VEGF in Patients with Advanced Hepatocellular Carcinoma Receiving Intra-arterial Chemotherapy

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Abstract. *Aim: Vascular endothelial growth factor (VEGF) is a primary driving force for both physiological and pathological angiogenesis and over-expression of VEGF has been detected in hepatocellular carcinoma (HCC). The aim of the present study was to clarify the usefulness of VEGF for monitoring the response to intra-arterial chemotherapy in patients with HCC. Patients and Methods: Seventy-three patients with liver cirrhosis (LC) and advanced HCC (aHCC) received hepatic arterial infusion chemotherapy (HAIC: leucovorin (LV) at 12 mg/h, cisplatin (CDDP) at 10 mg/h and 5-fluorouracil (5-FU) at 250 mg/22 h) via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneous drug delivery system. Results: i) Serum VEGF levels were higher in patients with progressive disease than those in patients with a partial response or stable disease. ii) VEGF levels were higher in patients with alcoholic LC than those in patients with hepatitis C-related or hepatitis B-related LC. iii) VEGF levels were higher in stage IVB patients than those in patients with stage III or IVA disease. iv) VEGF levels were significantly higher in patients with giant or confluent multinodular tumors than those in patients with multiple discrete nodules. v) Serum VEGF levels were higher in patients with vascular invasion than in patients without vascular invasion. Conclusion: Monitoring the serum VEGF level is useful for predicting the response of aHCC to HAIC, as well as for predicting metastasis, tumor type and vascular invasion.*

Sorafenib is an oral multikinase inhibitor with strong in vitro activity related to targeting the Raf/mitogen-activated protein kinase/extracellular signal-related kinase signaling pathway and has been used to treat advanced hepatocellular carcinoma (aHCC). In the Sorafenib HCC Assessment Randomized Protocol (SHARP) study, 602 patients (mainly Europeans) were randomized to receive sorafenib or placebo therapy. They had an Eastern Cooperative Oncology Group performance status of 0 - 2 and were all in Child-Pugh class A. The sorafenib group achieved a median overall survival time of 10.7 months *versus* 7.9 months for the placebo group (1). Sorafenib has also demonstrated significant clinical activity against HCC in phase II and phase III studies (2, 3), with sorafenib treatment achieving a longer median survival time and longer time to radiologic progression compared with placebo. However, Pinter *et al*. reported that complications of sorafenib therapy may occur more frequently in patients who are in Child-Pugh classes B or C (4). In the most recent Japan Society of Hepatology (JSH) treatment algorithm for HCC (2014 update), sorafenib and hepatic arterial infusion chemotherapy (HAIC) are recommended for HCC patients with Vp1, Vp2 (minor portal vein invasion) or Vp3 (portal invasion at the 1st portal branch). In contrast, sorafenib is not recommended for HCC patients with Vp4 (portal invasion at the main portal branch), whereas HAIC is recommended for such patients with tumor thrombus in the main portal brunch (5). Therefore, HAIC is still one of the few remaining options for treating aHCC in liver cirrhosis (LC) patients who are in Child-Pugh class A or B, as well as LC patients with Vp3 or Vp4. Improvement of implanted drug delivery systems has made it possible to perform repeated hepatic arterial infusion of anticancer agents in patients with aHCC, while HAIC has been found to improve both survival and quality of life (6). HAIC with 5-fluorouracil (5-FU) and cisplatin (CDDP), using an infuser pump and an implanted reservoir, has been shown to prolong the survival of patients with aHCC (6-8). We have also reported that the combination of intra-arterial low-dose 5-FU, CDDP and leucovorin (LV) prolongs the survival of aHCC patients (9) and that continuous intra-arterial infusion for 24 h is more effective than infusion for 6 h in patients with HCV-related LC and aHCC, although a 24-h infusion also causes stronger hematologic toxicity (10).

