Monitoring serum proangiogenic cytokines from hepatocellular carcinoma patients treated with sorafenib

Takuya Adachi[1], Kazuhiro Nouso[1,2], Koji Miyahara[3], Atsushi Oyama[1], Nozomu Wada[1], Chihiro Dohi[1], Yasuto Takeuchi[1], Tetsuya Yasunaka[1], Hideki Onishi[1], Fusao Ikeda[1], Shinichiro Nakamura[1], Hidenori Shiraha[1], Akinobu Takaki[1], Hiroyuki Takabatake[4], Shin-ichi Fujioka[5], Haruhiko Kobashi[6], Yoshitaka Takuma[3], Shouta Iwadou[3], Shuji Uematsu[3], Koichi Takaguchi[7], Hiroaki Hagihara[8], Hiroyuki Okada[1] on behalf of the Okayama Liver Cancer Group.

[1]Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, [2]Okayama City Hospital, [3]Hiroshima City Hospital, [4]Kurashiki Central Hospital, [5]Okayama Saiseikai General Hospital, [6]Okayama Red Cross General Hospital, [7]Kagawa Prefectural Central Hospital, [8]Sumitomo Besshi Hospital, Japan

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Address for correspondence:

Kazuhiro Nouso, M.D., Ph.D.

Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences,

2-5-1 Shikata-cho, Kita-ku, Okayama-City, Okayama 700-8558, Japan

Tel.: +81-86-235-7219

Fax: +81-86-225-5991

E-mail: nouso@cc.okayama-u.ac.jp

Abstract

Background: Several factors, including proangiogenic cytokines, have been reported as predictive markers for the treatment effect of sorafenib in patients with hepatocellular carcinoma (HCC); however, most of them were determined based on one-time measurements prior to treatment.

Methods: We consecutively recruited 80 advanced HCC patients who were treated with sorafenib prospectively. Serum levels of eight proangiogenic cytokines and the appearance of adverse events were monitored periodically, and their correlations with the prognoses of the patients were evaluated.

Results: Among six significant risk factors for overall survival in univariate analyses, high angiopoietin-2 (hazard ratio, 2.06), high hepatocyte growth factor (hazard ratio, 2.08), and poor performance status before the treatment (hazard ratio, 2.48) were determined as independent risk factors. In addition, high angiopoietin-2 at the time of progressive disease was a marker of short post-progression survival (hazard ratio, 4.27). However, there was no significant variable that predicted short progression-free survival except the presence of hepatitis B virus surface antigen.

Conclusions: Predictions of overall survival and post-progression survival were

possible by periodically measuring serum proangiogenic cytokines, especially angiopoietin-2, in patients with HCC treated with sorafenib.

Introduction

Hepatocellular carcinoma (HCC) is a hypervascular malignant tumour arising from liver parenchyma [1], and is the second leading cause of cancer-related death worldwide [2]. Advanced HCC is known for its poor prognosis [3 4]. Sorafenib is a standard therapy for advanced HCC because the survival benefits have been demonstrated in two randomized, placebo-controlled, double-blind phase III clinical trials [5 6]. Thereafter, most randomized studies of the new multikinase inhibitors, sunitinib, brivanib, and linifanib, or a combination of sorafenib and erlotinib did not reveal a better survival benefit or tolerability compared to sorafenib monotherapy [7]. Recently, regorafenib, lenvatinib, and cabozantinib have shown survival benefits [8 9 10 11]. Because several drugs are available for the treatment of advanced HCC, it is important to know the efficacies of the drugs in each patient prior to, or soon after starting treatment. Especially, early prediction of sorafenib efficacy is important, because it is still the most common treatment for advanced HCC.

Studies based on patient cohorts have identified several early surrogate markers, including changes in serum alpha-fetoprotein (AFP) levels after treatment, monitoring of the tumour blood supply with dynamic contrast-enhanced

magnetic resonance imaging, and the appearance of treatment-related adverse events. Amplification of FGF3/FGF4 or vascular endothelial growth factor (VEGF)A, and the increased expression of phospho-Mapk14 or phospho-Atf2 have also been reported as possible predictive markers that must be still validated [12].

