SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 12: 579-584, 2016

Therapeutic evaluation of sorafenib for hepatocellular carcinoma using contrast-enhanced ultrasonography: Preliminary result

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Received January 26, 2015; Accepted April 1, 2016

DOI: 10.3892/ol.2016.4669

Abstract. The present study aimed to determine the usefulness of contrast-enhanced ultrasonography (CEUS) with Sonazoid in evaluating the therapeutic response to sorafenib for hepatocellular carcinoma (HCC). In total, 26 patients with advanced HCC who received sorafenib and were followed up by CEUS were enrolled in the present study. CEUS was performed prior to and within 2-4 weeks of treatment, and the images of the target lesion in the post-vascular phase with a re-injection method were analyzed. The presence (+) or absence (-) of intratumoral necrosis and the intratumoral vascular architecture on micro-flow imaging (MFI) were compared prior to and subsequent to treatment. Target lesions that exhibited non-enhancement after re-injection were considered to indicate intratumoral necrosis. The intratumoral vascular architecture was classified into three groups, as follows: Vd, the intratumoral vessels visually narrowed or decreased; Vnc, the vessels remained unchanged; and Vi, the vessels were thickened or increased. Survival curves were estimated using the Kaplan-Meier method and compared using the log rank test between the intratumoral necrosis (+) and (-) groups, and among the Vd, Vnc and Vi groups. $P<0.05$ was considered to indicate a statistically significant difference. The number of patients in the intratumoral necrosis (+) and (-) groups was 8 and 18 patients, respectively, and the median survival time (MST) was 7.2 months [95% confidence interval (CI), 2.2-12.2] and 9.5 months (95% CI, 5.1-13.8), respectively ($P=0.44$). The MFI findings were observed in 11 patients in the Vd group, 10 patients in the Vnc group and 5 patients in the Vi group. The MSTs in the Vd, Vnc and Vi groups were 15.6 months (95% CI, 5.0-23.3), 11.0 months (95% CI, 3.5-17.6) and 3.6 months (95% CI: 1.2-6.0), respectively. The P-value for the differences between the Vd and Vnc groups, Vd and Vi groups, and Vnc and Vi groups were 0.78, 0.016 and 0.047, respectively, which indicated that the survival time decreased significantly in the Vi group. Evaluation of intratumoral vascular architecture using MFI demonstrates promise for assessing the therapeutic response to sorafenib in patients with HCC.

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Key words: sorafenib, hepatocellular carcinoma, contrast-enhanced ultrasonography, micro-flow imaging, therapeutic response

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-associated mortality worldwide (1). While local treatments, including surgical resection and radiofrequency ablation (RFA), lead to favorable outcomes for early-stage HCC (2,3), no effective treatment has been established for advanced HCC that is not amenable to surgical resection, and the prognosis of advanced HCC is poor.

Sorafenib (Nexavar; Bayer, Leverkusen, Germany) is an oral multi-targeted tyrosine kinase inhibitor (4-6) that is indicated for unresectable advanced HCC and significantly improves the progression-free and overall survival times of patients (7,8). Sorafenib has been widely used for the treatment of unresectable advanced HCC, but it is an expensive drug that has certain adverse effects, such as hand-foot skin reaction and diarrhea (7). In order for patients to continue treatment with sorafenib, it is essential to evaluate the early response to sorafenib. Sorafenib is characterized by antitumor effects, including tumor growth inhibition and antiangiogenic effects, which makes it challenging to evaluate the therapeutic effects using the conventional Response Evaluation Criteria in Solid Tumors (RECIST) (9). Alternative evaluation criteria, including tumor necrosis and intratumoral hemodynamics, such as the modified RECIST (10), Response Evaluation Criteria in Cancer of the Liver (11) and Choi criteria (12), have been recommended.

