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ORIGINAL ARTICLE

Serial serum VEGF-A, angiopoietin-2, and endostatin measurements in cirrhotic patients with hepatocellular carcinoma treated by transcatheter arterial chemoembolization

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KEYWORDS

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Vascular endothelial growth factor

Abstract Vascular endothelial growth factor (VEGF), angiopoietin-2, and endostatin have been reported to be related with angiogenesis of hepatocellular carcinoma (HCC). The potential feasibility of serial serum VEGF-A, angiopoietin-2, and endostatin measurements in cirrhotic patients with HCC treated by transcatheter arterial chemoembolization (TACE) was investigated. VEGF-A, angiopoietin-2, and endostatin serum level were determined by enzyme-linked immunosorbent assay 1 day before and 7 days after TACE in 40 patients. Then they were followed up for 3 months. The results showed that TACE could cause significant increase of VEGF-A ($p < 0.01$) and angiopoietin-2 ($p = 0.01$); whereas there was no significant change of endostatin ($p > 0.1$). Twenty-five patients with rapid growth of HCC within 3 months after TACE had higher proportion of American Joint Committee on Cancer HCC staging $>II$ and higher increase of VEGF-A after TACE than 15 patients without rapid growth (all $p < 0.05$). Stepwise logistic regression analysis revealed that VEGF-A >16.7 pg/mL 7 days after TACE selected by receiver operating characteristic curve analysis ($p < 0.05$) was the only independent predictor for rapid growth of HCC (odds ratio 6.33, 95% confidence interval: upper 26, lower 1.54, $p < 0.05$; sensitivity 76%, specificity 66.7%, accuracy 72.5%, positive predictive level 79.2%, negative predictive level 62.5%, $p < 0.01$). In conclusion, significant increases

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of serum level VEGF-A and angiopoietin-2 after TACE have been demonstrated from this study. Therefore, serial VEGF-A level 1 day before and 7 days after TACE may be used to predict rapid HCC growth.

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Introduction

Hepatocellular carcinoma (HCC) is a hypervascular tumor characterized by neovascularization. The vascular endothelial growth factor (VEGF) and angiopoietin families have been found to work in tandem with HCC angiogenesis [1]. The VEGF family has at least seven members, and VEGF-A is a key player in angiogenesis [2,3]. VEGF mRNA is usually significantly upregulated in HCC [4–10]. Increased serum or plasma VEGF levels have been shown to be correlated with microscopic venous invasion [11], distant metastasis [12], and overall survival in HCC [13–15]. Although there were some conflicting results [16], other studies showed that preoperative serum VEGF [17], circulating VEGF-A mRNA [18], or expression of VEGF-A mRNA in HCC tissue [19] play an important role in the prediction of postoperative recurrence of HCC. Moreover, high expression of VEGF-A mRNA in noncancerous liver remnants of HCC may be a significant biological indicator for the invasiveness of postoperative recurrence [20]. Angiopoietins have been identified as ligands for Tie-2 receptor and are thought to be important factors in vascular maturation and stability during angiogenesis [1,3]. Levels of angiopoietin-2 mRNA expressions in HCC [7], non-small cell lung cancer [21], and gastric cancer [22,23] have been reported to be significantly increased compared with adjacent noncancerous components. This increased expression may play a critical role in promoting tumor angiogenesis and progression [7,21,23]. High serum angiopoietin-2 levels have been shown to correlate with tumor invasion in esophageal squamous cell cancer [24] and recurrence of lung cancer [25]. Endostatin, the 20-kDa C-terminal fragment of collagen XVIII [26], is an angiogenic inhibitor [27] and has been shown to prevent angiogenesis and drive large tumors into dormancy [28]. Endostatin expressions are significantly stronger in adjacent nontumorous tissues than in HCC tissue, and the expressions in nontumorous tissues correlate with HCC stages and expressions of VEGF [29]. Serum endostatin is found to have a significantly inverse correlation with the angiogenic score of HCC [30]. However, it is still controversial that endostatin expression or its serum level has a significant role in predicting the prognosis of patients with HCC [13,29–31].

