Original Article

Impact of Serum Vascular Endothelial Growth Factor on Prognosis in Patients with Unresectable Hepatocellular Carcinoma after Transarterial Chemoembolization

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ABSTRACT

Objective: To investigate the expression level of serum vascular endothelial growth factor (VEGF) in patients with unresectable hepatocellular carcinoma (HCC) and its relationship with the clinicopathological characteristics, and to assess the impact of serum VEGF as a predictive factor for HCC prognosis during transarterial chemoembolization (TACE) treatments.

Methods: Serum VEGF levels were measured using enzyme-linked immunosorbent assay (ELISA) in 60 random patients who underwent TACE or transarterial infusion (TAI) for unresectable HCC between May and September 2008 and 12 healthy volunteers were also involved in this study to serve as control. All patients' clinicopathological features were retrospectively analyzed. Serum VEGF levels were correlated with clinicopathological features of the HCC patients. The patients' survival rates were analyzed with Kaplan-Meier survival curves and compared by the log-rank test. The prognostic significance of serum VEGF levels and factors related to survival rate were evaluated by univariate and multivariate analysis.

Results: The median serum VEGF level in the HCC patients was 285 pg/ml (range 14–1,207 pg/ml), significantly higher than that of healthy controls (P=0.021). The serum VEGF levels were significantly correlated with platelet counts (r=0.396, P=0.002) but not other clinicopathological features. Patients with serum VEGF level >285 pg/ml had worse overall survival compared with those with serum VEGF level <285 pg/ml (P=0.002). By multivariate analysis, the serum VEGF level was a significant prognostic factor.

Conclusion: High serum VEGF levels may predict poor prognosis of HCC after TACE. This study highlights the importance of tumor biomarker as a prognostic predictor in TACE therapy for HCC, which has an intrinsic problem of unavailability of histopathological prognostic features.

Key words: Hepatocellular carcinoma; Vascular endothelial growth factor; TACE; ELISA

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, which cause approximately 600,000 to 1,000,000 deaths annually[1]. It has been the second cancer killer in China since 1990s. As the disease is often advanced at the first manifestation, there is a 5-year survival rate of less than 5% without treatment. Surgical resection and liver transplantation are the only potentially curative therapies. However, only few patients (11.9%–30.1%) at clinical presentation of tumors are suitable candidates for surgery because of multicentricity or poor hepatic functional reserve due to pre-existing cirrhosis. In clinical practice, therefore, transarterial chemoembolization (TACE) or transarterial embolization (TAE) is considered standard palliative treatment[2-4]. However, TACE or TAE, as all currently available systemic options for HCC therapy, usually induce only short-termed disease stabilizations in majority of the patients, early identification of potential treatment responders would be useful to both oncologists and patients.

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Current determination of survival and prognosis in patients treated with TACE or TAE for unresectable HCC is mainly based on clinical assessment. Serum alpha-fetoprotein (AFP) as a well accepted tumor marker is only expressed by 60% of our patients. Thus, for more than one-third of the patients, AFP could not be linked to prognosis. Apart from well-known clinical factors related to tumor stage or liver function, remarkably few data are available upon other measurable prognostic or predictive factors for TACE or TAE treatment response in HCC.

Because HCC is a highly vascular tumor, it has been studied screening patients for markers of increased angiogenesis, which may be detected even before HCC, is clinically significant. Vascular endothelial growth factor (VEGF) has successfully been correlated with stimulation of angiogenesis and does therefore reflect functional tumor activity, which is otherwise often difficult to be assessed by conventional imaging modalities[5]. Tumor expression of VEGF has been shown to be related to microscopic venous invasion, metastasis spread and poor prognosis of HCC[6-8]. The elevation of VEGF in blood implies a promotion of tumor angiogenesis, and several studies have shown that high serum VEGF levels predicted poor survival results independent of clinicopathological features in patients with various types of cancer undergoing resection or receiving chemotherapy[9-11]. In this prospective study, we analyzed the serum VEGF levels in patients with HCC prior to TACE to determine the clinicopathological significance of VEGF, and to assess the clinical usefulness of VEGF as a predictor of outcome in patients undergoing TACE therapy for HCC.