A previous study reported the use of Arrival time parametric imaging (AtPI) (13) with contrast-enhanced ultrasonography (CEUS) using Sonazoid (Daiichi Sankyo, Tokyo, Japan) to evaluate early responses to sorafenib in patients with advanced HCC (14). In this method, based upon the color mapping images obtained by AtPI, the mean arrival time of the contrast agent at the target lesion from the starting point, which was a large artery near the lesion, was calculated to

obtain the difference between prior to and 2 weeks subsequent to the administration of sorafenib. Blood flow velocity was considered to have been reduced when the difference was ≥0 [mean time (MT) (+) group] and to have been increased when the difference was <0 [MT(-) group]. When the overall survival was compared between the groups, the survival time was significantly longer in the MT(+) group compared with the MT(-) group. Thus, MT, which is an index of objective evaluation by AtPI, may be useful for evaluating the early response to sorafenib.

In general, AtPI is used to evaluate any changes in intratumoral hemodynamics, such as changes in the blood flow velocity. In the present study, the presence or absence of intratumoral necrosis was compared with the intratumoral vascular architecture on micro-flow imaging (MFI) (15-17) (Toshiba Medical Systems, Otawara, Japan) in the post-vascular phase by CEUS with Sonazoid prior to and subsequent to sorafenib administration, to determine whether the visual changes may be applied to the evaluation of the therapeutic effects of sorafenib.

Materials and methods

Patient sample. In total, 94 patients with advanced HCC underwent treatment with sorafenib at Toho University Medical Center, Omori Hospital (Tokyo, Japan) between April 2009 and July 2013. Of the 94 patients, 45 patients agreed to participate the present study and CEUS was performed prior to sorafenib administration. However, of the 45 patients, 26 patients were able to take sorafenib for more than 4 weeks, and were followed up by CEUS with Sonazoid prior to and 2-4 weeks subsequent to the sorafenib administration. Therefore, these 26 patients were enrolled in the present study. They were all male patients with a mean age of 69.3±6.9 years. The underlying liver diseases were hepatitis B in 2 patients, hepatitis C in 16 patients, alcoholic hepatitis in 7 patients and other in 1 patient. The Child-Pugh classification (18) was A for 21 patients and B for 5 patients. The median α-fetoprotein (AFP) level prior to administration was 639.4 ng/ml, and the median level of des-γ-carboxy prothrombin (DCP) was 617 mAU/ml. The initial dose of sorafenib was 800 mg/day for 4 patients, 400 mg/day for 14 patients and 200 mg/day for 8 patients. The present study was approved by the Ethical Review Board of Toho University Medical Center, Omori Hospital. Informed consent was obtained from all patients for inclusion in the current study.

CEUS. CEUS was performed prior to and 2-4 weeks subsequent to the administration of sorafenib. One lesion that could be followed throughout the study period was selected. Ultrasonography (US) was used in each patient to standardize evaluations, and CEUS was performed in the same cross-section, under the same conditions, at all time points. The ultrasound equipment used in the present examination was an SSA-790A ultrasound (Toshiba Medical Systems, Otawara, Japan) with a Toshiba PVT-375BT convex probe (3.75-MHz center frequency). The imaging mode was wideband harmonic imaging (pulse subtraction), with transmission and reception frequencies of 1.8 and 3.5 MHz, respectively. The mechanical index (MI) for acoustic output was set to 0.2; the dynamic range was set to 60-65 dB. A single focus point was set at the deep site of the lesion, and a bolus intravenous injection of 0.5 ml Sonazoid was administered via a left cubital venous line followed by flushing with 10 ml normal saline. In the post-vascular phase 10-15 min subsequent to the injection of Sonazoid, 0.5 ml Sonazoid was intravenously injected for the target lesion using the re-injection method (19) to compare the presence or absence of intratumoral necrosis 60-90 sec subsequent to the re-injection, in addition to the intratumoral vascular architecture on the MFI findings prior to and subsequent to the administration of sorafenib.

