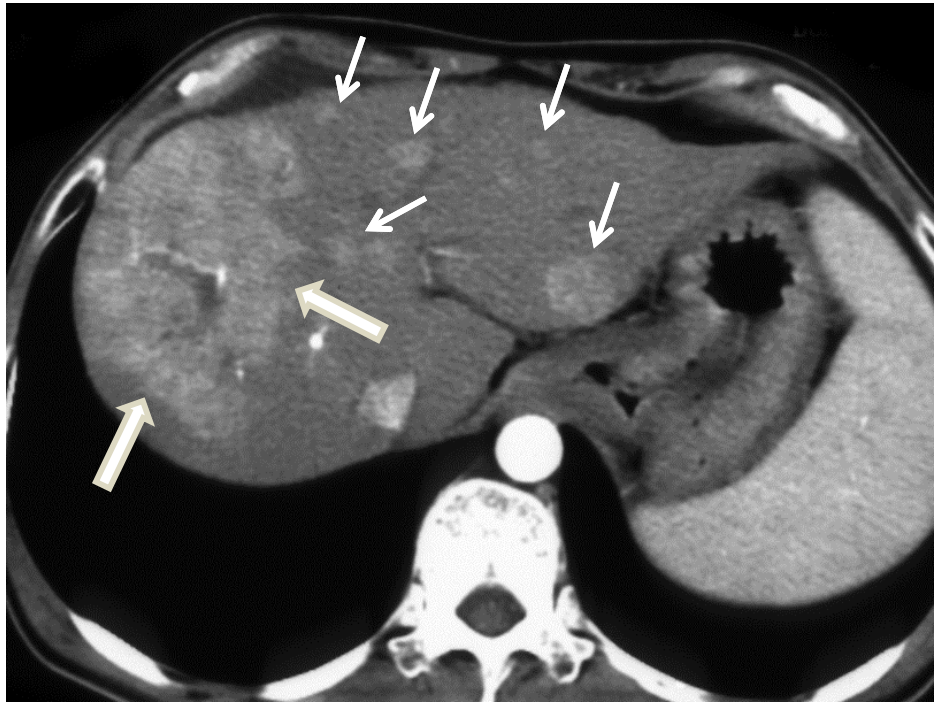


Fig. 3

(a)



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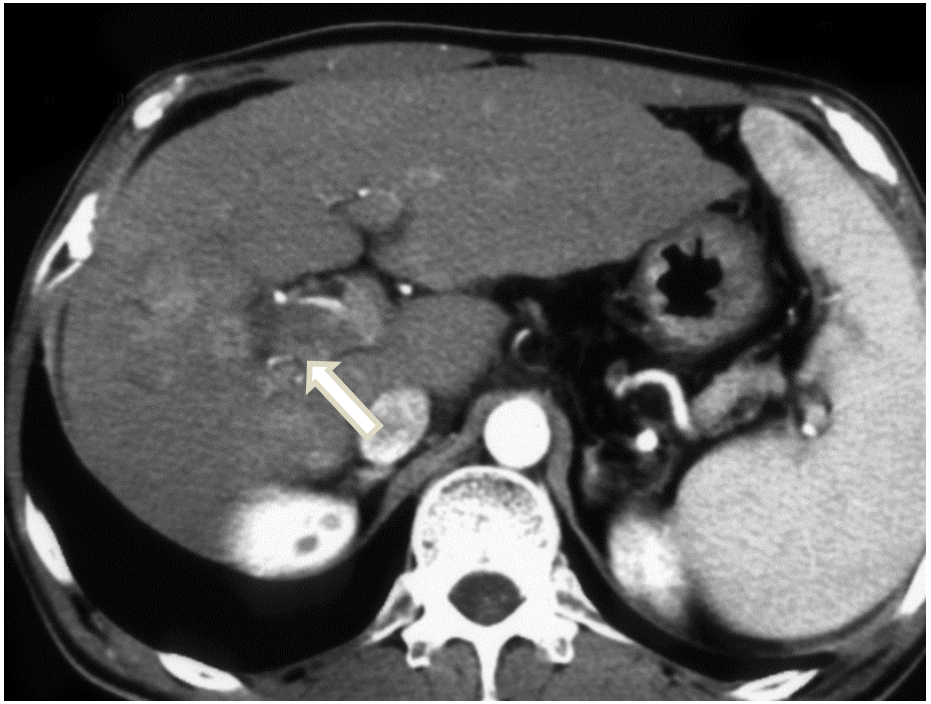
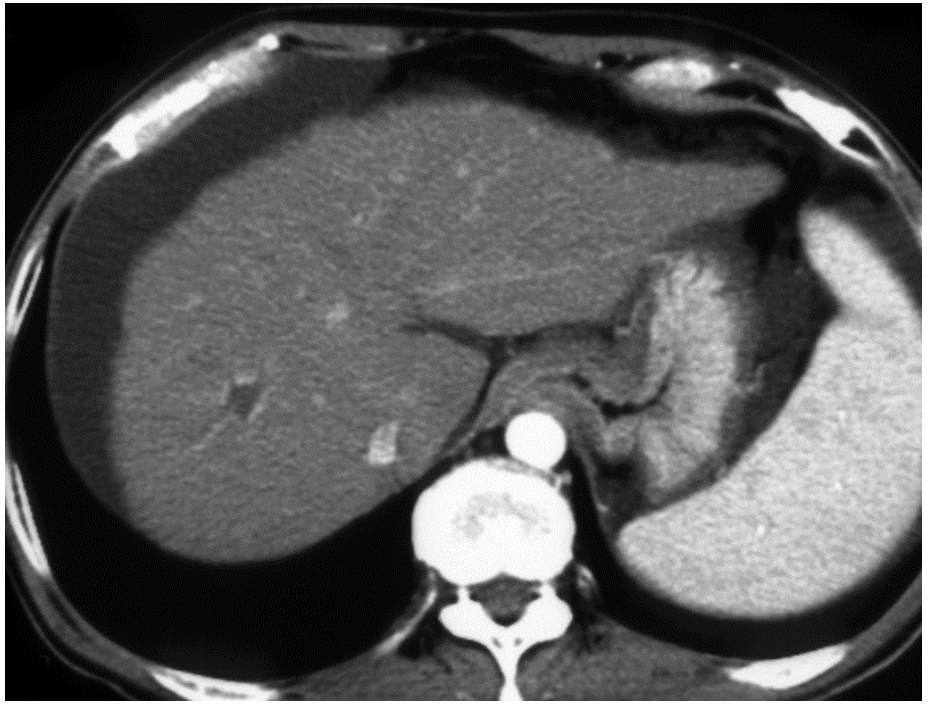