We previously reported that high expression of angiopoietin-2 (Ang-2) or high numbers of elevated cytokines in the serum were associated with poor progression-free survival (PFS) and overall survival (OS) in advanced HCC patients treated with sorafenib [13]. Llovet et al. also reported the possibility of Ang2 and VEGF as predictors of survival. However, none of the biomarkers measured before starting sorafenib has been validated in terms of predicting the response to sorafenib [14].

In the present study, we sequentially examined the expressions of cytokines as well as adverse events prospectively, to reveal the significance of measuring proangiogenic cytokines as predictors of treatment efficacy and survival in patients with advanced HCC who received sorafenib treatment.

Materials and methods

Patient characteristics and diagnosis of HCC

Between January 2013 and January 2016, we enrolled 80 consecutive patients with advanced HCC, who were treated with sorafenib at our institute or collaborating hospitals (Hiroshima City Hospital, Kurashiki Central Hospital, Kagawa Prefectural Central Hospital, Sumitomo Besshi Hospital, Okayama Red Cross General Hospital, and Okayama Saiseikai General Hospital) in this prospective study. Diagnosis of HCC was confirmed based on hyperattenuation in the arterial phase and hypoattenuation in the portal/venous phase [15]. Written informed consent for drawing blood and using it for this study was obtained from all patients. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, was approved by the ethics committees of the institutes involved, and was registered at UMIN (UMIN000009771).

Treatments and follow-ups

Forty-one patients started sorafenib treatment at 400 mg bid, 35 patients were treated at 400 mg sid, four patients were treated with 400 mg QOD. A reduced starting dose was sometimes chosen by doctors because of the possibility of

low tolerance resulting from low body weight and/or old age. The dose reduction of sorafenib was carried out according to the protocol recommended by the pharmaceutical company.

The patients were followed-up until June 2017. They were checked bimonthly by routine surveillance imaging, such as dynamic computed tomography or magnetic resonance imaging, in addition to periodic blood tests that included AFP and des-gamma-carboxyprothrombin (DCP). All patients had at least one untreated target lesion that could be measured in one dimension, and the treatment effects were evaluated according to the Modified Response Evaluation Criteria in Solid Tumors (mRECIST) guideline [16].

Data collection

The clinical information of consenting patients was abstracted from medical records. Variables included age, sex, markers for hepatitis virus infection, Eastern Cooperative Oncology Group performance status (ECOG PS), Child-Pugh grade, and serum laboratory tests, such as AFP and DCP. The HCC parameters of size, number of lesions, presence of macroscopic vascular invasion (MVI) and extrahepatic spread were collected before starting the sorafenib treatment. The

adverse events within 1 month after starting sorafenib treatment were also examined.

Measurement of cytokines

Serum was collected before starting sorafenib treatment, after 2 weeks, after 4 weeks, at the time of the first imaging evaluation (after 8 weeks), and at the time of progressive disease (PD). The blood samples were centrifuged for 10 minutes at 15,000 × *g*, and the supernatants were frozen immediately and stored at -30°C until use. The samples were assayed to determine the concentration of follistatin (FST), granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), leptin, platelet-derived growth factor BB (PDGF-BB), platelet endothelial cell adhesion molecule-1 (PECAM-1), Ang-2, and VEGF using a BioPlex 200 System (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's protocols. The samples were tested in duplicate, and the mean value was used for the analysis.

Statistical analysis

The cytokine data and the characteristics of the patients were compared

with the PFS and OS. The PFS and OS were calculated from the first day of sorafenib treatment. The relationship between post-progression survival (PPS) and the expression level of each cytokine at PD was also analysed. Wilcoxon's rank sum test was used to compare continuous data. Fisher's exact test was used to compare categorical data. Survival was estimated by the Kaplan-Meier method and compared using the log-rank test. Cox's proportional hazards model was used to analyse the hazard ratio (HR) and 95% confidence interval (CI). Factors exhibiting significance using univariate analyses were further analysed by multivariate analyses. To avoid the effect of multicollinearity, the HR of cytokine was examined separately using multivariate analyses. For statistical analyses, $P<0.05$ was considered significant. All statistical analyses were performed using JMP Pro statistical software (version 12, SAS Institute, Cary, NC, USA).