Transcatheter arterial chemoembolization (TACE) is a palliative method for the management of HCC [32–36]. This procedure induces extensive ischemic necrosis in HCC. Ischemia strongly correlates with increased expression of angiogenic factors and stimulates angiogenesis. TACE can also increase VEGF expression in the residual surviving HCC tissues [37]. Li et al. [38] showed that plasma VEGF levels were significantly elevated in patients with HCC on the first post-TACE day. Then VEGF levels decreased gradually on the third day and had no statistical difference with pre-TACE levels on the seventh day post-TACE. This indicates that persistent elevation of VEGF level 7 days after TACE

may be of clinical significance. On the other hand, the possible influence of TACE on serum angiopoietin-2 and endostatin levels has not been clarified. This prospective study was to investigate the potential utilities of serial serum VEGF-A, angiopoietin-2, and endostatin level 1 day before and 7 days after TACE in cirrhotic patients with HCC.

Patients and methods

Patients

A total of 40 cirrhotic patients with newly discovered HCC planned to receive the first session of TACE were included (Table 1). Two patients with chronic hepatitis B infection started lamivudine therapy 2 months after TACE because of persistently elevated serum alanine aminotransferase (ALT, normal upper limit ≤ 40 IU/L) levels more than five times of normal upper limit. No patient with chronic hepatitis C infection received combined interferon and ribavirin therapy during the study periods. The diagnosis of HCC was based on fine-needle aspiration cytology and/or biopsy. HCC staging was based on the American Joint Committee on Cancer TNM staging system [39]. The reasons for 14 patients with HCC staging I to receive TACE rather than operative resection or local ablation therapy were because of larger tumor sizes (>5 cm) and ages >70 years in three patients and the larger tumor (5 cm) located near hepatic hilum in two patients. For the remaining nine patients with HCC staging I, the decisions to receive TACE alone were made by the patients after being informed the risks and benefits of TACE, operative resection, and local ablation therapy. The diagnosis of liver cirrhosis was based on a coarse and contracted liver on abdominal ultrasonography with at least one of the following two positive findings including (1) esophageal or cardiac varices found by endoscopic examination and (2) the presence of portal collateral circulation with or without splenomegaly on computed tomography. Chronic hepatitis B or C was diagnosed by persistently positive serum hepatitis B surface antigen or antihepatitis C antibody for more than 6 months. The etiology of cirrhosis and HCC was considered to be alcoholic in origin when patients had a history of alcohol consumption for more than 120 g alcohol per day for more than 10 years and no other known causes of chronic hepatitis. Child-Pugh classification was applied to record the hepatic reserved function. This study was approved by the institutional review board of our hospital (ClinicalTrials.gov NCT00834028). Each patient was given informed consent to participate in this study.

Methods

All patients received serum VEGF-A, angiopoietin-2, and endostatin measurements and complete blood count 1 day before and 7 days after TACE. The liver function test and

Table 1 Characteristics of 40 cirrhotic patients with newly discovered HCC planned to receive the first session of transcatheter arterial chemoembolization

Characteristics	Child-Pugh Class A	Child-Pugh Class B	Total
No. of patients	33	7	40
Sex (male/female) ^a	28/5	7/0	35/5
Age (yr) ^a	63, 44–77	57, 51–68	63, 44–77
Etiology^a			
Chronic hepatitis B	17	4	21
Chronic hepatitis C	12	3	15
Alcohol	2	0	2
Unknown	2	0	2
HCC staging^a			
I	14	0	14
II	9	3	12
IIIA	6	1	7
IIIC	3	2	5
IV	1	1	2

^a A *p* value >0.05.

Data are given as the median and range. HCC staging is based on the American Joint Committee on Cancer TNM staging system. The Wilcoxon rank sum test, Fisher exact test, or Chi-square test was applied for statistical analysis.

HCC = hepatocellular carcinoma.

serum alpha-fetoprotein (AFP) measurement were also concomitantly performed. Data obtained 7 days after TACE minus data obtained before TACE were used to calculate the differences of data in serial comparisons.