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Table I. *Seventy-three patients with liver cirrhosis and advanced HCC.*

	Control	CR/PR	SD	PD	*p*
No. of patients	28	21	32	20	
Mean age	61.4±9	68.4±6	67.7±8	64.7±8	0.401
Gender (M/F)	17/11	19/2	28/4	17/3	0.869
Child-Pugh classification (A/B/C)	16/7/5	11/10/0	11/17/4	8/8/4	0.234
Stage (III/IVA/IVB)		4/14/3	3/25/4	2/11/7	0.198
Type of HCC (multiple/diffuse/giant)		15/5/1	19/7/6	10/6/4	0.294
Vascular invasion (Vp3/Vp4)		2/4	6/4	3/4	0.572

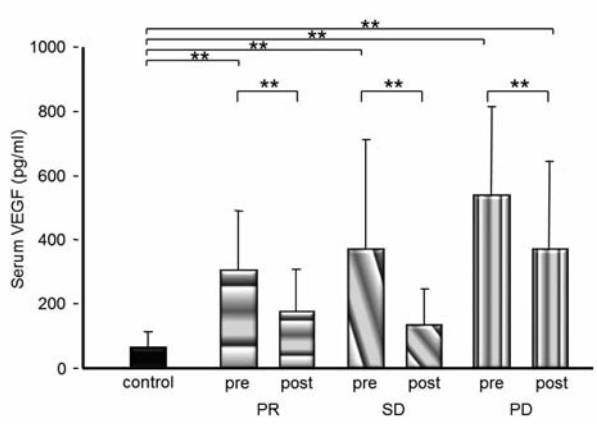

Figure 1. *Serum level of VEGF and the etiology of aHCC in LC patients treated by HAIC.* $^{**}p<0.01$

Vascular endothelial growth factor (VEGF) is a primary driving force for both physiological and pathological angiogenesis (11) and its overexpression has been observed in HCC (12, 13). The circulating VEGF level is reported to be correlated with the stage of HCC and the highest levels are observed in patients with metastasis (14). In addition, VEGF may be a significant predictor of the response to treatment and the clinical outcome of HCC (15-17). Accordingly, the present retrospective study was performed to assess the usefulness of VEGF for monitoring the response to HAIC in patients with aHCC.

Patients and Methods

Patients. Seventy-three adult Japanese patients who had aHCC and LC (hepatitis B virus (HBV)-, hepatitis C virus (HCV)- or alcohol-related) received HAIC at our hospital between 2004 and 2011. Their tumors were classified as inoperable on the basis of computed tomography (CT) findings. Blood samples were collected in the early morning before and after chemotherapy. The control group consisted of 28 adult Japanese patients with HCV-related LC and no evidence of HCC.

Chemotherapy. HAIC involved 24-h intra-arterial infusion of LV at 12 mg/h, CDDP at 10 mg/h and 5-FU at 250 mg/m^2/22 h. Continuous infusion was performed into the proper hepatic artery every 5 days for 4 weeks *via* a catheter connected to a subcutaneously implanted drug delivery system (9, 10).

In each patient, an intra-arterial catheter was inserted *via* the femoral artery and was attached to a subcutaneous reservoir (18). The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the anticancer agents. Written informed consent was obtained from each patient.

Serum VEGF measurement. Venous blood samples were drawn into a serum separator tube and centrifuged at 1,800 ×g for 10 min to obtain serum samples that were stored at –80˚C. Serum VEGF concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions (19). The VEGF concentration in each sample was measured in duplicate by an investigator who was blinded to the clinical information of the patients.

Response and endpoints. The response of each tumor was assessed using the Response Evaluation Criteria in Solid tumors (RECIST) (20) from CT scans obtained at baseline and 4 weeks after treatment.

The primary endpoint of this study was the response rate according to World Health Organization (WHO) criteria, while the secondary endpoint was the toxicity of HAIC.

Statistical Analysis. Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS, version 11.0; SPSS, Chicago, IL, USA). Results are expressed as the mean±standard deviation (SD). The Wilcoxon's signed rank sum test was used to compare patient's characteristics within each group, while the Dunnett's test was employed for comparisons between the control and treated groups. A probability of less than 0.05 was considered to indicate statistical significance.