Fig. 3

(c)



(d)

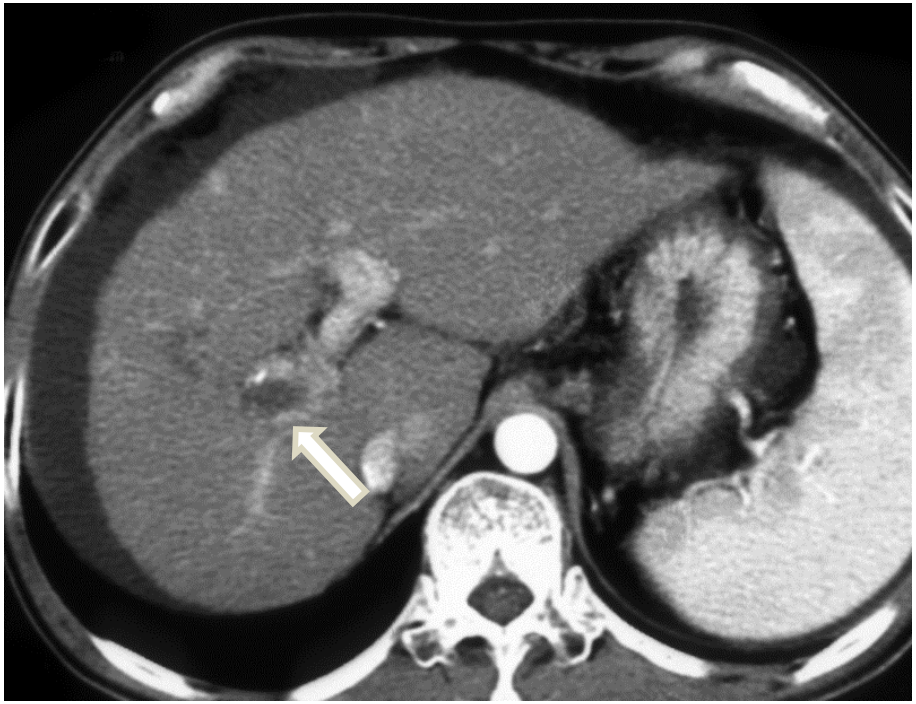


Fig. 4

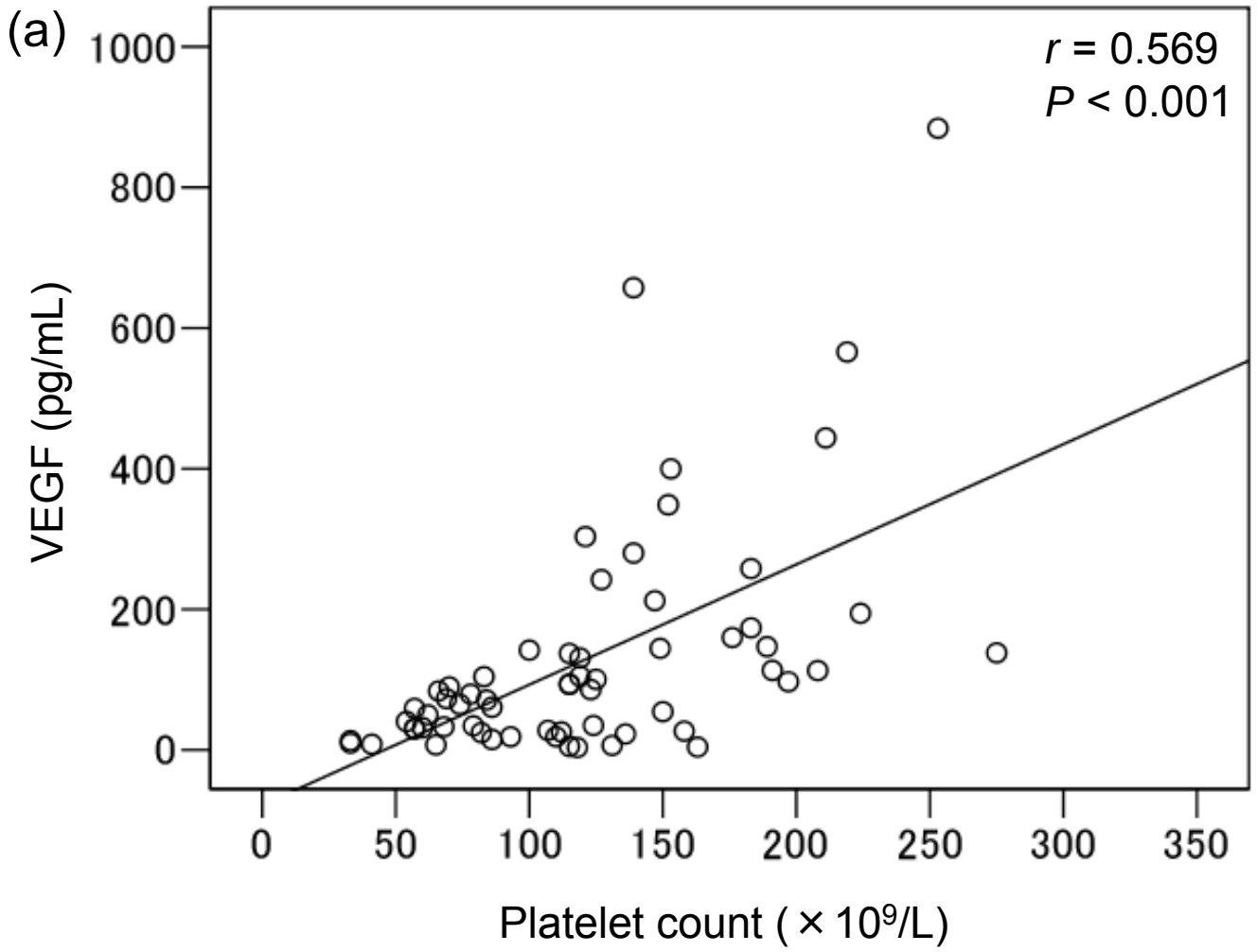

(a)
r = 0.569
P < 0.001
VEGF (pg/mL)
1000
800
600
400
200
0
0
50
100
150
200
250
300
350
Platelet count (× 10^9/L)