MATERIALS AND METHODS

Patients

Between May and September 2008, serum VEGF levels were measured using enzyme-linked immunosorbent assay (ELISA) in 60 random patients with diagnosed HCC undergoing at least one course of TACE or transarterial infusion (TAI) in Beijing Cancer Hospital. Diagnosis was based on histological or cytological examination by ultrasound guided biopsy for 28 patients. For the remaining 32 patients, diagnosis was based on the guidelines of American Association for the Study of Liver Diseases (AASLD) criteria. Indications for TACE or TAI were: (1) patient's refusal resection; (2) unresectable tumors because of multicentricity, topography conditions or poor hepatic functional reserve; (3) positive margin or vascular invasion; (4) recurrence after resection or locoregional therapy [i.e. radiofrequency ablation (RFA)]; and (5) persistent high AFP level after hepatectomy. Exclusion criteria for treatment were poor liver function, portal obstruction of at least three segmental branches, advanced cardiac or pulmonary disease and severe renal function impairment.

Of the 60 patients, 27 patients did not undergo TACE or TAI before blood collection, the remaining 33 patients underwent at least one course of TACE or TAI before blood collection. Each patient's disease was staged using Barcelona Clinic Liver Cancer's (BCLC) scheme: 17 patients had Stage A, 28 Stage B, and 15 Stage C tumors. To determine the severity of underlying cirrhosis, the Child-Pugh classification was used: 51 patients had Child's A class cirrhosis and 9 Child's B class. The type of the tumor was considered as paucifocal with no more than three distinct lesions, and as multifocal with more than three lesions and infiltrated or diffused nonfocal lesions. Of the 12 patients after resection, 9 patients received TACE because of HCC recurrence, and 3 patients received TAI because of HCC vascular invasion. Two patients received TACE because of HCC recurrence after RFA.

The study was approved by the institutional review board of the hospital and conducted according to the standards of the Declaration of Helsinki, and informed written consent was obtained from all patients before their treatment.

TACE Procedure

The chemoembolization procedure consisted of injecting iodized oil (Lipiodol; Laboratoire Andre Guerbet, Aulnaysous-Bois, France) mixed with epirubicin hydrochloride (20–60 mg; Main Luck Pharmaceutical, Shenzhen, China) as an emulsion into segmental or subsegmental tumor-feeding arteries. Sometimes subsequent embolization was obtained by using Spongostan particles (Jinling, Nanjing, China) as an alternative in some cases. For those with hepatic arteriovenous fistula, Spongostan particles were used to block the fistula before iodized oil was used. Before or after iodized oil embolization, two to three chemotherapeutic regimens of 5-fluorouracil (750–1,000 mg; Xudong Haipu, Shanghai, China), cisplatin (50–100 mg; Keding, Nanjing, China) or carboplatin (200–300 mg; Qilu, Jinan, China), and mitomycin (8–12 mg; Kyowa Hakko Kogyo, Tokyo, Japan) were diluted in 50–100 ml sodium chloride solution or glucose injection and infused through the catheter. The TAI procedure was adopted when the tumor mass was too superficial, and the arterial anatomy precluded a super selective injection, or when significant arteriovenous fistulas or intrahepatic portal thrombosis presented. In each case, the decision to repeat TACE or TAI was based on computed

tomography (CT) results. The therapeutic course was repeated for: (1) perilesional recurrence of the tumor; (2) sharp disappearance of iodized oil; (3) daughter lesions; (4) intrahepatic metastasis; and (5) no changes after more than 12 months since the last treatment course. Each patient received 1–21 courses of locoregional therapy. The most common post-embolization complications were anorexia, nausea, transient fever, abdominal pain, and increased alanine aminotransferase levels, which were controlled with symptomatic treatments. No chemoembolization-related deaths were recorded.

Assay of Serum VEGF Level

Peripheral venous blood samples were taken from the 60 patients with HCC before TACE treatment, and from 12 sex- and age-matched healthy controls. Blood samples were drawn into serum separator tubes and centrifuged at 3,000 r/min for 10 min, then stored at –80°C until analysis. The levels of serum VEGF were quantified by the Quantikine human VEGF immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). The assay adopts the quantitative sandwich enzyme immunoassay technique that uses immobilized murine monoclonal antibody and horseradish peroxidase-linked polyclonal antibody, both of which are specifically against human VEGF and exhibit no marked cross-reactivity with other angiogenic factors.

The assay was performed according to manufacturer's instructions. The VEGF level was determined by interpolation to a standard curve generated from standards of VEGF recombinant protein provided in the kit. All samples were assayed in duplicate by an investigator blinded to the clinical data. The sensitivity of the assay for VEGF was 9 pg/ml and the coefficients of variation of intraassay and interassay measurement were in the range given by the manufacturer (4.5%–6.7% and 6.2%–8.8%, respectively).