Results

The patients were divided into three groups based on the response to HAIC. Twenty-one of the 73 patients showed a complete or partial response (PR group) and 32 patients had stable disease (SD group), while 20 patients showed no response (progressive disease (PD) group). There were 19 men and 2 women aged 59 to 83 years (mean±SD: 68.4+6 years) in the PR group, 28 men and 4 women aged 54 to 80 years (67.7±8 years) in the SD group and 17 men and 3 women aged 51 to 77 years (64.7±8 years) in the PD group. In the PR group, 13 patients had HCV-related LC (C-LC), two patients had HBV-related LC (B-LC) and six patients had alcoholic LC (ALC). In the SD group, 16 patients had C-LC, 6 patients had B-LC and 10 patients had ALC, while the PD group included 8 patients with C-LC, 9 patients with

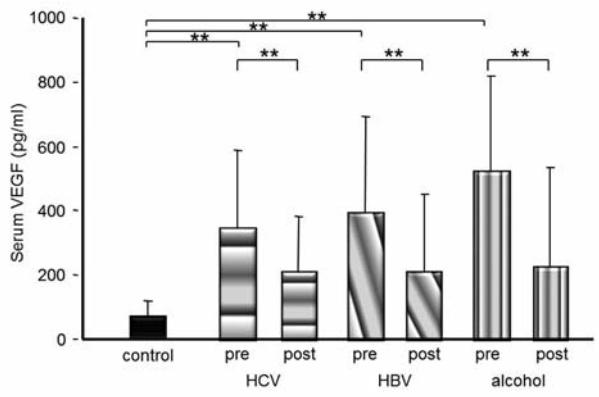

Figure 2. *Serum level of VEGF and the etiology of LC in patients with aHCC treated by HAIC.* $^{**}p<0.01$.

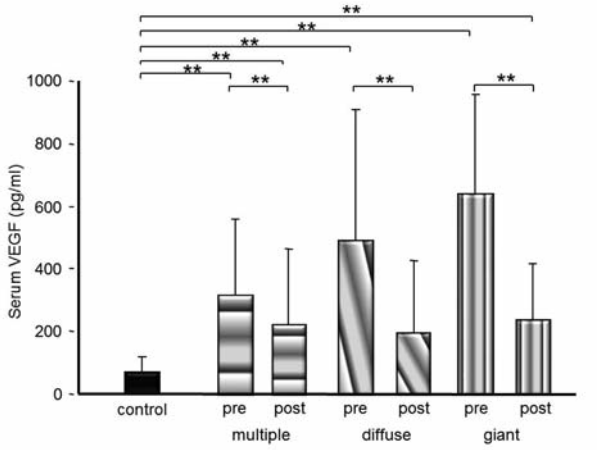

Figure 3. *Serum level of VEGF and the tumor type in LC patients with aHCC treated by HAIC.* $^{**}p<0.01$

B-LC and 3 patients with ALC. The Child-Pugh class was A for 11 patients in the PR group, 11 patients in the SD group and 8 patients in the PD group, while it was B for 10, 17 and 8 patients, respectively, and C for 4 patients each from the SD and PD groups. In the PR group, four patients had stage III disease, 14 patients had stage IVA disease and three patients had stage IVB disease. In the SD group, 3 patients had stage III disease, 25 patients had stage IVA disease and 4 patients had stage IVB disease, while the respective numbers were 2, 11 and 7 in the PD group. In the PR group, 15 patients had multiple HCC, five patients had diffuse HCC and one patient had giant HCC. In the SD group, 19 patients had multiple HCC, 7 patients had diffuse HCC and 6 patients had giant HCC, while the respective numbers in the PD group were 10, 6 and 4 patients. In the PR group, two patients had involvement of major branches of the portal vein and four patients had thrombus in the portal trunk, while the respective numbers were 6 and 4 in the SD group and 3 and 4 in the PD group. The control group was composed of 17 men and 11 women aged 48-78 years (61.4±9 years). The Child-Pugh class was A for 16 patients, while it was B for 7 patients and C for 5 patients (Table I).