Fig. 4

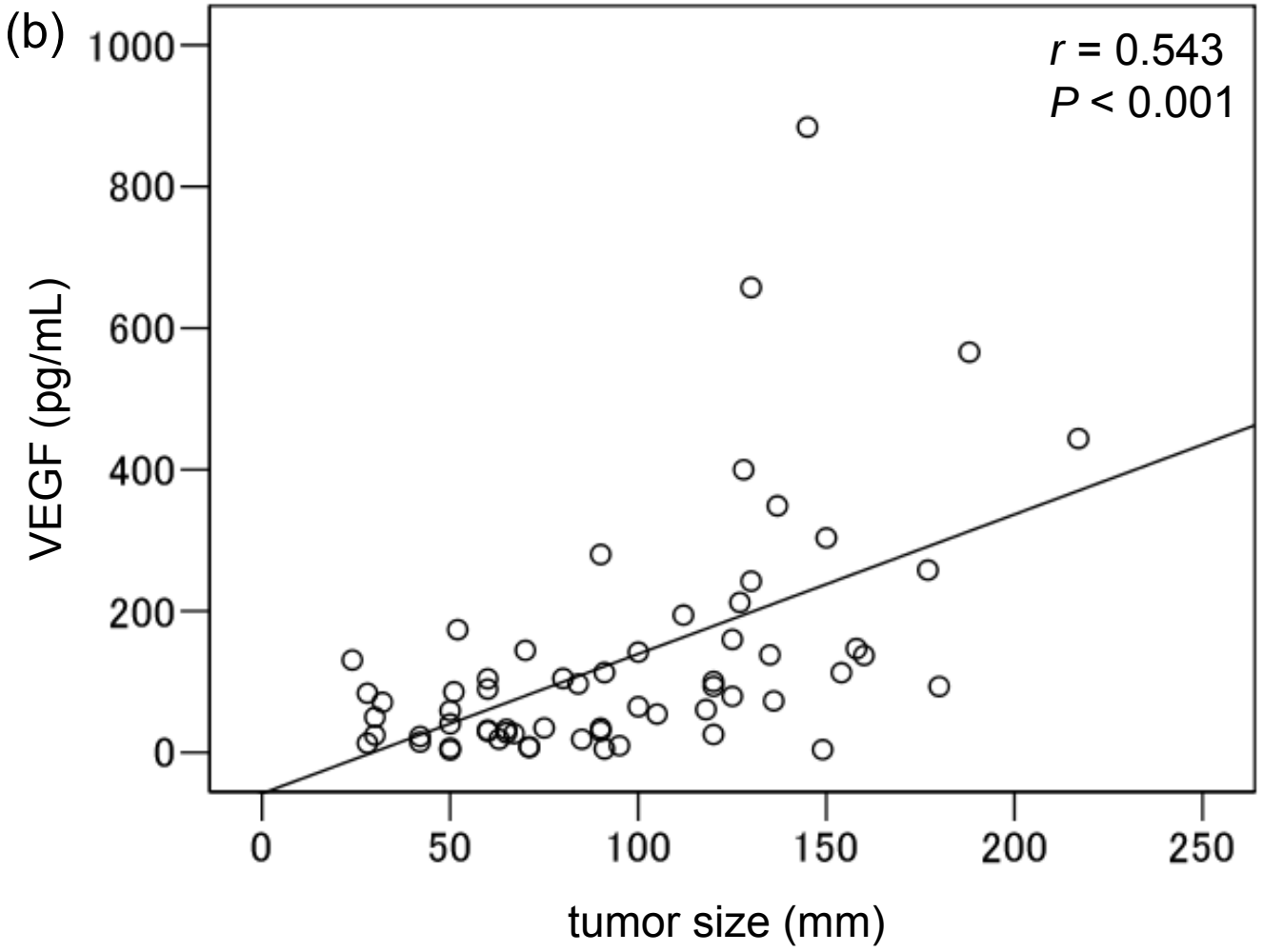

(b)
r = 0.543
P < 0.001
VEGF (pg/mL)
1000
800
600
400
200
0
0
50
100
150
200
250
tumor size (mm)