MFI. MFI was introduced by Sugimoto *et al* (15). Briefly, maximum-hold processing started immediately subsequent to the burst scan. The burst scan consisted of high MI (1.3-1.6) scanning for 5 frames. Low MI (0.16-0.30) scanning was commenced again, immediately subsequent to the MI burst scanning, to visualize the fresh microbubble contrast agent flowing into the scanning volume. The maximum intensity holding sequence was started simultaneously with flash replenishment low MI imaging, which stored and exhibited the maximum brightness at each pixel. The accumulation time for each MFI sequence was 5-7 sec, depending on the perfusion of the target tissue. Target lesions that exhibited non-enhancement (cystic-like) subsequent to re-injection were considered to indicate intratumoral necrosis. The intratumoral vascular architecture shown on the MFI findings was classified into three groups, as follows: Vd group, in which intratumoral vessels visually narrowed or decreased (Fig. 1); Vnc group, in which intratumoral vessels remained unchanged; and Vi group, in which intratumoral vessels were thickened or increased (Fig. 2). The classification was performed by 2 ultrasonography specialists, who classified the MFI findings independently without knowledge of the clinical characteristics or therapeutic course of each patient. The observers reviewed the images and clips stored on the hard disc for offline analysis.

Statistical analysis. The degree of inter-observer agreement was calculated using the κ-statistic. In general, a κ-statistic value >0.75 is considered to indicate excellent agreement, 0.4-0.75 indicates good agreement and <0.4 indicates poor agreement (20). In the Vd, Vnc and Vi groups, the following 12 patient and tumor background characteristics were reported: Mean age; gender; underlying liver disease, consisting of hepatitis B, hepatitis C, alcoholic hepatitis and other; Child-Pugh classification (A or B); previous treatment, such as transarterial chemoembolization, RFA and surgical resection; median AFP level; median DCP level; Barcelona Clinic Liver Cancer (BCLC) staging classification (B or C) (21); presence or absence of portal vein tumor thrombus (PVTT); presence or absence of extrahepatic metastasis; median duration of sorafenib administration; and presence or absence of intratumoral necrosis (Table I).

The survival time was calculated using the Kaplan-Meier method, in order to compare times between the intratumoral necrosis (+) and (-) groups, and among the Vd, Vnc and Vi groups based on the MFI findings. The survival time was calculated from the start of sorafenib administration to the final follow-up or mortality.

Statistical analysis was performed using SPSS version 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). P<0.05 were considered to indicate a statistically significant difference.

Table I. Characteristics of the patients.

Variables	Vd group, n	Vnc group, n	Vi group, n
Total	11	10	5
Age, years[a]	66.9±6.8	70.2±6.2	72.8±8.2
Gender			
Male	11	10	5
Female	0	0	0
Etiology			
Alcohol	3	2	2
HBV	1	0	1
HCV	6	8	2
Other	1	0	0
Child Pugh classification			
A	10	8	3
B	1	2	2
Previous treatment			
Yes	8	8	4
TACE	6	7	4
RFA	4	6	2
Surgical resection	3	2	0
No	3	2	1
Median AFP, ng/ml	151.9	1,153.8	13,276.0
Median DCP, mAU/ml	553.0	1,444.5	681.0
BCLC			
B	5	8	3
C	6	2	2
PVTT			
Yes	2	1	0
No	9	9	5
Extrahepatic metastasis			
Yes	2	0	0
No	9	10	5
Median period of sorafenib administration, months (range)	15.6 (5.7-33.3)	9.7 (3.6-51.3)	3.6 (1.8-11.5)
Intratumoral necrosis			
Yes	4	3	1
No	7	7	4

[a]Mean ± standard deviation. HBV, hepatitis B virus; HCV, hepatitis C virus; TACE, trancecatheter arterial chemoembolization; RFA, radiofrequency ablation; AFP, α-Fetoprotein; PIVKA II, protein induced by vitamin K absence-II; BCLC, barcelona clinic liver cancer; PVTT, portal vein tumor thrombus.

Results

The mean initial dose of sorafenib, median observation period and median survival time (MST) in all 26 patients were 400±196 mg/day, 9.4 months (range, 3.1-51.3 months) and 9.5 months (95% CI, 5.7-13.0), respectively. The concordance rate (κ-statistic) of the MFI findings between the 2 observers was 0.865, which was considered excellent agreement. The MFI findings were observed in 11 patients in the Vd group, 10 patients in the Vnc group, and 5 patients in the Vi group.