Serum level of VEGF and tumor response. Figure 1 shows the serum level of VEGF in relation to the response of aHCC treated to HAIC. The VEGF level was significantly lower after chemotherapy (PR group: 159.8±109 pg/ml; SD group: 176.5±260 pg/ml; PD group: 281.9±245 pg/ml) than before chemotherapy (PR group: 317.8±220 pg/ml; SD group: 352.2±338 pg/ml; PD group: 471.3±310 pg/ml) in each response group ($p<0.01$ by the Wilcoxon's signed rank sum test). Before chemotherapy, the serum level of VEGF was significantly higher in each group of aHCC patients than in the control group (64.4±58 pg/ml) ($p<0.01$ by the Dunnett's test). After chemotherapy, the VEGF level of the PD group was still significantly higher than that of the control group ($p<0.01$, Dunnett's test) but there was no significant difference of VEGF levels between the control group and the PR or SD groups (Figure 1). These results indicate that the serum level of VEGF was not normalized after chemotherapy in the PD group, although it was significantly decreased by HAIC.

Serum level of VEGF and the etiology of LC. Figure 2 shows the serum level of VEGF in relation to the etiology of LC. The VEGF level was significantly lower after chemotherapy (HCV: 184.5±139 pg/ml; HBV: 213.3±251 pg/ml; alcohol: 218.8±333 pg/ml) than before chemotherapy (HCV: 313.0±216 pg/ml; HBV: 390.1±304 pg/ml; alcohol: 468.2±434 pg/ml) in each etiologic sub-group ($p<0.01$ Wilcoxon's signed rank sum test). There was no significant difference of the serum VEGF level after chemotherapy between the control group and each etiologic subgroup, although the VEGF level in each subgroup of aHCC patients was significantly higher than that of the control group before chemotherapy ($p<0.01$, Dunnett's test) (Figure 2). These results indicate the serum level of VEGF was not related to the etiology of LC in patients receiving HAIC for aHCC.

Serum level of VEGF and tumor type. Figure 3 displays the serum level of VEGF in relation to the type of HCC. In patients with each type of tumor, VEGF levels were significantly lower after chemotherapy (multiple: 224.1±265 pg/ml; diffuse: 107.7±83 pg/ml; giant: 261.5±201 pg/ml) than before chemotherapy (multiple: 310.0±259 pg/ml; diffuse: 318.1±283 pg/ml; giant: 714.6±310 pg/ml) ($p<0.01$, Wilcoxon's signed rank sum test). Before chemotherapy, the VEGF level of the patients with each tumor type was significantly higher than that in the control group ($p<0.01$,

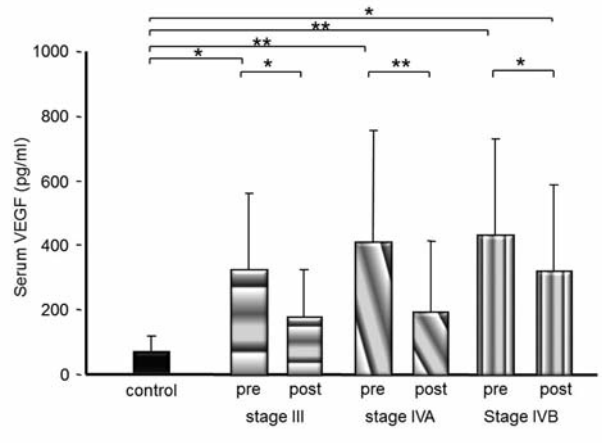

Figure 4. *Serum level of VEGF and the tumor stage in LC patients with aHCC treated by HAIC. *p<0.05, **p<0.01.*