Fig. 5

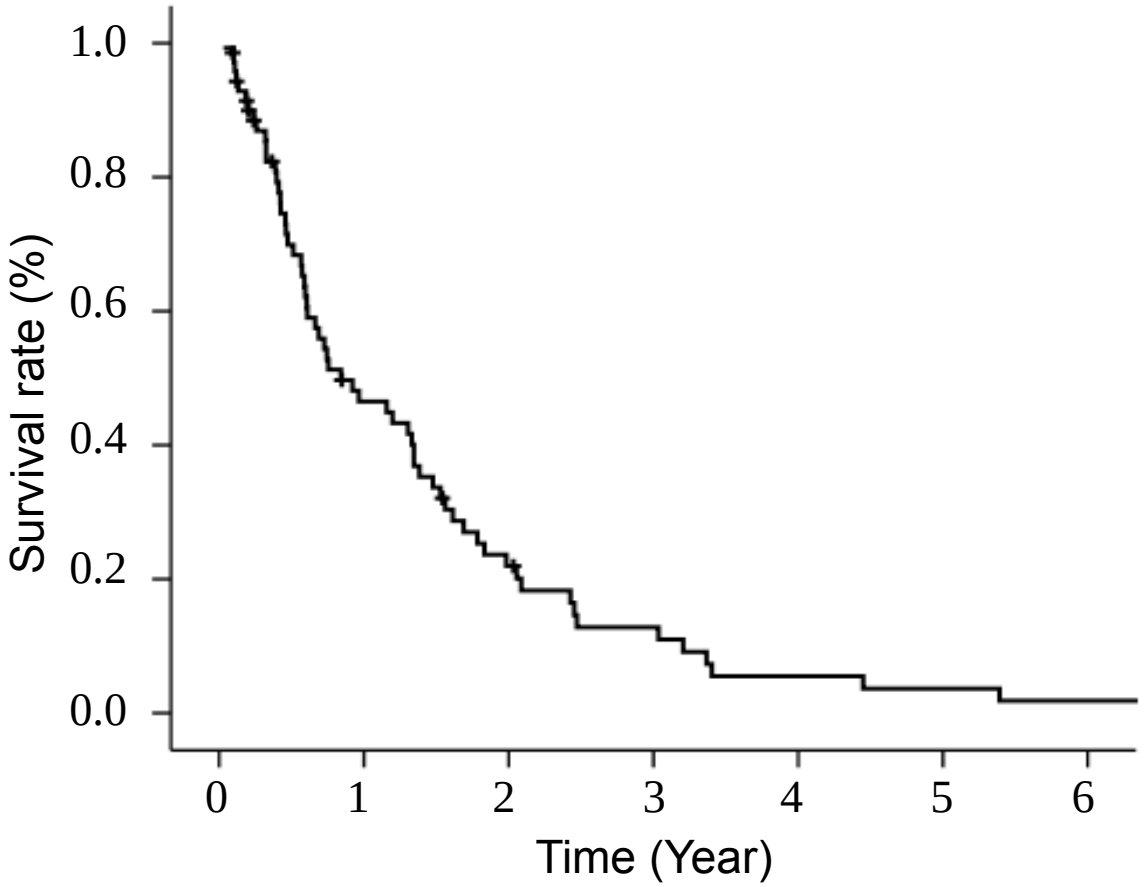


Fig. 6

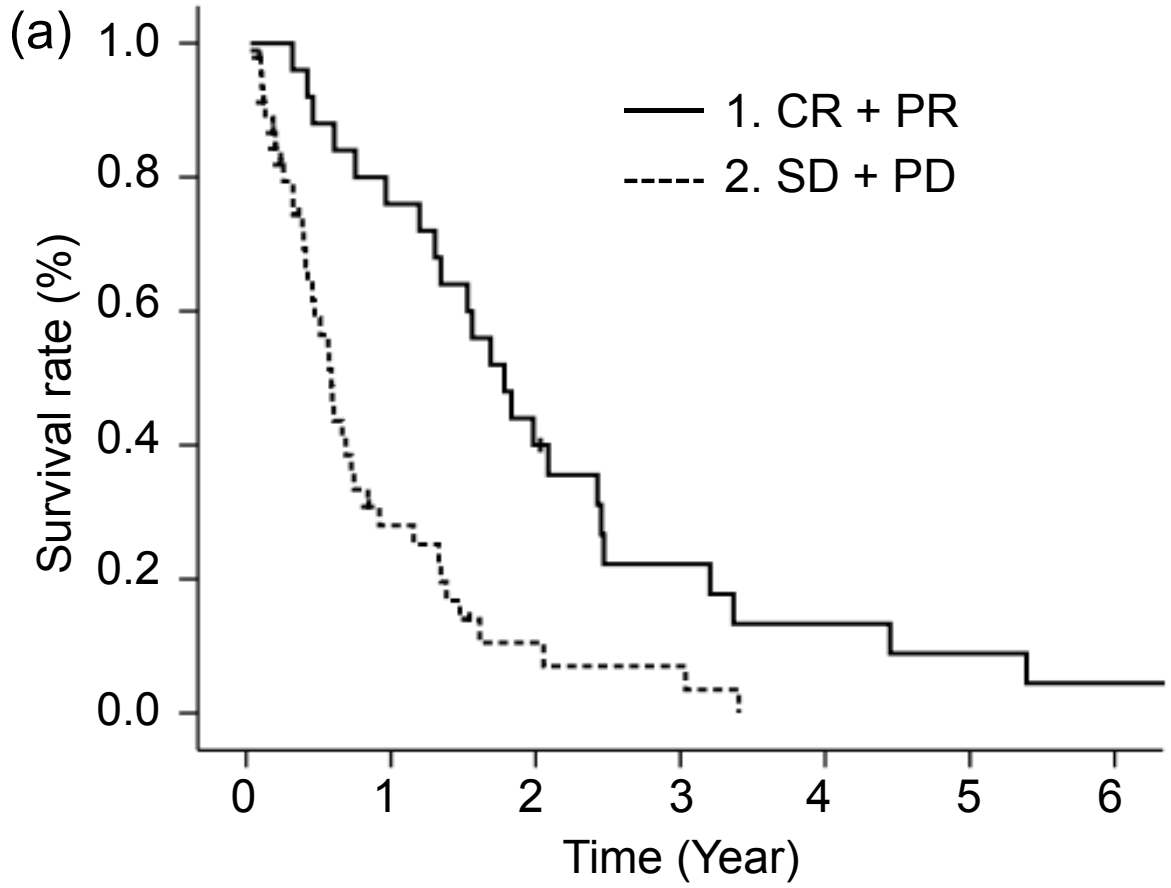

(a)
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate (%)
1. CR + PR
2. SD + PD
0
1
2
3
4
5
6
Time (Year)