Results

Characteristics of the patients

The median age of the patients was 72 years and 64 (80%) were male (Table 1). Liver function was preserved and performance status (PS) was good in most of the patients. The percentage of Child-Pugh A and ECOG PS:0 were 86.3% and 81.3%, respectively. Approximately two-thirds (63.8%) of the patients had multiple tumours ≧5) in the liver, 51.3% had tumours≧30 mm in diameter, and MVI was observed in 40.0% of the patients. Distant metastases or lymph node metastases was observed in 62.5% of the patients. The percentage of advanced stage HCC was higher in this cohort compared to a previous report by Miyahara et al. [13].

The median PFS was 93 days, and the median OS was 318 days. At the first evaluation (2 months after starting the treatment), 5 (6.3%) patients had a partial response (PR), 26 (32.5%) had a stable disease (SD), and 36 (45%) had PD. Of these, 10 (12.5%) patients were able to maintain a SD for more than 1 year.

Treatment effects and proangiogenic cytokines

The median cytokine concentrations before sorafenib treatment were as

follows: 530.0 pg/mL for FST, 16.3 pg/mL for G-CSF, 1,449.3 pg/mL for HGF, 3,661.3 pg /mL for leptin, 2,569.2 pg/mL, for PDGF-BB, 6054.9 pg/mL for PECAM-1, 376.2 pg/mL for Ang-2, and 156.7 pg/mL for VEGF. The cytokine levels but FST and VEGF were not different among patients with different etiologies (Supplemental Table 1).We previously reported in our retrospective study that all proangiogenic cytokines (FST, G-CSF, HGF, leptin, PDGF-BB, PECAM-1, Ang-2, and VEGF) before sorafenib treatment were higher in PD patients than in non-PD patients [13]. In this prospective study, median values of the PD group were also higher than those in the non-PD group, except FST and PECAM-1; however, the differences were not statistically significant (Figure 1).

Risk factors for PFS

We divided the expression of cytokines into two groups by the median and examined their risks for PFS. No cytokine before the therapy (Table 2) or after 2 or 4 weeks (data not shown) was correlated with PFS using univariate analyses. Among 13 variables including the patients' characteristics, tumour factors, and adverse events within 4 weeks, only the presence of hepatitis B surface antigen was correlated with a short PFS (HR, 3.23; 95%CI, 1.63–6.13; P=0.001).

Risk factors for OS

Univariate analysis revealed that high HGF (HR, 2.08; 95%CI, 1.23–3.59; P=0.006) and high Ang-2 (HR,1.90; 95%CI, 1.12–3.25; P=0.017) before sorafenib treatment were risk factors for survival, in addition to four clinical parameters, which were poor ECOG PS (HR, 2.23; 95%CI, 1.14–4.07; P=0.020), large tumour number (≥5) (HR, 1.79; 95%CI, 1.04–3.23; P=0.035), high DCP (>100 mAU/mL) (HR, 2.46; 95%CI 1.31–5.04; P=0.004), and no hand foot syndrome within 30 days (HR,1.85; 95%CI, 1.08–3.18; P=0.024) (Table 3). Multivariate analyses of these factors revealed that poor ECOG PS (HR, 2.48; 95%CI, 1.14–5.22; P=0.022), high HGF (HR, 2.08; 95%CI, 1.11–3.97; P=0.021), and high Ang-2 (HR, 2.06; 95%CI, 1.12–3.84; P=0.018) were independent risk factors for survival (Table 4, Figure 2).

PPS and cytokine expression

Because serum concentrations of Ang-2 and HGF before sorafenib treatment were closely correlated with the OS, the correlations between the levels at the time of PD and PPS were analysed. No correlation was observed between HGF at PD and PPS; however, the PPS of patients with high Ang-2 at PD was

significantly shorter than that with low Ang-2 (P<0.001, Figure 3).