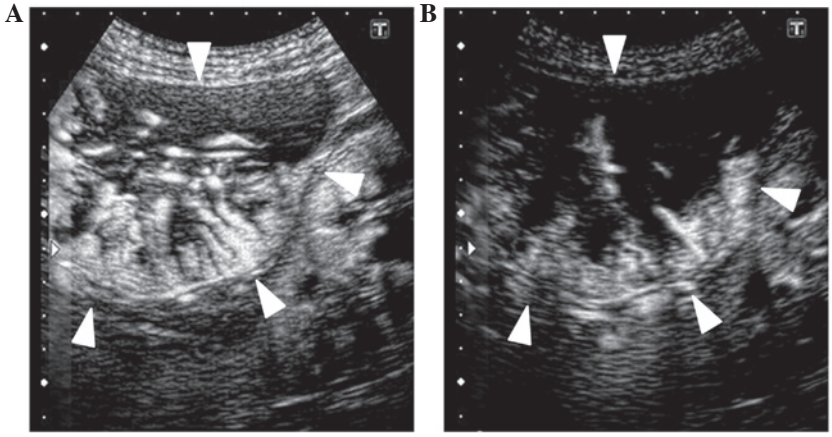

Figure 1. Representative images of the Vd group. The patient was a 70-year-old man with chronic hepatitis C virus. Sorafenib administration (400 mg/day) was started for advanced hepatocellular carcinoma. Contrast-enhanced ultrasonography was performed for giant tumor in S6 (arrowheads) (A) prior to and (B) 4 weeks subsequent to the sorafenib administration. The intratumoral vascular architecture on the micro-flow imaging was narrowed and decreased subsequent to therapy compared with the vascular architecture prior to therapy.

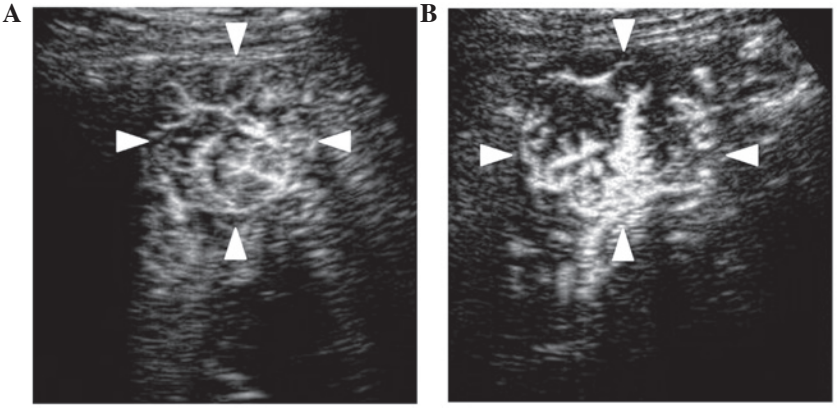

Figure 2. Representative imaging of the Vi group. The patient was a 79-year-old man with hepatitis B virus cirrhosis. Sorafenib administration (400 mg/day) was started for advanced hepatocellular carcinoma. Contrast-enhanced ultrasonography was performed for a tumor in S8 (arrowheads) (A) prior to and (B) 4 weeks subsequent to the sorafenib administration. The intratumoral vascular architecture on the micro-flow imaging findings was thickened and increased subsequent to therapy compared with the vascular architecture prior to therapy.

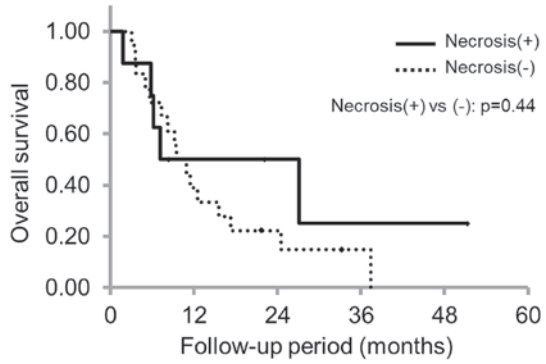

Figure 3. Comparison between cumulative overall of the necrosis (+) and necrosis (-) groups.