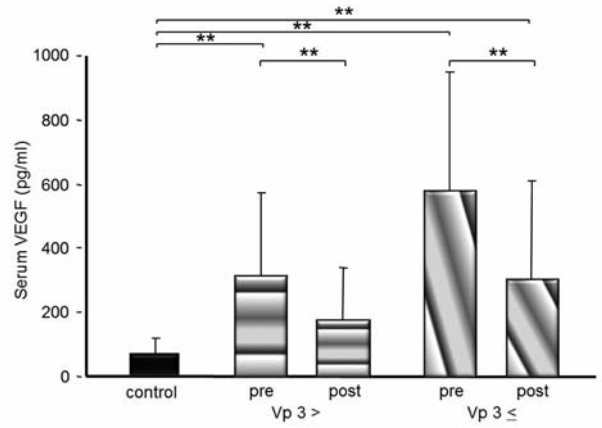

Figure 5. *Serum level of VEGF and vascular invasion in LC patients with aHCC treated by HAIC. **p<0.01*

Dunnett's test). After chemotherapy, VEGF levels were still significantly higher in the patients with multiple and giant tumors than in the control group ($p<0.01$, Dunnett's test) but there was no significant difference of VEGF between the control group and the patients with diffuse tumors (Figure 3). These results indicate that VEGF was not normalized after chemotherapy in patients with multiple and giant tumors, although it was significantly decreased by HAIC.

Serum level of VEGF and tumor stage. Figure 4 summarizes serum VEGF levels in relation to the tumor stage. In patients with tumors of each stage, the serum level of VEGF was significantly lower after chemotherapy (stage III: 177.6±145 pg/ml; stage IVA: 160.1±209 pg/ml; stage IVB: 320.1±268 pg/ml) than before chemotherapy (stage III: 323.0±237 pg/ml; stage IVA: 361.3±318 pg/ml; stage IVB: 431.8±298 pg/ml) ($p<0.01$, Wilcoxon's signed rank sum test). Before chemotherapy, serum VEGF levels were significantly higher in patients with tumors of each stage than in the control group ($p<0.01$, Dunnett's test). After chemotherapy, VEGF levels were still significantly higher in patients with stage of IVB tumors than in the control group ($p<0.01$, Dunnett's test) but there were no significant differences of VEGF between the control group and patients with stage III or stage IVA tumors (Figure 4). These results indicate that the serum VEGF level was not normalized after chemotherapy in patients with stage IVB tumors, although it showed a significant decrease after HAIC.

Serum level of VEGF and vascular invasion. Figure 5 summarizes the relation between the serum level of VEGF and vascular invasion. Serum VEGF levels were significantly lower after chemotherapy (Vp3>: 166.7±161 pg/ml; Vp<3: 282.3±321 pg/ml) than before chemotherapy (Vp3>: 312.2±266 pg/ml; Vp≤3: 517.1±335 pg/ml) in patients from each vascular invasion subgroup ($p<0.01$ by the Wilcoxon's signed rank sum test). Before chemotherapy, the serum level of VEGF was significantly higher in both vascular invasion subgroups than in the control group ($p<0.01$ by the Dunnett's test). After chemotherapy, the serum VEGF level the Vp3≤ subgroup was still significantly higher than that of the control group ($p<0.01$ by the Dunnett's test) but there was no significant difference of VEGF levels between the control group and the Vp3> sub-group (Figure 5). These results indicate that VEGF was not normalized after chemotherapy in the Vp3≤ subgroup, although it decreased significantly after HAIC.

Discussion

The advent of molecular-targeting agents has revolutionized the therapeutic strategy for LC patients with aHCC as liver transplantation is almost completely unavailable. However, it was reported that HAIC is a potentially useful treatment procedure in patients with aHCC even after failure of sorafenib for the reason that it was well tolerated and exhibited moderate antitumor activity (21). HAIC may still occupy the important location as one way of the treatment in LC patients with aHCC. We reported that the serum levels of VEGF might be a useful predictor of the presence of HCC in patients with C-LC, while serum levels of alpha-fetoprotein and VEGF can predict the tumor type and vascular invasion, respectively (22). Therefore, investigation of the monitoring of VEGF in LC patients with aHCC receiving HAIC was considered to be worthwhile. In the present retrospective study, we assessed the usefulness of VEGF for monitoring the response of aHCC. Serum levels of VEGF were significantly lower after chemotherapy than before chemotherapy in each response