Changes of serum Ang-2 level during the treatment

To know the relationship between treatment effect and Ang-2 changes during sorafenib treatment, we compared the serum Ang-2 levels before the treatment and at the time of the evaluation in PD patients and non-PD patients. Ang-2 level was significantly increased in PD patients (median: from 372.7 to 777.6 pg/mL, p=0.013); however, no changes were observed in non-PD cases (median: from 390.2 to 474.8 pg/mL, p=0.71).

Discussion

In this prospective cohort study, we examined serum proangiogenic cytokines periodically in patients with advanced HCC who received sorafenib treatment. There was no cytokine that could predict drug response and PFS. However, high HGF and Ang-2 as well as a poor performance status before treatment were significantly correlated with a short OS. In addition, patients with high Ang-2 at the time of PD showed a short PPS. These results indicated that measuring serum proangiogenic cytokines, especially Ang-2 at appropriate times, was helpful in predicting the prognoses of patients.

VEGF and Ang-2 are known to be produced by cancer cells and play important roles in regulating tumour angiogenesis [17]. Angiopoietin-1 (Ang-1) is a counterpart of Ang-2 that is predominantly expressed in support cells of large blood vessels as well as stromal, endothelial, and tumour cells Ang-1 recruits pericytes and smooth muscle cells and stabilizes vascular networks. Ang-2 is an agonist and antagonist of Ang-1, which is expressed during vascular remodelling, and prevents vascular stability. Consequently, Ang-2 helps VEGF to stimulate endothelial cells, resulting in neovascularization of the tumour [18]. Immunohistochemical examination of HCC has revealed that increased Ang-2 expression was associated with tumour dedifferentiation, and the expression was higher in hypervascular

HCC patients than in hypovascular HCC patients [19]. We observed in this study that high Ang-2 before treatment was a marker for a short survival and at the time of PD was a marker for a short PPS. These results indicated that the majority of serum Ang-2 expressed in HCC patients was tumour-derived and could be a marker of the angiogenic potential of HCC, independent of sorafenib treatment. These findings were consistent with the results of our previous report and with the SHARP study by Llovet et al., although they reported the relationship between OS and Ang-2 level using only one time point, which was before sorafenib treatment [14].

There are several reports showing that the decrease of AFP during sorafenib treatment correlated with better prognosis [20] [21]. In this study, the percentage of non-PD in patients whose AFP decreased over 20% at 4 weeks of the treatment was higher than that in patients who did not show the decrease (66.7% vs. 31.4%, P=0.02). The result was consistent with the published reports of retrospective studies. The same relationship might be observed with other factors including Ang-2 so that further examination is necessary in future.

HGF and activation of its transmembrane tyrosine kinase receptor, cellular MET (cMET), has been implicated in cellular invasion and metastases through induction of increased proliferation, migration, and angiogenesis [22]. A positive

correlation between high serum HGF levels and short OS has been reported in HCC patients [23]; however, the relationship was observed only between expression of HGF before sorafenib treatment and OS in the study. The expression at the time of PD did not correlate with PPS. There are several possible reasons for this discrepancy. First, cMET is often downregulated in HCC patients [24], so HGF may not be able to promote the growth of HCC in all patients. Second, HGF is also produced by stromal cells such as stellate cells in addition to cancer cells [25]. Moreover, the serum level is sometimes elevated in patients with hepatitis [26], indicating that HGF does not always reflect the tumour burden. Recently, elevated HGF expression as an autocrine cMET activation mechanism in acquired resistance to sorafenib was reported [27]. Further analysis with local HGF levels might provide another perspective.

We previously conducted a retrospective study and reported that all proangiogenic cytokine concentrations (FST, G-CSF, HGF, leptin, PDGF-BB, PECAM-1, Ang-2, and VEGF) examined in this study before sorafenib treatment were higher during PD than during non-PD periods [13]. However, we did not observe these differences prospectively. In the present study, fewer patients were examined and more advanced HCC (a higher percentage of MVI, larger tumour sizes, and

higher tumour numbers) were included. There were many patients with higher cytokine levels when compared to our previous study. These differences might have decreased the correlations between cytokine levels and the treatment effects.