Follow-up

After discharge, the patients were followed up routinely in the outpatient clinic. Clinical data were obtained by close follow-up every 1–3 months after chemoembolization until November 2009 or until patient expire. Follow-up included serum AFP level measurement, complete blood count, biochemical liver and kidney function tests, and ultrasonography (US) examination. Spiral CT triphasic scanning was performed when a recurrence was suspected due to an increase in AFP level from a decreased baseline after treatment, and it was also performed at least every 6 months even if the AFP level remained low or the tumor did not secrete AFP. Magnetic resonance imaging was performed additionally if necessary. Chemoembolization was repeated if the initial lesions seemed to revascularize or new lesions appeared. The defined endpoint was nonsurvival. By the time of censoring of data for analysis, follow-up was complete for all patients, and the last patient in the cohort had been followed up for 402 days.

Statistical Analysis

Continuous data were expressed as median and range or $\bar{x}\pm s$. Groups were compared by the Mann-Whitney U test or t test for continuous data. Correlations of continuous data were performed by the Pearson rank correlation coefficient (r). Survival rates were estimated by the Kaplan-Meier method and compared by the log rank test. Multivariate analysis was performed by the Cox proportional hazard model. All statistical analyses were performed with SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). A P value <0.05 was considered statistically significant.

RESULTS

Characteristics of Patients

Baseline clinical characteristics of the 60 patients are shown in Table 1. Of the 60 patients, 7 patients (11.7%) underwent only one course of TACE or TAI, while the remaining 53 patients (88.3%) underwent 2–21 courses of TACE or TAI. The mean interval time of two courses was 32–371 days. Forty-eight patients (80.0%) had hepatitis B virus (HBV)-related HCC, 8 patients (13.3%) had hepatitis C virus (HCV)-related HCC, and 4 patients (6.7%) had coinfection of the two hepatitis viruses-related HCC. All patients had evidence of underlying cirrhosis. The study population included 46 patients with unresectable HCC, 9 patients with recurrence after resection, 1 patient with recurrence after RFA, 3 patients with positive margin or vascular invasion after resection and 1 patient with continued high AFP level after RFA. There were no TACE or TAI-related deaths.

Correlation of Serum VEGF Levels and Clinicopathological Features

The median serum VEGF level of the 60 patients with HCC was 285 pg/ml (range 14–1,207 pg/ml), significantly higher than that of the 12 healthy controls (125 pg/ml, 26–311 pg/ml) (P=0.021). No significant difference of serum VEGF levels was observed between 33 patients with previous TACE and 27 patients without previous TACE (median, 284 vs. 298 pg/ml, P=0.345). The serum VEGF levels in patients with BCLC stage C were higher than those in patients

Table 1. Baseline clinical data

Characteristic	Data
Sex, *n* (%)	
Male	55 (91.7%)
Female	5 (8.3%)
Age (year), median (range)	62 (37–84)
Viral etiology	
Hepatitis B, *n* (%)	48 (80.0%)
Hepatitis C, *n* (%)	8 (13.3%)
Hepatitis B and C, *n* (%)	4 (6.7%)
Child-Pugh class, *n* (%)	
A	51 (85.0%)
B	9 (15.0%)
BCLC stage, *n* (%)	
A	10 (16.7%)
B	34 (56.7%)
C	16 (26.7%)
Serum AFP (ng/ml), median (range)	86.36 (1.04–124,961.00)
Serum albumin (g/L), $\bar{x}\pm s$	39.79±4.131
Serum bilirubin (μmol/L), median (range)	14.40 (5.1–42.1)
Serum ALT (IU/L), median (range)	41.50 (12–210)
Serum AST (IU/L), median (range)	42.50 (18–174)
Serum GGT (IU/L), median (range)	101.5 (18–656)
Platelet count ($\times10^9$/L), $\bar{x}\pm s$	160.92±83.41
Diameter of the largest tumor	
<10 cm, *n* (%)	40 (83.3%)
≥10 cm, *n* (%)	10 (16.7%)
Portal vein tumor thrombus	
Yes, *n* (%)	10 (16.7%)
No, *n* (%)	50 (83.3%)
Arteriovenous fistula	
Yes, *n* (%)	6 (10.0%)
No, *n* (%)	54 (90.0%)
Extrahepatic spread	
Yes, *n* (%)	7 (11.7%)
No, *n* (%)	53 (88.3%)
Number of tumors, *n* (%)	
Paucifocal	26 (43.3%)
Multifocal	34 (56.7%)