TACE was carried out by infusion of the mixture of chemotherapeutic agents (epirubicin; Pharmacia & Upjohn S.p.A, Milan, Italy, with or without mitomycin-C; Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan) and lipiodol (Lipiodol Ultra-Fluid; Guerbet, Aulnay-sous-Bois, France) into the lesion, followed by the embolization of the supplying arteries using various sizes of nonabsorbable Embosphere Microspheres (BioSphere Medical, Inc., Rockland, MA, USA) or absorbable agents (Avitene Microfibrillar Collagen Hemostat; MedChem Products, Inc., Woburn, MA, USA, or gelfoam particles of 1–2 mm in size, Pharmacia & Upjohn Company, Kalamazoo, MI, USA). The doses of chemotherapeutic agents and the amount of lipiodol depended on the size of the tumor. The choice of using nonabsorbable or absorbable embolic agent depended on the sizes and patterns of the supplying arteries. The procedure of TACE was completed when all detectable supplying arteries of the tumor and arteriportal shunt were embolized as detected by immediate post-TACE angiography. The whole procedure of TACE was carried out by an independent radiologist who did not know the contents of the study. All patients were followed for 3 months. The liver function test and serum AFP measurement were performed at an interval of 1 month, abdominal ultrasonography was performed 2–3 months after TACE, chest X-ray and computed tomography or magnetic resonance imaging were performed 3 months after TACE. Angiography was performed when evidence of

intrahepatic recurrence of HCC existed. The results of TACE were classified into rapid growth and without rapid growth of HCC after TACE in 2 groups. Rapid growth of HCC was defined as increase of tumor size >1 cm and/or development of intrahepatic or metastatic nodule, which was not detected on previous imaging studies.

All patients fasted overnight for blood sampling. The serum used for the study was collected by centrifugation of blood immediately withdrawn from the patient and stored at –20°C for further investigation. Serum levels of VEGF-A, angiopoietin-2, and endostatin were determined by enzyme-linked immunosorbent assay (VEGF-A; IBL-Hamburg, Hamburg, Germany and angiopoietin-2, endostatin; GlobalSpec, Inc., New York, NY, USA). The experimental procedures were carried out following the manufacturer's protocols. The detection limit for VEGF-A using this method was 32 pg/mL. The level below 32 pg/mL was defined as undetectable. The level of 32 pg/mL was used in patients with undetectable serum VEGF-A level for statistical calculation. The detection limits for angiopoietin-2 and endostatin were 8.29 pg/mL and 0.023 ng/mL, respectively.

Statistical analysis

Data were analyzed using JMP 7.0 software (SAS Institute, Cary, NC, USA). All data for continuous variables were expressed as median and range. The Wilcoxon rank sum test was used to compare the difference between medians of continuous variables. Because there were large variations in platelet counts and VEGF-A, angiopoietin-2, endostatin, and ALT levels among patients, the nonparametric two-sided sign test rather than the paired *t* test was used for the comparison of paired medians. The Fisher exact test or Chi-square test was used to compare proportions between groups. Correlation analysis was investigated by the calculation of a correlation coefficient (*r*). A receiver operating characteristic curve was used to establish the cutoff level that provided the maximal diagnostic accuracy. Stepwise logistic regression was used for multivariate analysis. The statistical significance was defined as *p* < 0.05.

Results

Data before TACE

There was no significant correlation among VEGF-A, angiopoietin-2, endostatin, AFP, and ALT levels.

VEGF-A

Patients with HCC staging >II showed higher VEGF-A level than patients with HCC staging ≤II (88.74 pg/mL, 32–751.9 pg/mL vs. 32 pg/mL, 32–1535.64 pg/mL, *p* < 0.05). This level did not show significant difference between Child-Pugh Class A and B. Twenty patients showed undetectable VEGF-A, and the remaining 20 patients showed detectable VEGF-A levels (Table 2). Patients with detectable VEGF-A levels had significantly higher proportion of HCC staging >II and higher angiopoietin-2 levels

Table 2 Comparison between patients with and without detectable serum vascular endothelial growth factor-A levels before transcatheter arterial chemoembolization