Although we analysed the data of prospectively collected samples, there were some limitations in the study. First, we could not fix the starting dose of sorafenib treatment. Because this study was conducted as part of daily practice in multiple centres, the dose was prescribed by the doctors in charge. This might have lowered the power of the tests. Second, no restrictions of treatments prior to sorafenib and after PD were defined. In addition, we did not directly compare the usefulness of the biomarkers between patients treated with sorafenib and the placebo.

Nevertheless, we clearly demonstrated that the prediction of OS and PPS in patients with HCC who received sorafenib was possible by measuring serum proangiogenic cytokine levels at appropriate times. Among these cytokines, Ang-2 was the most important predictor. Further study with other new molecular target drugs will be necessary to confirm the usefulness of measuring proangiogenic cytokines to select the proper drug for the treatment of advanced HCC patients.

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References

1. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118-27.
2. Petrick JL, Braunlin M, Laversanne M, et al. International trends in liver cancer incidence, overall and by histologic subtype, 1978-2007. Int J Cancer 2016;139:1534-45.
3. Llovet JM. Updated treatment approach to hepatocellular carcinoma. J Gastroenterol 2005;40:225-35.
4. Ji Y, Wang Z, Li Z, et al. Angiotensin II induces angiogenic factors production partly via AT1/JAK2/STAT3/SOCS3 signaling pathway in MHCC97H cells. Cell Physiol Biochem 2012;29:863-74.
5. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
6. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.
7. Ch'ang HJ. Optimal combination of antiangiogenic therapy for hepatocellular carcinoma. World J Hepatol 2015;7:2029-40.

8. Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;389:56-66.

9. Kudo M. Systemic Therapy for Hepatocellular Carcinoma: 2017 Update. Oncology 2017;93 Suppl 1:135-146.

10. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391:1163-1173.

11. Nault JC, Galle PR, Marquardt JU. The role of molecular enrichment on future therapies in hepatocellular carcinoma. J Hepatol 2018.

12. Shao YY, Hsu CH, Cheng AL. Predictive biomarkers of sorafenib efficacy in advanced hepatocellular carcinoma: Are we getting there? World J Gastroenterol 2015;21:10336-47.

13. Miyahara K, Nouso K, Morimoto Y, et al. Pro-angiogenic cytokines for prediction of outcomes in patients with advanced hepatocellular carcinoma. Br J Cancer 2013;109:2072-8.

14. Llovet JM, Pena CE, Lathia CD, et al. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer

Res 2012;18:2290-300.

15. Kim SH, Lee WJ, Lim HK, et al. Prediction of viable tumor in hepatocellular carcinoma treated with transcatheter arterial chemoembolization: usefulness of attenuation value measurement at quadruple-phase helical computed tomography. J Comput Assist Tomogr 2007;31:198-203.

16. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52-60.

17. Sugimachi K, Tanaka S, Terashi T, et al. The mechanisms of angiogenesis in hepatocellular carcinoma: angiogenic switch during tumor progression. Surgery 2002;131:S135-41.

18. Bupathi M, Kaseb A, Janku F. Angiopoietin 2 as a therapeutic target in hepatocellular carcinoma treatment: current perspectives. Onco Targets Ther 2014;7:1927-32.

19. Torimura T, Ueno T, Kin M, et al. Overexpression of angiopoietin-1 and angiopoietin-2 in hepatocellular carcinoma. J Hepatol 2004;40:799-807.

20. Personeni N, Bozzarelli S, Pressiani T, et al. Usefulness of alpha-fetoprotein response in patients treated with sorafenib for advanced hepatocellular carcinoma. J Hepatol 2012;57:101-7.

21. Kawaoka T, Aikata H, Murakami E, et al. Evaluation of the mRECIST and alpha-fetoprotein ratio for stratification of the prognosis of advanced-hepatocellular-carcinoma patients treated with sorafenib. Oncology 2012;83:192-200.

22. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res 2013;19:2310-8.