BCLC: barcelona Clinic Liver Cancer; AFP: alpha-fetoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyltransferase.

with BCLC stage A and B (median, 349 vs. 212 pg/ml, *P*=0.001). The serum VEGF levels in patients with portal vein tumor thrombus were higher than those in patients without portal vein tumor thrombus (median, 424 vs. 249 pg/ml, *P*=0.011). The serum VEGF levels in patients with HCC ≥10 cm in diameter were higher than those in patients with HCC <10 cm in diameter (median, 497 vs. 249 pg/ml, *P*=0.047). The serum VEGF levels in female patients (median, 654 pg/ml; range, 210–1,207 pg/ml) were higher than those in male patients (median, 284 pg/ml; range, 14–1,152 pg/ml), but the difference was not statistically significant (*P*=0.056). The serum VEGF levels in patients with extrahepatic spread (median, 326 pg/ml; range, 197–545 pg/ml) were higher than those in patients without extrahepatic spread (median, 252 pg/ml; range, 14–1,207 pg/ml), but the difference was not statistically significant (*P*=0.119). The serum VEGF levels in patients with arteriovenous fistula (median, 395 pg/ml; range, 197–496 pg/ml) were higher than those in patients without arteriovenous fistula (median, 256 pg/ml; range, 14–1,207 pg/ml), but the difference was not statistically significant (*P*=0.092). No significant difference of serum VEGF levels was observed between patients with Child-Pugh class A cirrhosis and those with Child-Pugh class B cirrhosis (median, 285 vs. 259 pg/ml, *P*=0.909), between patients with no more than three distinct lesions and those with more than three lesions (median, 285 vs. 275 pg/ml, *P*=0.479). A significant positive correlation between serum VEGF levels and PLT levels was observed (*r*=0.396, *P*=0.002) (Figure 1). There were no significant correlations between serum VEGF levels and age (*r*=–0.041, *P*=0.755), serum AFP (*r*=0.197, *P*=0.132), ALT (*r*=–0.032, *P*=0.808), AST (*r*=0.170, *P*=0.195), GGT (*r*=0.161, *P*=0.220), bilirubin (*r*=–0.080, *P*=0.545), or albumin (*r*=0.015, *P*=0.911) levels.

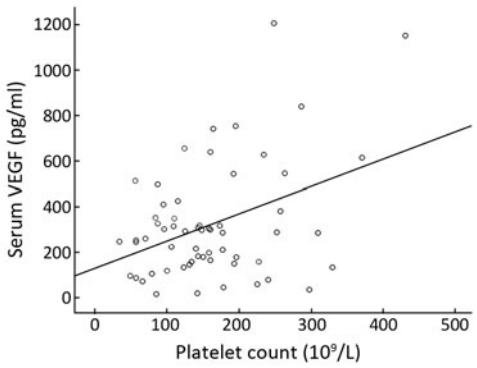

Figure 1. Scatter plot showing the correlation between the serum VEGF levels and platelet count (*r*=0.396, *P*=0.002).

Long-Term Survival after TACE or TAI

Figure 2 shows the overall survival of the 60 patients from the time of first TACE or TAI, with a median follow-up of 17.54 months (range, 0.23–82.59 months). The 0.5-, 1-, 1.5-, and 2-year overall survival rates were 85%, 73%, 68%, and 57%, respectively. By the time of censoring of data for analysis, 26 (43.3%) of 60 patients had died. Of these 26 patients, 11 patients (42.3%) died of tumor progression, 5 patients (19.2%) died of liver failure, 8 patients (30.8%) died of upper gastrointestinal bleeding, and 2 patients (7.7%) died of intraabdominal bleeding following tumor rupture.

Prognostic Significance of Serum VEGF Levels

All continuous variables were dichotomized by

using the respective median or mean values as the cutoff. Patients with high serum VEGF levels had significantly worse overall survival compared with those with low serum VEGF levels (*P*=0.002) (Figure 3), in accordance with previous studies[12]. Besides high serum VEGF levels, significant unfavorable prognostic factors for overall survival included female (*P*=0.002), extrahepatic spread (P=0.005), portal vein thrombosis (*P*<0.001), arteriovenous fistula (*P*=0.008) and high serum AFP levels (*P*=0.002). Significant prognostic factors in the univariate analyses were entered into multivariate analyses. Table 2 shows the results of multivariate analyses of prognostic factors for overall survival. Serum VEGF level (*P*=0.020), serum AFP level (*P*=0.009), extrahepatic spread (*P*=0.005) and sex (*P*=0.011) were independent prognostic factors of the overall survival.