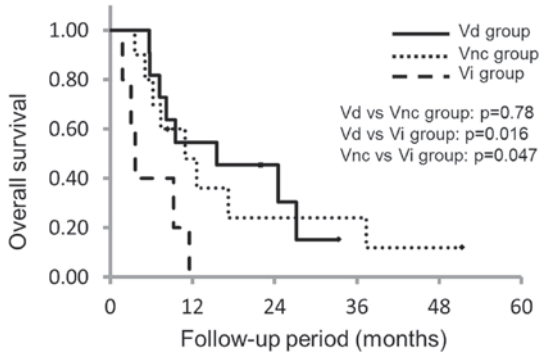

Figure 4. Comparison between cumulative overall survival in the Vd, Vnc, and Vi groups based on the micro-flow imaging findings.

Of the 26 patients, 8 patients showed intratumoral necrosis and 18 patients did not, and the MST was 7.2 months (95% CI, 2.2-12.2) and 9.5 months (95% CI, 5.1-13.8) in the patient with and without intratumoral necrosis, respectively. There were no statistically significant differences between the groups (P=0.44; Fig. 3). Based upon the MFI findings, the MST in the Vd, Vnc and Vi groups was 15.6 months (95% CI, 5.0-23.3), 11.0 months (95% CI, 3.5-17.6) and 3.6 months (95% CI, 1.2-6.0), respectively. The P-values for the differences between the Vd and Vnc groups, Vd and Vi groups, and Vnc and Vi groups were 0.78, 0.016 and 0.047, respectively, which indicated that the survival time decreased significantly in the Vi group (Fig. 4).

Discussion

MFI was developed as combination of the flash-replenishment sequence and the maximum intensity holding sequence was expected to make it possible to visualize vascular structures with high spatial resolution and vascular continuity (15-17).

Moschouris *et al* (22) reported the therapeutic effect of sorafenib based on the findings of non-contrast-enhanced US and CEUS using SonoVue (Bracco, Milan, Italy) prior to and subsequent to sorafenib administration, but there has been no comparison study using CEUS with Sonazoid; in particular, MFI on imaging prior to and subsequent to sorafenib administration. The present study compared the presence or absence of intratumoral necrosis, as well as the intratumoral vascular architecture, based upon MFI findings in the post-vascular phase by CEUS using Sonazoid, prior to and subsequent to sorafenib administration, and determined whether the visual changes may be applied to evaluation of therapeutic effects of sorafenib.

According to Moschouris *et al* (22), who studied 21 patients with HCC by US and CEUS using SonoVue, intratumoral bleeding or necrosis occurred in 50% of patients undergoing sorafenib treatment, showing the therapeutic efficacy of treatment, which was visible as a cystic transformation by US. In general, necrosis may be attributed to the characteristic therapeutic effects of sorafenib. The present comparison between the intratumoral necrosis (+) and (-) groups suggested a tendency towards prolongation of survival in the intratumoral necrosis (+) group, but there was no statistically significant difference in the survival time between the 2 groups. However, the small sample size and the short observation period of the present study may have contributed to this finding. A future study should increase the number of patients and the duration of the observation period.

Tanaka *et al* (23) and Sato *et al* (24) classified the MFI findings based on the MFI pattern classification, in order to allow comparison with the histological examination of HCC tumor biopsy and surgically excised specimens. Tanaka *et al* (23) reported that the MFI pattern classification may aid the evaluation of the differentiation grade of HCC. In addition, Sato *et al* (24) reported that the MFI pattern classification may be an individual predictive factor of a poor prognosis in patients with HCC. These studies suggest that the MFI findings of the intratumoral vascular architecture may aid the evaluation of the differentiation grade of HCC.