23. Vejchapipat P, Tangkijvanich P, Theamboonlers A, et al. Association between serum hepatocyte growth factor and survival in untreated hepatocellular carcinoma. J Gastroenterol 2004;39:1182-8.

24. Santoro A, Rimassa L, Borbath I, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol 2013;14:55-63.

25. Liu WT, Jing YY, Yu GF, et al. Hepatic stellate cell promoted hepatoma cell invasion via the HGF/c-Met signaling pathway regulated by p53. Cell Cycle 2016;15:886-94.

26. Panasiuk A, Pogorzelska J, Prokopowicz D. [Growth factors in chronic hepatitis C]. Przegl Epidemiol 2007;61:559-66.

27. Firtina Karagonlar Z, Koc D, Iscan E, et al. Elevated hepatocyte growth factor

expression as an autocrine c-Met activation mechanism in acquired resistance to sorafenib in hepatocellular carcinoma cells. Cancer Sci 2016;107:407-16.

Table 1. Characteristics of hepatocellular carcinoma patients

Variables		
Median age, year (range)	72	(42-86)
Sex		
Male	64	(80%)
Female	16	(20%)
Viral infection		
HBsAg-positive	15	(18.8%)
HCVAb-positive	38	(47.5%)
Others	27	(33.8%)
ECOG performance status		
0	65	(81.3%)
1	15	(18.8%)
Child-Pugh grade		
A	69	(86.3%)
B	11	(13.8%)
Intrahepatic tumour		
Tumour number (≧5)	51	(63.8%)
Tumour size (≧30 mm)	41	(51.3%)
Macroscopic vascular invasion	32	(40.0%)
Extrahepatic spread		
Lymph node	17	(21.3%)
Distant metastasis	40	(50.0%)
Lymph node and/or distant metastasis	50	(62.5%)
Tumour markers, median (range)		
AFP (ng/mL)	259	(2.4-415825)
DCP (mAU/mL)	532	(10-485520)

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; ECOG, Eastern Cooperative Oncology Group; HBsAg, hepatitis B virus surface antigen; HCVAb, anti-hepatitis C virus antibody.

Table 2. Cytokines and clinical parameters for predicting progression-free survival (univariate analysis)

Variables	Hazard Ratio	95% Confidence Interval	P-value
FST (>530.0 pg/mL)	0.86	0.51-1.42	0.560
G-CSF (>15.4 pg/mL)	0.83	0.50-1.38	0.487
HGF (>1449.3 pg/mL)	1.47	0.88-2.43	0.135
Leptin (>3661.3 pg/mL)	0.83	0.50-1.38	0.492
PDGF-BB (>2569.2 pg/mL)	1.04	0.63-1.73	0.849
PECAM-1 (>6054.9 pg/mL)	1.03	0.62-1.73	0.887
Ang-2 (>357.7 pg/mL)	1.45	0.86-2.45	0.158
VEGF (>156.1 pg/mL)	0.89	0.53-1.49	0.679
Age (>72 years)	1.13	0.67-1.88	0.636
Sex (male)	1.25	0.68-2.46	0.478
ECOG PS (≥1)	1.67	0.87-2.99	0.113
HBsAg (positive)	3.23	1.63-6.13	0.001
HCVAb (positive)	0.86	0.51-1.46	0.589
Child-Pugh grade B	0.84	0.37-1.70	0.664
Tumour size (≧30 mm)	1.30	0.78-2.16	0.308
Tumour number (≧5)	1.47	0.87-2.57	0.151
Macroscopic vascular invasion	1.29	0.76-2.15	0.330
Extrahepatic spread	0.79	0.48-1.33	0.383
AFP (>100 ng/mL)	1.50	0.90-2.52	0.112
DCP (>100 mAU/mL)	1.24	0.72-2.22	0.439
Hand foot syndrome	0.91	0.55-1.54	0.748
Diarrhoea	0.98	0.40-2.05	0.964
Hypertension	1.25	0.69-2.17	0.431
Other adverse events	1.06	0.63-1.79	0.822

Note: Proangiogenic cytokines measured before starting sorafenib treatment were divided into two groups using the median. Adverse events within 1 month from starting sorafenib were listed (hand foot syndrome, diarrhoea, hypertension, and other adverse events).