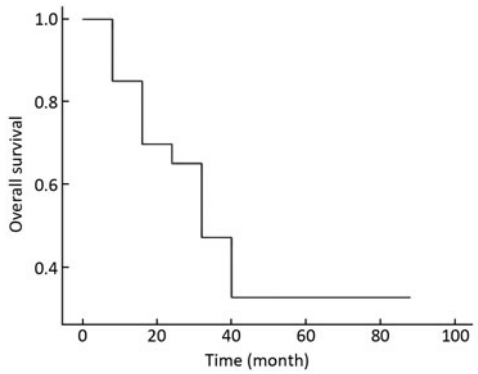

Figure 2. Overall survival curves after the first TACE or TAI in the 60 patients.

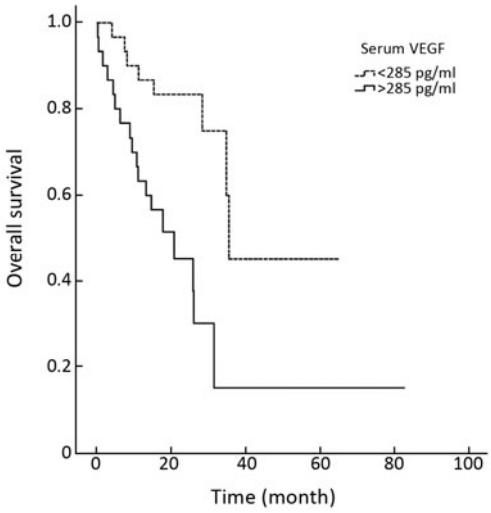

Figure 3. Prognostic influence of serum VEGF level on overall survival after TACE or TAI, using the median serum VEGF level 285 pg/ml of the whole study population as the cutoff value for low and high serum VEGF levels.

Table 2. Prognostic factors for overall survival by multivariate analysis

Variable	Risk ratio (95% CI)	*P*
Overall survival		
Serum VEGF level (>285 vs. <285 pg/ml)	2.865 (1.178–6.971)	0.020
Serum AFP (>86.36 vs. <86.36 ng/ml)	3.394 (1.359–8.473)	0.009
Extrahepatic spread (Yes vs. No)	4.659 (1.584–13.700)	0.005
Sex (Male vs. Female)	0.215 (0.066–0.707)	0.011

95% CI: 95% confidence interval; VEGF: vascular endothelial growth factor; AFP: alpha-fetoprotein.

DISCUSSION

Although the effect of TACE in improving the long-term survival of patients with unresectable HCC remains controversial, satisfactory outcome can be achieved by the careful selection of candidates based on the guidelines of the AASLD[13]. As shown in this study, the 3-year overall survival rate of all 60 random patients was 31%, in accordance with previous studies[14].

Angiogenesis is a cascade of linked and sequential steps that ultimately lead to the neovascularization of tumors. VEGF was initially identified in 1983 as a protein secreted by tumor cells[15]. It is one of the most potent angiogenic factors expressed in various human cancers[16, 17]. As an endothelial growth factor, VEGF stimulates endothelial cell proliferation, thus inducing the budding of new blood vessels around the growing tumor masses. Recently, the expression of VEGF in patients with various malignancies has been made possible by measuring circulating VEGF concentrations with the ELISA. Studies have shown that high serum VEGF levels predicted poor survival results independent of clinicopathological features in patients with different types of cancer undergoing resection or receiving chemotherapy[9-11].

HCC is a highly vascular tumor characterized by neovascularization and high propensity for venous invasion. Significant HCC growth is dependant on angiogenesis, and an increase in tumor dimension beyond 0.5 mm will induce the proliferation of vascular endothelial cells[18]. Overexpression of VEGF has been documented in HCC[7]. A study has demonstrated that VEGF has an autocrine effect in stimulating cancer cell proliferation in HCC in addition to its angiogenic effect, further underscoring the importance of VEGF in HCC[19]. A strong VEGF expression was observed in the tissue of HCC and closely associated with tumor progression and metastasis[20]. However, a major problem in prognostic

prediction after TACE for HCC is the lack of histopathological features such as microscopic vascular invasion or intrahepatic metastasis, which are the most important prognostic factors after resection or transplantation for HCC[21-23]. Previously, there has been debate regarding whether the circulating VEGF levels reflect tumor expression of VEGF. Studies have demonstrated that high pretreatment circulating VEGF levels predicted poor response and survival in patients undergoing TACE for advanced HCC[24, 25].