group. After HAIC, however, the VEGF level of the PD group was still significantly higher than that of the control group, while there was no significant difference of VEGF levels between the control group and the PR or SD groups. El-Assal *et al*. reported lower VEGF protein expression in HCC than in the corresponding nontumorous liver (23). However, it was also reported that the vascular endothelial cells in tumor tissues show strong immunostaining for VEGF, whereas those in nontumorous tissues do not show appreciable staining and that tumorous vascular endothelial cells are the main targets of VEGF released from HCC cells (24, 25). In addition, Mise *et al*. reported that VEGF is involved in neovascularization and infiltration of cancer cells into the tumor capsule in HCC patients (26). We found that the serum level of VEGF was not normalized after HAIC in the PD group suggesting that changes of VEGF are useful for predicting the response of aHCC to HAIC. However, the serum level of VEGF was significantly lower after HAIC than before chemotherapy in the PD group, although it was not normalized. Paracrine communication between hepatocytes and hepatic sinusoidal endothelial cells *via* VEGF and its receptor has been reported (27). We previously reported that HAIC might cause hepatotoxicity that induces fibrosis without releasing aminotransferases and suggested that HAIC could possibly damage endothelial cells (28). In the present study, VEGF levels might have decreased after chemotherapy in the PD group due to the toxicity of HAIC on endothelial cells.

Furthermore, we examined factors related to normalization of VEGF by HAIC. We found that the serum level of VEGF was not normalized after HAIC in patients with stage IVB, multiple or giant tumors and those with Vp3≤ vascular invasion. It was reported that early HCC (Edmondson-Steiner grade 1) is occasionally hypovascular on angiography or CT arteriography (29) and that the number of arteries in a hepatic nodule increases during progression from adenomatous hyperplasia to atypical adenomatous hyperplasia and then HCC (30). Stroescu *et al*. reported that over-expression of VEGF was more frequent in large HCCs than small HCCs and that VEGF expression was markedly stronger in patients with poorly-differentiated HCC (31). These reports support our results that the serum level of VEGF was not normalized after HAIC in patients who had multiple or giant tumors. Stroescu *et al*. also indicated that multiple or giant HCC show much more neovascularization than diffuse HCC. Shim *et al*. reported that a marked increase in the serum level of VEGF at 1-2 days after transcatheter arterial chemoembolization for HCC was associated with distant metastasis or vascular invasion (32), while Stroescu *et al*. reported that over-expression of VEGF in patients with poorly differentiated HCC was correlated with a high recurrence rate and short postoperative survival (31). In the present study, the serum VEGF level of patients who had stage IVB tumors or had Vp3≤ vascular invasion was not normalized after treatment of HAIC and this might have been because of neovascularization in extrahepatic tumors that HAIC did not reach. Also, our results indicated that stage IVB tumors and tumors with Vp3≤ vascular invasion show more prominent neovascularization than stage III and IVA tumors or those with Vp3> vascular invasion.

Conclusion

Our results suggest that monitoring the serum VEGF level is useful for predicting the response, metastasis, tumor type and vascular invasion in LC patients receiving HAIC for aHCC.

References

1 Llover JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D and Bruix J: SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med *359*: 378-390, 2008

2 Moreno-Aspitia, A, Morton RF, Hillman DW, Lingle WL, Rowland KM Jr, Wiesenfeld M, Flynn PJ, Fitch TR and Perez EA: Phase II trial of sorafenib in patients with metastatic breast cancer previously exposed to anthracyclines or taxanes: North Central Cancer Treatment Group and Mayo Clinic Trial N0336. J Clin Oncol *27*: 11-15, 2009.

3 Abou-Alfa GK, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M and Saltz LB: Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol *24*: 4293-4300, 2006.