Fig. 6

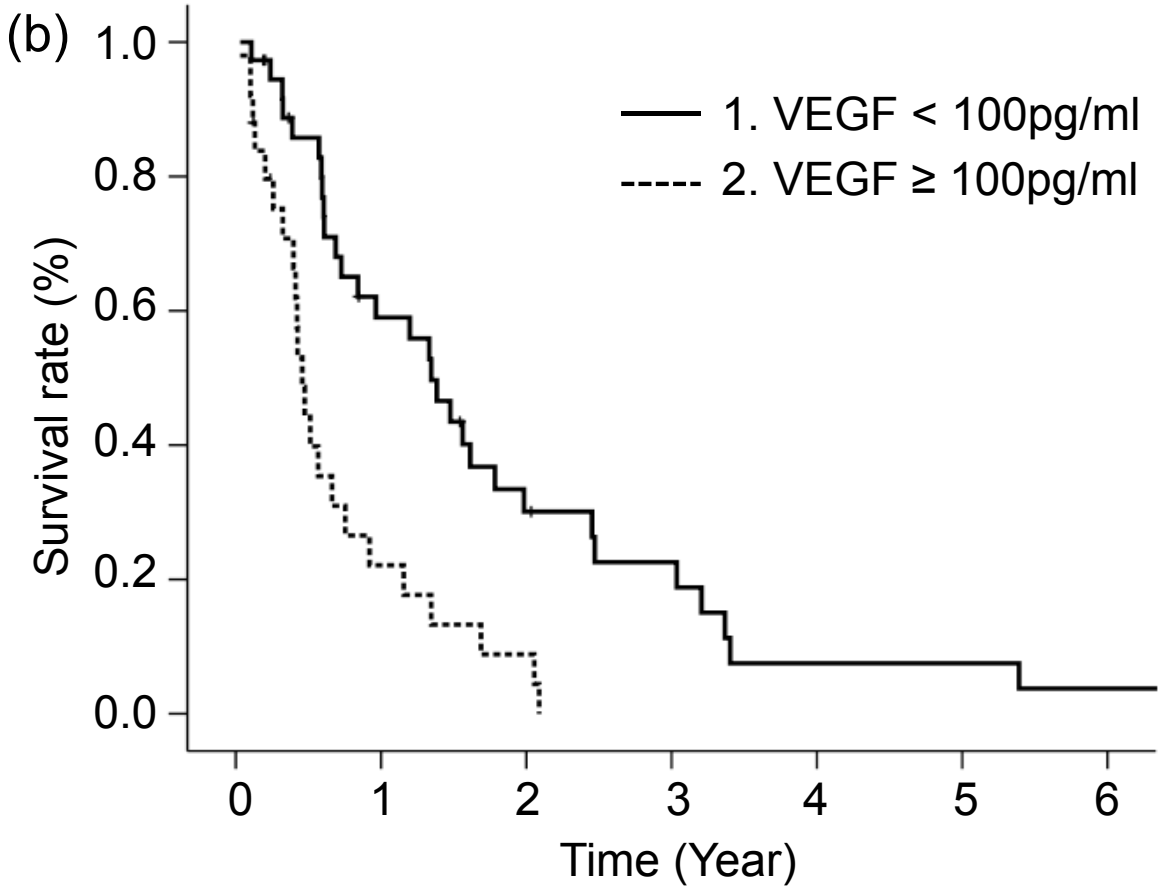