	Detectable (n = 20)	Undetectable (n = 20)	p
Age (yr)	62.5, 44–77	64, 53–76	>0.05
Sex (male/female)	18/2	17/3	>0.05
Child-Pugh Class (A/B)	15/5	18/2	>0.05
HCC staging (\leq II/ $>$ II)	10/10	16/4	<0.05
Angiopoietin-2 (pg/mL)	4974.6, 3301.9–9413.6	3921.9, 3041.6–6717.4	<0.05
Endostatin (ng/mL)	170.74, 139.94–213.36	164.59, 132.53–276.67	>0.05
AFP (ng/mL)	21.8, 3.2–8529.1	19.95, 1.05–1.502	>0.05
ALT (IU/L)	50, 14–186	42.5, 18–176	>0.05
Platelet counts ($\times 10^3/\mu\text{L}$)	135.5, 52–282	113.5, 53–235	>0.05

Data are given as the median and range. HCC staging is based on the American Joint Committee on Cancer TNM staging system. The Wilcoxon rank sum test, Fisher exact test, or Chi-square test was applied for statistical analysis. The normal ranges for platelet counts were $172\text{--}450 \times 10^3/\mu\text{L}$.

AFP = alpha-fetoprotein (normal upper limit ≤ 20 ng/mL); ALT = alanine aminotransferase (normal upper limit ≤ 40 IU/L); HCC = hepatocellular carcinoma.

than patients with undetectable VEGF-A levels. VEGF-A levels did not show significant correlation with platelet counts and age.

Angiopoietin-2

Patients with HCC staging $>$ II showed significantly higher angiopoietin-2 levels than patients with HCC staging \leq II (5326.3 pg/mL, 3301.9–9413.6 pg/mL vs. 3983.35 pg/mL, 3041.6–6717.4 pg/mL, $p < 0.05$). Patients with Child-Pugh Class B showed higher angiopoietin-2 levels than those with Child-Pugh Class A (6651.5 pg/mL, 3640.5–9413.6 pg/mL vs. 3981.1 pg/mL, 3041.6–6576.2 pg/mL, $p < 0.01$). Angiopoietin-2 levels did not show significant correlation with platelet counts and age.

Endostatin

Endostatin levels did not show significant difference between patients with HCC staging \leq II and $>$ II and between patients with Child-Pugh Class A and B. Endostatin levels showed significant correlation with platelet counts in all patients ($r = 0.38$, $p < 0.05$), in patients with Child-Pugh Class A ($r = 0.44$, $p < 0.05$), and in Child-Pugh Class A patients with undetectable VEGF-A ($r = 0.74$, $p < 0.001$). Endostatin levels also showed significant correlation with

age in patients with Child-Pugh Class A ($r = 0.36$, $p < 0.05$) and in Child-Pugh Class A patients with detectable VEGF-A levels ($r = 0.61$, $p < 0.05$).

Alterations of data after TACE

TACE caused significant increases of VEGF-A, angiopoietin-2, and ALT levels and significant decrease of platelet counts (Table 3). There was no significant correlation between differences of ALT levels and differences of platelet counts.

VEGF-A

Eighteen patients with detectable VEGF-A levels before TACE and seven patients with undetectable VEGF-A levels before TACE showed increased VEGF-A levels after TACE. Patients with detectable VEGF-A levels before TACE had higher incidence of increased VEGF-A levels after TACE (18 of 20) than patients with undetectable VEGF-A levels before TACE (7 of 20) ($p < 0.01$). Differences of VEGF-A levels showed negative correlation with differences of ALT levels in all patients ($r = -0.34$, $p < 0.05$) and in patients with Child-Pugh Class A ($r = -0.42$, $p < 0.05$). Differences of VEGF-A levels showed negative correlation with differences of platelet counts in all patients ($r = -0.5$, $p = 0.001$) and in patients with Child-Pugh Class B ($r = -0.9$, $p < 0.01$),

Table 3 Changes of data after transcatheter arterial chemoembolization in 40 patients