4 Pinter M, Sieghart W, Graziadei I, Vogel W, Maieron A, Königsberg R, Weissmann A, Kornek G, Plank C and Peck-Radosavljevic M: Sorafenib in unresectable hepatocellular carcinoma from mild to advanced stage liver cirrhosis. Oncologist *14*: 70-76, 2009.

5 Kudo M, Matsui O, Izumi N, Iijima H, Kadoya M, Imai Y, Okusa T, Miyama S, Tsuchiya K, Ueshima K, Hiraoka A, Ikeda M, Ogasawara S, Yamashita T, Minami T and Yamakado K: JSH consensus-based clinical practice guidelines for the management of hepatocellular carcinoma: 2014 update by the liver cancer study group of Japan. Liver Cancer *3*: 458-468, 2014.

6 Toyoda H, Nakano S, Kumada T, Takeda I, Sugiyama K, Osada T, Kirishima S, Suga T and Takahashi M: The efficacy of continuous local arterial infusion of 5-fluorouracil and cisplatin through an implanted reservoir for severe advanced hepatocellular carcinoma. Oncology *52*: 295-299, 1995.

7 Murata K, Shiraki K, Kawakita T, Yamamoto N, Okano H, Nakamura M, Sakai Takahisa, Deguchi M, Ohmori S and Nakano T: Low-dose chemotherapy of cisplatin and 5-fluorouracil or doxorubicin *via* implanted fusion port for unresectable hepatocellular carcinoma. Anticancer Res *23*: 1719-1722, 2003.

8 Okuda K, Tanaka M, Shibata J, Ando E, Ogata T, Kinoshita H, Eriguchi N, Aoyagi S and Takikawa K: Hepatic arterial infusion chemotherapy with continuous low dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. Oncology Report *6*: 587-591, 1999.