FST, follistatin; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte

growth factor; PDGF-BB, platelet-derived growth factor BB; PECAM-1, platelet endothelial cell adhesion molecule-1; Ang-2, angiopoietin-2; VEGF, vascular endothelial growth factor. Other abbreviations were shown in Table 1.

Table 3. Cytokines and clinical parameters for predicting overall survival (univariate analysis)

Variables	Hazard ratio	95% Confidence interval	P-value
FST (>530.0 pg/mL)	1.15	0.68-1.95	0.591
G-CSF (>15.4 pg/mL)	1.05	0.62-1.78	0.844
HGF (>1449.3 pg/mL)	2.08	1.23-3.59	0.006
Leptin (>3661.3 pg/mL)	1.01	0.59-1.70	0.963
PDGF-BB (>2569.2 pg/mL)	0.95	0.56-1.62	0.872
PECAM-1 (>6054.9 pg/mL)	1.03	0.61-1.77	0.888
Ang-2 (>357.7 pg/mL)	1.90	1.12-3.25	0.017
VEGF (>156.1 pg/mL)	1.18	0.70-2.02	0.525
Age (>72 years)	1.14	0.67-1.93	0.616
Sex (male)	1.07	0.57-2.19	0.829
ECOG PS (≥1)	2.23	1.14-4.07	0.020
HBsAg (positive)	1.94	0.99-3.56	0.051
HCVAb (positive)	0.99	0.58-1.68	0.991
Child-Pugh grade B	1.26	0.52-2.63	0.572
Tumour size (≧30 mm)	1.61	0.95-2.72	0.075
Tumour number (≧5)	1.79	1.04-3.23	0.035
Macroscopic vascular invasion	1.54	0.89-2.60	0.113
Extrahepatic spread	1.16	0.68-2.00	0.578
AFP (>100 ng/mL)	1.64	0.96-2.86	0.067
DCP (>100 mAU/mL)	2.46	1.31-5.04	0.004
HFS	0.54	0.31-0.92	0.024
Diarrhoea	0.86	0.33-1.87	0.734
Hypertension	0.92	0.47-1.66	0.795
Other adverse events	1.02	0.60-1.78	0.926

Note and Abbreviations were the same as listed in Table 2.

Table 4. Prognostic factors for overall survival (multivariate analysis)

Variables	Hazard ratio	95% Confidence interval	P-value
HGF (>1449.3 pg/mL)	2.08	1.11-3.97	0.021
Ang-2 (>357.7 pg/mL)	2.06	1.12-3.84	0.018
ECOG PS (≧1)	2.48	1.14-5.22	0.022
Tumour number (≧5)	1.48	0.80-2.82	0.205
DCP (>100 mAU/mL)	2.09	0.98-4.52	0.054
HFS	0.82	0.44-1.43	0.379

Note and Abbreviations were the same as listed in Table 2.

Figure legends

Figure 1. Serum cytokine levels in patients with progressive disease (PD) and non-PD. Median values of the PD group were greater than those of non-PD except FST and PECAM-1; however, the differences were not statistically significant.

Horizontal bars in the boxes and the numbers indicate the median.

FST, Follistatin; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; PDGF-BB, platelet-derived growth factor BB; PECAM-1, platelet endothelial cell adhesion molecule-1; Ang-2, angiopoietin-2; VEGF, vascular endothelial growth factor.

Figure 2. Survival of advanced HCC patients. Survival of the patients were short when hepatocyte growth factor (HGF) was high (A), angiopoietin-2 (Ang-2) was high (B), and performance status was poor (C).

Figure 3. Cytokines and post-progression survival. No difference of post-progression survival was observed regardless of the level of serum hepatocyte growth factor (HGF) at the time of progressive disease (PD) (A). However, post-progression survival was significantly shorter in patients with high angiopoietin-2 (Ang-2) at the time of PD (B).