	Increase	Decrease	No change	p
VEGF-A (pg/mL)	25 (59.2, 14.1–230.7)	2 (6.08, 4.3–7.86)	13	<0.01
Angiopoietin-2 (pg/mL)	29 (606.1, 59–17643.3)	11 (298.7, 13.8–942)	0	0.01
Endostatin (ng/mL)	22 (11.65, 0.23–42.88)	18 (6.55, 0.12–51.02)	0	>0.1
ALT (IU/L)	35 (106, 1–485)	5 (9, 8–30)	0	<0.01
Platelet counts ($\times 10^3/\mu\text{L}$)	4 (2, 1–6)	36 (24, 1–154)	0	<0.01

Parentheses indicate median and range of changed data. The nonparametric two-sided sign test was used for statistical analysis.

ALT = alanine aminotransferase; VEGF = vascular endothelial growth factor.

but not in patients with Child-Pugh Class A ($p > 0.05$). Differences of VEGF-A levels had positive correlation with differences of angiopoietin-2 levels in all patients ($r = 0.59$, $p < 0.0001$) and in patients with Child-Pugh Class A ($r = 0.35$, $p < 0.05$) or B ($r = 0.86$, $p < 0.05$).

Angiopoietin-2

There was no significant correlation between differences of angiopoietin-2 levels and differences of ALT levels. Differences of angiopoietin-2 levels showed negative correlation with differences of platelet counts in all patients ($r = -0.7$, $p < 0.0001$) and in patients with Child-Pugh Class B ($r = -0.99$, $p < 0.0001$), but not in patients with Child-Pugh Class A ($p > 0.05$).

Endostatin

Differences of endostatin levels showed positive correlation with differences of ALT levels in all patients ($r = 0.33$, $p < 0.05$) and in patients with Child-Pugh Class A ($r = 0.37$, $p < 0.05$). There was no significant correlation between differences of endostatin levels and differences of platelet counts.

Follow-up

Twenty-five patients showed rapid growth of HCC within 3 months after TACE (Table 4) (Fig. 1A–C). Patients with rapid growth of HCC had significantly higher proportion of

HCC staging $>II$ and higher increment of VEGF-A levels after TACE than patients without rapid growth of HCC. There was only marginally significant difference of post-TACE VEGF-A levels between patients with and without rapid growth of HCC (127.6 pg/mL, 32–1628.86 pg/mL vs. 46.1 pg/mL, 32–485.29 pg/mL, $p = 0.057$). Difference of ALT levels or platelet counts caused by TACE did not showed significant difference between patients with and without rapid growth of HCC (89 IU/L, 314 to -8 IU/L vs. 106 IU/L, 485 to -30 IU/L, $p > 0.05$ for ALT; $-26 \times 10^3/\mu\text{L}$, 2 to $-154 \times 10^3/\mu\text{L}$ vs. $-20 \times 10^3/\mu\text{L}$, 6 to $-48 \times 10^3/\mu\text{L}$, $p > 0.05$ for platelet counts). Stepwise logistic regression analysis using statistical significantly items in Table 4, includes (1) increase of VEGF-A level >16.7 pg/mL selected by receiver operating characteristic curve (Fig. 2) and (2) HCC staging $>II$ as variables showed that only increase of VEGF-A level >16.7 pg/mL was an independent predictor for rapid growth of HCC (odds ratio 6.33, 95% confidence interval: upper 26, lower 1.54, $p < 0.05$; sensitivity 76%, specificity 66.7%, accuracy 72.5%, positive predictive level 79.2%, negative predictive level 62.5%, $p < 0.01$).

Discussion

The biologically active VEGF may be stored in platelet to prevent circulating VEGF from inducing the development of new blood vessels except at sites where coagulation takes place [40]. Application of serum rather than plasma samples to measure VEGF-A levels can be influenced by release of VEGF-A from destructed platelets during the

Table 4 Comparison between patients with and without rapid growth of HCC after TACE