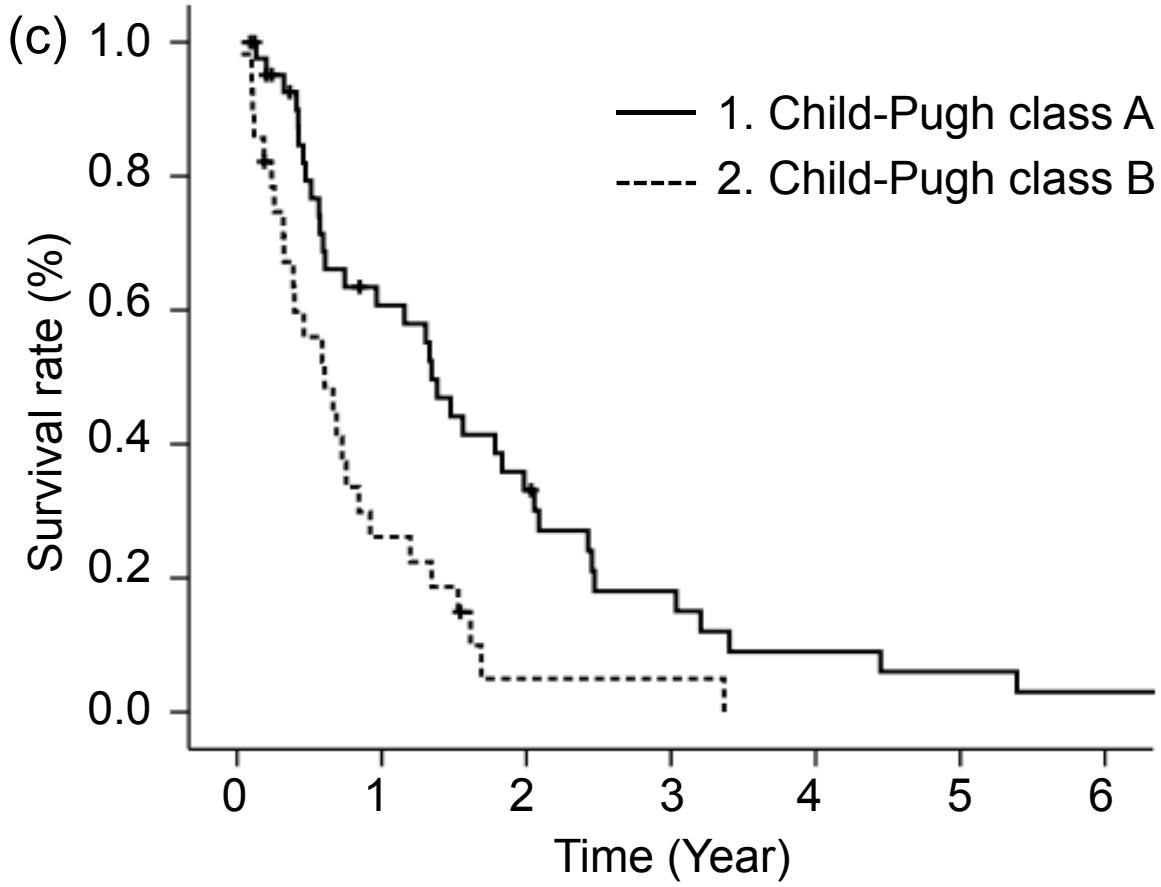
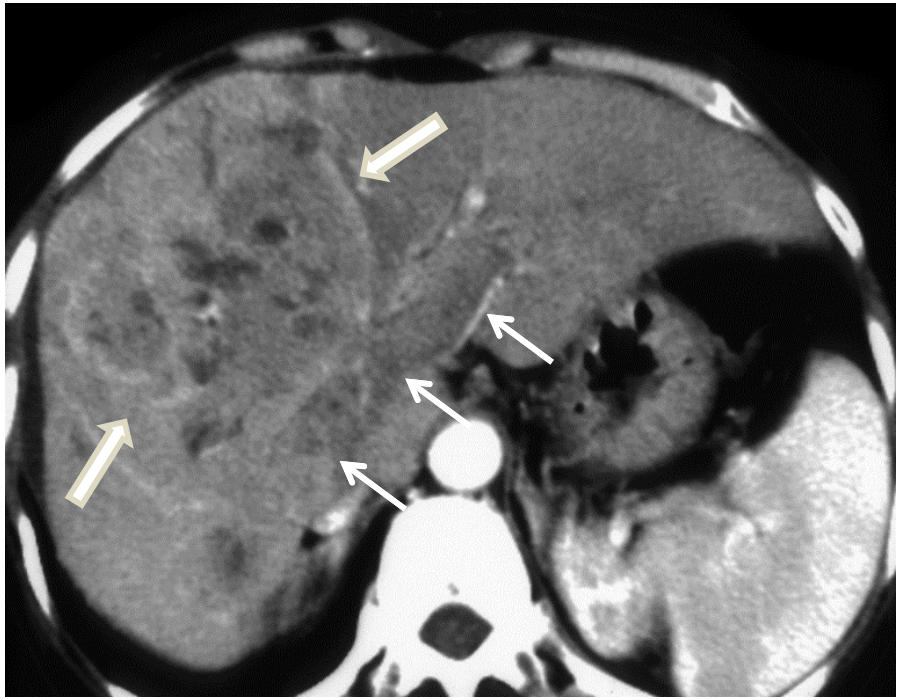