We found that serum VEGF was markedly elevated in the majority of patients with HCC received TACE treatment, and the increase was closely related to a more advanced BCLC stage of diseases. This finding suggested that HCC cells were an important source of serum VEGF. But the lower serum VEGF levels in HCC patients overlapped considerably with those in normal controls, thus limiting the application of VEGF as a tumor marker in the early detection of HCC.

Portal vein involvement, arteriovenous fistula and extrahepatic metastasis, which were confirmed as adverse prognostic factors, can reflect the ability of tumor cells to invade blood vessels. In the current study, we found that high serum VEGF levels were associated with the presence of portal vein tumor thrombus. Although the difference was not statistically significant, the serum VEGF levels in patients with arteriovenous fistula and extrahepatic spread were respectively higher than those in patients without arteriovenous fistula and extrahepatic spread. These results indicated that serum VEGF may be used as a tumor marker in detecting vascular invasive phenotypes of HCC. Tumor size was an important prognostic factor of overall survival in previous studies[26, 27]. As shown in this study, HCC ≥10 cm in diameter was associated with high serum VEGF levels. Tumor multiplicity was also an adverse prognostic factor, but no significant difference of serum VEGF levels was observed between patients with no more than three distinct lesions and those with more than three lesions in our study.

The positive correlation of serum VEGF levels with platelet count in this study is consistent with the findings of previous studies[28, 29]. This is attributable to the fact that most VEGF in the peripheral circulation is carried in the platelets, and the VEGF is released when the platelets are activated during blood clotting. It has been postulated that platelets may serve as a scavenger or storage of VEGF from the tumor, and the release of VEGF from the platelets at distant metastasis may play a central role in the process of hematogenous dissemination of cancers[30]. A study has demonstrated that increased levels of bone marrow-derived endothelial progenitor cells in the peripheral circulation of patients with advanced HCC, and a high correlation between serum VEGF levels and levels of circulating endothelial progenitor cells were observed[31]. It has been suggested that platelets aggregate at metastatic sites, due to factors released from metastatic cells and vascular invasion, resulting in microthrombosis, tumor adhesion, and may release VEGF to the circulation[32, 33]. Therefore, we considered that in metastatic HCC patient platelet activation was a part of the sources of serum VEGF.

Poor liver function was commonly identified as an adverse prognostic factor in previous studies[34-39]. However, as shown in this study, tumor progression including vascular invasion, extrahepatic metastasis and advanced BCLC stage rather than liver failure was the main adverse prognostic factor after TACE. In present study, no correlation was found between serum VEGF and AFP, ALT, AST, GGT, bilirubin, or albumin, suggesting that they had different mechanisms of production, and serum VEGF might be an independent predictive factor.

The current study shows that high serum VEGF level was one of independent unfavorable prognostic factors for overall survival and suggests that serum VEGF may be a useful biomarker for prognostic prediction in patients with advanced HCC treated with TACE.

Although the current study suggests that serum VEGF level may be a useful prognostic biomarker for TACE therapy of HCC, further studies with larger patient populations are needed to validate its prognostic value and determine the optimum cutoff value. Furthermore, the biological significance of circulating VEGF in cancer patients remains to be clarified.

The findings of this study may have some therapeutic implications. The prognostic significance of serum VEGF suggests that antiangiogenic therapy targeting VEGF may be a potentially effective adjuvant therapy after TACE of HCC. An anti-VEGF monoclonal antibody has been approved for treatment of cancer patients[38]. Promising results that use the anti-VEGF antibody in combination with systemic chemotherapy in treating advanced HCC have recently been reported[39]. Furthermore, several VEGF receptor antagonists currently in clinical trials have been shown to inhibit growth and metastasis of HCC in preclinical studies[24]. It is worthwhile to investigate the efficacy of such anti-VEGF therapies in the adjuvant setting after TACE therapy for HCC, and to study whether pretreatment serum VEGF levels may help select patients who are more likely to benefit from such therapies.

In conclusion, this study shows that a high serum VEGF level predicts poor overall survival after TACE of HCC. This may be of particular value because of the unavailability of conventional histopathological prognostic features inherent to the TACE nature of the treatment.

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