	Rapid growth of HCC (+) ($n = 25$)	Rapid growth of HCC (–) ($n = 15$)	p
Age (yr)	65, 44–77	63, 55–76	>0.05
Sex (male/female)	22/3	13/2	>0.05
Child-Pugh Class (A/B)	20/5	13/2	>0.05
HCC staging ($\leq II$ / $>II$)	13/12	13/2	<0.05
Data before TACE			
VEGF-A (pg/mL)	57.79, 32–1535.64	32, 32–493.14	>0.05
Angiopoietin-2 (pg/mL)	4209.2, 3104.3–9413.6	4337.3, 3041.6–6651.5	>0.05
Endostatin (ng/mL)	167.04, 137.7–276.67	170.3, 132.53–227.04	>0.05
AFP (ng/mL)	32.5, 1.05–8529.1	14.1, 3.2–1022.7	>0.05
ALT (IU/L)	47, 14–171	44, 19–186	>0.05
Platelet counts ($\times 10^3/\mu\text{L}$)	135, 52–282	118, 53–233	>0.05
Differences of data caused by TACE			
VEGF-A (pg/mL)	-43.21 , -230.7 –(0)	0, -107.1 –(7.857)	<0.05
Angiopoietin-2 (pg/mL)	-391.4 , -17643.3 –(942)	-248.4 , -1055.9 –(637.9)	>0.05
Endostatin (ng/mL)	-2.14 , -23.26 –(51.02)	-2.22 , -42.88 –(9.85)	>0.05
ALT (IU/L)	-89 , -314 –(8)	-106 , -485 –(30)	>0.05
Platelet counts ($\times 10^3/\mu\text{L}$)	26, -2 –(154)	20, -6 –(48)	>0.05

Data are given as the median and range. HCC staging is based on the American Joint Committee on Cancer TNM staging system. The data obtained before TACE minus the data obtained 3 days after TACE were used to calculate the differences of data in serial determinations. The Wilcoxon rank sum test, Fisher exact test, or Chi-square test was applied for statistical analysis. The normal ranges for platelet counts were 172 – $450 \times 10^3/\mu\text{L}$.

AFP = alpha-fetoprotein (normal upper limit ≤ 20 ng/mL); ALT = alanine aminotransferase (normal upper limit ≤ 40 IU/L); HCC = hepatocellular carcinoma; TACE = transcatheter arterial chemoembolization; VEGF = vascular endothelial growth factor.

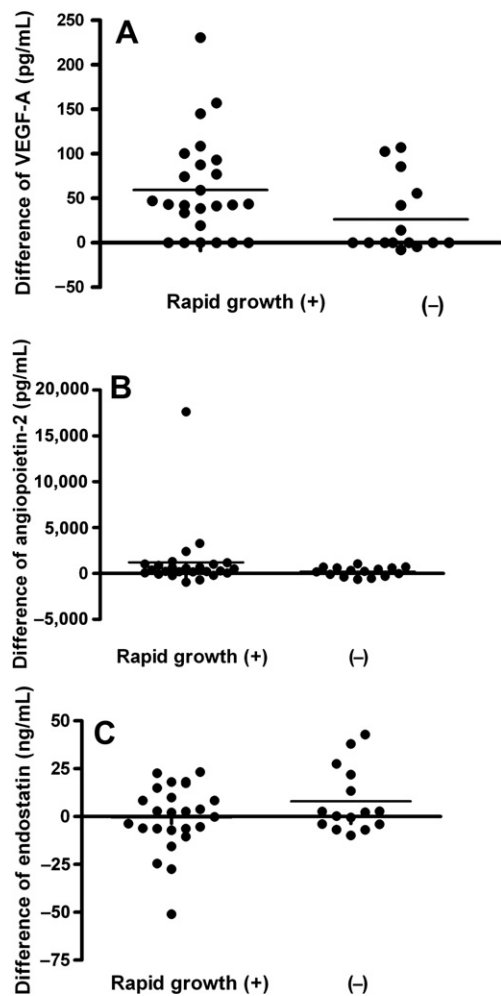


Figure 1. (A–C) Comparison of the differences of VEGF-A (1A), angiopoietin-2 (1B), and endostatin (1C) levels between patients with and without rapid growth of hepatocellular carcinoma. There was a significant difference in VEGF-A levels ($p < 0.05$). Data obtained 7 days after transcatheter arterial chemoembolization minus data obtained before transcatheter arterial chemoembolization were used to calculate the differences of data in serial determinations. VEGF-A = vascular endothelial growth factor-A.