(b)
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate (%)
1. VEGF < 100pg/ml
2. VEGF ≥ 100pg/ml
0
1
2
3
4
5
6
Time (Year)

Fig. 6


(c)
1.0
0.8
0.6
0.4
0.2
0.0
Survival rate (%)
1. Child-Pugh class A
2. Child-Pugh class B
0
1
2
3
4
5
6
Time (Year)

Supplemental File

(a)



(b)

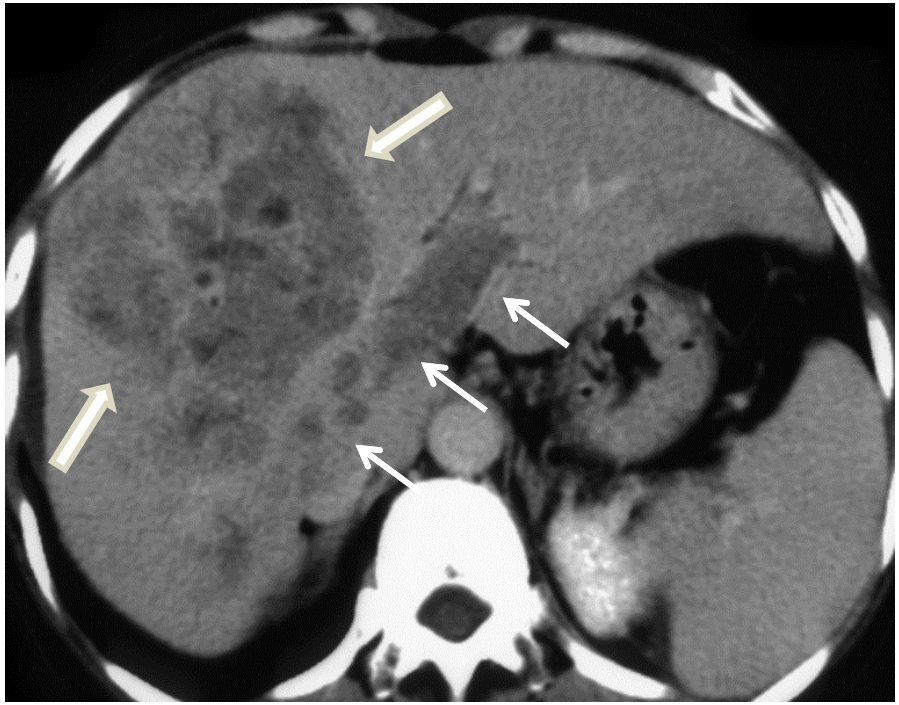


Table 1: Baseline Clinical Characteristics

Patient characteristics	
Gender (male/female)	56/15
Age (years)	65 (44-85)
HCV infection (+/-)	42/29
HBV infection (+/-)	19/52
Child-Pugh class (A/B)	43/28
Platelet count ($\times 10^9$/L)	117 (33-275)
VEGF (pg/mL)	81.6 (3.4-884.2)
AFP (ng/mL) (<1000/≥1000)	35/36
DCP (AU/mL) (<1000/≥1000)	20/46
Previous treatment (yes/no)	26/45
Tumor characteristics	
Maximum tumor size (mm)	90 (24-217)
Macroscopic findings (nodular/infiltrative)	33/38
Tumor location (unilobular/bilobular)	47/24
Grade of portal vein invasion (trunk/first or second branch)	22/49
Hepatic vein invasion (present /absent)	15/56

HCV= hepatitis C virus; HBV= hepatitis B virus; VEGF= vascular endothelial growth factor; AFP= alpha-fetoprotein; DCP= des-gamma-carboxy prothrombin.

Table 2: Comparison of Patient Characteristics Based on Therapeutic Effect

	CR + PR group (n=25)	SD + PD group (n=46)	*P* value
Gender (male/female)	17/8	39/7	0.108
Age (years)	68 (50-81)	65 (44-85)	0.426
Child-Pugh class (A/B)	18/7	25/21	0.146
Platelet count ($\times 10^9$/L)	86 (33-253)	119 (33-275)	0.238
VEGF (pg/mL)	31.6 (3.4-884.2)	97.0 (4.1-657.4)	0.014
AFP (ng/mL) (<1000/≥1000)	10/15	25/21	0.248
DCP (AU/mL) (<1000/≥1000)	9/16	11/35	0.280
Previous treatment (yes/no)	9/16	17/29	0.936
Maximum tumor size (mm)	75 (30-149)	91 (24-217)	0.147
Macroscopic finding (nodular/infiltrative)	12/13	21/25	0.850
Tumor location (unilobular/bilobular)	11/14	13/33	0.181