9 Nagai H and Sumino Y: Therapeutic strategy of advanced hepatocellular carcinoma by using combined intra-arterial chemotherapy. Recent Pat Anticancer Drug Discov *3*: 220-226, 2008.
10 Nagai H, Kanayama M, Higami K, Momiyama K, Ikoma A, Okano N, Matsumaru K, Watanabe M, Ishii K, Sumino Y and Miki K: Twenty-four hour intra-arterial infusion of 5-fluorouracil, cisplatin, and leucovorin is more effective than 6-hour infusion for advanced hepatocellular carcinoma. World J Gastroenterol Jan 14; *13(2)*: 280-284, 2007.
11 Semela D and Dufour JF: In: Vascular endotherial growth factor signaling. In: Signaling Pathways in Liver Diseases. Dufour JF, Calvien PA (eds.). Springer-Verlag Berlin Germany, pp. 91-104, 2005.
12 Miura H, Miyazaki T, Kuroda M, Oka T, Machinami R, Kodama T, Shibuya M, Makuuchi M, Yazaki Y and Ohnishi S: Increased expression of vascular endotherial growth factor in human hepatocellular carcinoma. J Hepatol *27*: 854-861, 1997.
13 Yamaguchi R, Yano H, Iemura A, Ogasawara S, Haramaki M and Kojiro M: Expression of vascular endothelial growth factor in human hepatocellular carcinoma. Hepatology *28*: 68-77, 1998.
14 Jinno K, Tanimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Doi T, Endo H, Yamashita T and Okada Y: Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. J Gastroenterol *33*: 376-382, 1998.
15 Poon RT, Ng IO, Lau C, Zhu LX, Yu WC, Lo CM, Fan ST and Wong J: Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: A prospective study. Ann Surg *233*: 227-235, 2001.
16 Chao Y, Li CP, Chau GY, Chen CP, King KL, Lui WY, Yen SH, Chang FY, Chan WK and Lee SD: Prognostic significance of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin in patients with resectable hepatocellular carcinoma after surgery. Ann Surg Oncol *10*: 355-362, 2003.
17 Poon RT, Lau C, Yu WC, Fan ST and Wong J: High serum levels of vascular endothelial growth factor predict poor response to transarterial chemoembolization in hepatocellular carcinoma: a prospective study. Oncol Report *11*: 1077-1084, 2004.
18 Iwamiya T, Sawada S and Ohta Y: Repeated arterial infusion chemotherapy for inoperable hepatocellular carcinoma using an implantable drug delivery system. Cancer Chemother Pharmacol *33*: S134-138, 1994.
19 Kong SY, Park JW, Lee JA, Park JE, Park KW, Hong EK and Kim CM: Association between vascular endothelial growth factor gene polymorphisms and survival in hepatocellular carcinoma patients. Hepatology *46*: 446-55, 2007.
20 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*: 228-247, 2009.
21 Terashima T, Yamashita T, Arai K, Sunagozaka H, Kitahara M, Nakagawa H, Kagaya T, Mizukoshi E, Honda M and Kaneko S: Feasibility and efficacy of hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma after sorafenib. Hepatol Res *44*: 1179-1185, 2014.
22 Mukozu T, Nagai H, Matsui D, Kanekawa T and Sumino Y: Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. Anticancer Res 33:1013-1021, 2013.
23 El-Assal ON, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T and Nagasue N: Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. Hepatology *27*: 1554-1562, 1998.
24 Plate KH, Breier G, Weich HA and Risau W: Vascular endotherial growth factor is a potential angiogenesis factor in human gliomas in vivo. Nature *359*: 845-848, 1992.
25 Brown LF, Berse B, Tognazzi K, Manseau EJ, Van De Water L, Senger DR, Dvorak HF and Rosen S: Vascular permeability factor mRNA and protein expression in human kidney. Kidney Int *42*: 1457-1461, 1992.
26 Mise M, Arii S, Higashituji H, Furutani M, Niwano M, Harada T, Ishigami S, Toda Y, Nakayama H, Fukumoto M, Fujita J and Imamura M: Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology *23*: 455-464, 1996.
27 Yamane A, Seetharam L, Yamaguchi S, Gotoh N, Takahashi T, Neufeld G and Shibuya M: A new communication system between hepatocytes and sinusoidal endothelial cells in liver through vascular endothelial growth factor and Flt tyrosine kinase receptor family (Flt-1 and KDR/Flk-1). Oncogene *9*: 683-2690, 1994.
28 Nagai H, Matsui T, Kanayama M, Momiyama K, Shiozawa K, Wakui N, Shinohara M, Watanabe M, Iida K, Ishii K, Igarashi Y and Sumino Y: Hepatotoxicity of intra-arterial combination chemotherapy in patients with liver cirrhosis and advanced hepatocellular carcinoma. Cancer Chemother Pharmacol *66*: 1123-1129, 2010.
29 Takayasu K, Shima Y, Muramatsu Y, Goto H, Moriyama N, Yamada T, Makuuchi M, Yamasaki S, Hasegawa H, Okazaki N, Hirohashi S and Kishi K: Angiography of small hepatocellular carcinomas: analysis of 105 resected tumors. Am J Roentgenol *147*: 525-529, 1986.
30 Ueda K, Terada T, Nakamura Y and Matsui O: Vascular supply in adenomatous hyperplasia of the liver and hepatocellular carcinoma: a morphometric study. Hum Pathol *23*: 619-626, 1992.
31 Stroescu C, Dragnea A, Ivanov B, Pechianu C, Herlea V, Sgarbura O, Popescu A and Popescu I: Expression of p53, Bcl-2, VEGF, Ki67 and PCNA and prognostic significance in hepatocellular carcinoma. J Gastrointestin Liver Dis *17*: 411-417, 2008.
32 Shim JH, Park JW, Kim JH, An M, Kong SY, Nam BH, Choi JI, Kim HB, Lee WJ and Kim CM: Association between increment of serum VEGF level and prognosis after transcatheter arterial chemoembolization in hepatocellular carcinoma patients. Cancer Sci *99*: 2037-2044, 2008.

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