collection of sample. This implies that the results of serum VEGF-A measurements can be influenced by platelet counts. Despite these potential pitfalls in serum VEGF-A measurement, previous studies showed that serum VEGF levels appeared to have significant predictive power for estimating overall survival in HCC and might be useful for defining prognosis in HCC [11,13–15]. This may be because of significantly strong correlation between serum and plasma VEGF levels [41]. Moreover, only measurement of serum rather than plasma VEGF-A levels can reflect the total amount of VEGF-A produced by the patient. Therefore, serum VEGF-A levels were adopted for analysis in the present study.

These results were in concordant with the previous studies that patients with more advanced HCC had higher serum VEGF levels [11,13–15] and also higher serum angiopoietin-2 levels compared with the patients with less

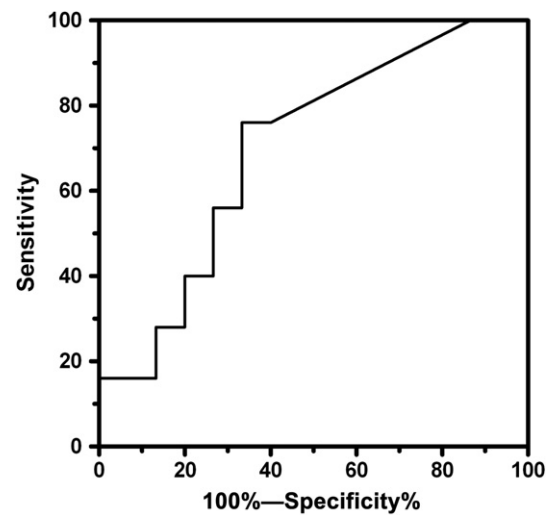


Figure 2. A receiver operating characteristic curve was used to establish the cutoff level of increased vascular endothelial growth factor-A level 7 days after transcatheter arterial chemoembolization to predict rapid growth of hepatocellular carcinoma within 3 months after transcatheter arterial chemoembolization with maximal diagnostic accuracy ($p < 0.05$).

advanced HCC. Although VEGF and angiopoietin-2 have been found to work cooperatively [1], our results did not reveal significant correlation between serum VEGF-A and angiopoietin-2 levels before TACE. This might be deduced from the present research that showed no significant difference of VEGF-A levels between patients with Child-Pugh Class A and B. On the contrary, high serum angiopoietin-2 levels were more frequently observed in patients with Child-Pugh Class B than in patients with Child-Pugh Class A. No correlation between serum VEGF-A levels and platelet counts may be because of half of our patients with undetectable VEGF-A levels before TACE. On the other hand, our results did not show significant association between serum endostatin levels and HCC staging. This result was in accordance with two previous studies [13,31], but different from one study [30]. This can be explained by that the serum endostatin levels are influenced by some confounding factors such as age, platelet counts, and serum VEGF-A levels of the patients as demonstrated in the present study.

Increase of serum ALT levels after TACE may partially reflect the damage of HCC and probably the adjacent nontumorous part of the liver caused by this procedure. Increase of ALT levels after TACE was found to correlate with decrease of VEGF-A levels and increase of endostatin levels. This implies that increase of tumor destruction by TACE decreases VEGF-A production from tumor, and increase of tissue damage may stimulate endostatin production. However, TACE did not cause significant difference in changes of serum endostatin levels. Different degrees of tissue damage caused by TACE among different patients may be the explanation. On the other hand, the present study showed that TACE actually caused significant increase rather than decrease of VEGF-A levels. This result was similar to the previous studies [42,43]. Increase of VEGF expression in the residual HCC tissues because of

TACE-induced ischemia may be the main explanation [37]. Negative correlation between differences of VEGF-A levels and differences of platelet counts suggests that release of VEGF-A from destructed platelets caused by TACE may be another explanation. Although the possible influence of TACE on serum angiopoietin-2 levels has not been clarified, the present results also showed increased angiopoietin-2 levels after TACE. Because there was also a negative correlation between differences of angiopoietin-2 levels and differences of platelet counts, TACE-induced ischemia and release of angiopoietin-2 from destructed platelets caused by TACE may be explanations.