Grade of portal vein invasion (main trunk/first or second branch)	7/18	15/31	0.688
Hepatic vein invasion (present /absent)	3/22	12/34	0.228

CR= complete response; PR= partial response; SD= stable disease; PD= progressive disease; VEGF= vascular endothelial growth factor; AFP= alpha-fetoprotein; DCP= des-gamma-carboxy prothrombin.

Table 3: Independent Predictors of VEGF by Multiple Logistic Regression Analysis

	OR (95%Cl)	*P* value
Platelet count ($\geq 120\times10^9$/L)	9.85 (2.38-40.77)	0.002
Maximum tumor size ($\geq$ 100 mm)	4.60 (1.13-19.01)	0.034
Tumor location (bilobular)	8.01 (1.28-50.30)	0.026

VEGF= vascular endothelial growth factor; 95% CI= 95% confidence interval; OR = Odds ratio.

Table 4: Univariate and Multivariate Analyses of Survival in Hepatocellular Carcinoma Patients

	Univariate		Multivariate	
	HR (95% Cl)	*P* value	HR (95% Cl)	*P* value
Gender (male)	1.42 (0.77-2.62)	0.272		
Age (> 65)	0.83 (0.50-0.84)	0.497		
Child-Pugh class (B)	2.34 (1.37-4.01)	0.002	1.81 (1.01-3.25)	0.046
Platelet count ($\times 10^9$/L) (≥120)	2.08 (1.24-3.49)	0.006		
VEGF (≥ 100 pg/mL)	2.94 (1.63-5.30)	<0.001	2.42 (1.33-4.38)	0.004
AFP (ng/mL) (≥1000)	1.01 (0.60-1.68)	0.980		
DCP(AU/ml) (≥1000)	0.86 (0.49-1.50)	0.590		
Previous treatment (yes)	0.85 (0.49-1.48)	0.569		
Maximum tumor size (mm) (≥ 100)	2.25 (1.35-3.76)	0.002		
Macroscopic finding (infiltrative)	1.42 (0.85-2.38)	0.178		
Tumor location (bilobular)	1.89 (1.09-3.27)	0.023		
Grade of portal vein invasion (trunk)	2.87 (1.61-5.12)	<0.001		
Grade of hepatic vein invasion (present)	1.31 (0.72-2.39)	0.385		
Therapeutic effect (SD + PD)	2.93 (1.68-5.13)	<0.001	2.46 (1.31-4.62)	0.005

HR= hazard ratio; 95% CI= 95% confidence interval; SD= stable disease; PD= progressive disease; VEGF= vascular endothelial growth factor; AFP= alpha-fetoprotein; DCP= des-gamma-carboxy prothrombin.