The American Joint Committee on Cancer TNM staging system used in the present study was reported to provide the most effective means of assessing the prognosis of patients after curative resection of HCC [44]. However, our results did not show that TNM HCC staging was an independent predictor for rapid growth of HCC after TACE. The major reason is that all HCC staging systems can only be used to estimate the degree of tumor invasion at the time of discovery. Patients with higher HCC staging having worse prognosis than patients with lower HCC staging is because of no satisfactory treatment for patients with higher HCC staging. These staging systems are not used to predict the speed of tumor growth. The characteristics of residual cancer cells after treatment and the presence of stimulating or inhibiting factors of the patient are major determinants for rapid growth of HCC after TACE. Patients with slow growing cancer cells do show slow growth of HCC after TACE regardless of the effects of TACE unless the characteristics of cancer cells are changed caused by TACE. Therefore, the present study did not choose items such as size of tumors, number of tumors, vascular invasion, and technique for TACE to predict rapid growth of HCC. Increase of serum VEGF levels after TACE can directly promote angiogenesis in the residual HCC tissue [37,38]. Although the present study showed that patients with detectable serum VEGF-A levels before TACE had higher incidence of increased serum VEGF-A levels after TACE than patients without detectable VEGF-A levels, there was no significant cutoff serum VEGF-A level before TACE to predict rapid growth of HCC after TACE. This is caused by the influence of diverse effects of TACE on different patients with HCC. Shim and et al. [43] showed that the related change in serum VEGF/platelet counts ratio 1–2 days after TACE from baseline >0.5 was significantly correlated with newly developed extrahepatic metastases 1 and 6 months after TACE. It is demonstrated that negative correlation between differences of platelet counts and differences of VEGF-A or angiopoietin-2 levels caused by TACE mainly originated from Child-Pugh Class B but not from Child-Pugh Class A. This implies using platelet counts to correct for VEGF levels may be affected by Child-Pugh class. Therefore, we adopt another method to predict rapid growth of HCC after TACE. The serum or plasma VEGF levels 1–2 days after TACE in most patients are elevated [38,42,43]. Because VEGF can be stored in circulating platelets, which have life span of around 3 days, measurement of total VEGF production within 5 days after TACE should theoretically consider the influence by the platelet counts. The influence of TACE-induced ischemia on VEGF production will subside around 7 days after TACE [38].

This implies that measurement of serum VEGF-A level 7 days after TACE alone may be used to reflect the characteristics of residual cancer cells in the production of VEGF-A. However, the results showed that the range of VEGF-A levels before TACE was quite wide. Decreased post-TACE VEGF-A levels in patients with high pre-TACE levels may still be higher than increased post-TACE VEGF-A levels in patients with low pre-TACE levels. Therefore, measurement of serum VEGF-A level 7 days after TACE alone cannot actually reflect the influence of TACE on the production of VEGF-A from the residual cancer cells. On the contrary, comparison between serum VEGF-A levels before and 7 days after TACE can attenuate the impact from variations in baseline VEGF-A levels and reflect the influence of TACE on the production of VEGF-A from the residual cancer cells. Patients with remarkable increase of serum VEGF-A levels 7 days after TACE have risk of rapid growth of HCC after TACE and should be closely followed.

The present results failed to show difference of angiopoietin-2 as predictor for rapid growth of HCC within 3 months after TACE. Small number of patients in the present study may be one of the reasons. Because patients with Child-Pugh Class B had significantly higher angiopoietin-2 levels than patients with Child-Pugh Class A, influence of different degrees of Child-Pugh classification among patients may be another reason. Further study may be necessary to clarify this issue.

In conclusion, the present results indicate that TACE causes significant increases of serum VEGF-A and angiopoietin-2 levels. The pattern of change in endostatin was not significantly influenced by TACE. Serial serum VEGF-A measurements 1 day before and 7 days after TACE may have potential to predict rapid growth of HCC after TACE.

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