Furthermore, the present study demonstrated that the survival time was significantly worse in the Vi group, in which the intratumoral vascular architecture increased on the MFI imaging subsequent to sorafenib administration, compared with the Vd and Vnc groups. There was no difference in survival time between the Vd group, in which the intratumoral vessels decreased visually, and the Vnc group, in which intratumoral vessels were not changed. It is possible that the aforementioned changes in the vascular architecture are consistent with those in the study by Jain (25), but the anti-angiogenic effect of sorafenib may change the intratumoral vascular architecture during the clinical course. The present study also suggested that the change in the intratumoral vascular architecture may predict the therapeutic efficacy of sorafenib.

Limitations of the present study include the small sample size, short observation period, evaluation by examination of a single crosssectional ultrasonography image and a single target lesion, and the non-quantitative nature of the analysis. Evaluation of a single cross-sectional ultrasonography image and a single target lesion may be controversial when considering the multicentric carcinogenesis of HCC (26,27). However, previous studies investigating the early evaluation of the therapeutic effects of sorafenib by AtPI using CEUS also demonstrated significant results, regardless of the evaluation of a single cross-sectional US image of a single target lesion (14). Furthermore, while the present study was a non-quantitative analysis, the visual changes in the MFI findings may be objective, as the concordance rate (κ-statistic) of the MFI findings between the 2 observers was 0.865, which was considered to indicate excellent agreement. The findings of the present study suggested that the comparison of the intratumoral vascular architecture using MFI prior to and subsequent to sorafenib administration may be applied to the evaluation of the therapeutic effects of sorafenib. Notably, sorafenib is an expensive drug that has certain adverse effects (7). Therefore, a change from sorafenib to other early treatments, according to the classification of MFI findings, may be considered.

In conclusion, the findings of the present study suggested that visual evaluation of the intratumoral vascular architecture using MFI may be applied to the evaluation of the therapeutic effect of sorafenib, even when the sample size is small. The early evaluation of the therapeutic effects of sorafenib in patients with HCC using CEUS with Sonazoid may be of use.

Acknowledgements

The authors wish to thank Takahide Kudo, Kenichi Maruyama and the staff of Department of Clinical Functional Physiology, Toho University Medical Center, Omori Hospital.

References

1. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55: 74-108, 2005.
2. Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ and Lau WY: A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 243: 321-328, 2006.
3. Yi HM, Zhang W, Ai X, Li KY and Deng YB: Radiofrequency ablation versus surgical resection for the treatment of hepatocellular carcinoma conforming to the Milan criteria: Systemic review and meta-analysis. Int J Clin Exp Med 7: 3150-3163, 2014.
4. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, *et al*: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64: 7099-7109, 2004.
5. Chang YS, Adnane J, Trail PA, Levy J, Henderson A, Xue D, Bortolon E, Ichetovkin M, Chen C, McNabola A, *et al*: Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. Cancer Chemother Pharmacol 59: 561-574, 2007.
6. Hotte SJ and Hirte HW: BAY 43-9006: Early clinical data in patients with advanced solid malignancies. Curr Pharm Des 8: 2249-2253, 2002.
7. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, *et al*: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359: 378-390, 2008.

8. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, *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 10: 25-34, 2009.
9. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
10. Lencioni R and Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 30: 52-60, 2010.
11. Kudo M, Kubo S, Takayasu K, Sakamoto M, Tanaka M, Ikai I, Furuse J, Nakamura K and Makuuchi M; Liver Cancer Study Group of Japan (Committee for Response Evaluation Criteria in Cancer of the Liver, Liver Cancer Study Group of Japan): Response evaluation criteria in cancer of the liver (RECICL) proposed by the liver cancer study group of Japan (2009 revised version). Hepatol Res 40: 686-692, 2010.
12. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA and Benjamin RS: Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: Proposal of new computed tomography response criteria. J Clin Oncol 25: 1753-1759, 2007.
13. Watanabe M, Shiozawa K, Takahashi M, Wakui N, Otsuka Y, Kaneko H, Tanikawa K, Shibuya K, KamiyamaN and Sumino Y: Parametric imaging using contrast-enhanced ultrasound with Sonazoid for hepatocellular carcinoma. J Med Ultrasonics 37: 81-86, 2010.
14. Shiozawa K, Watanabe M, Kikuchi Y, Kudo T, Maruyama K and Sumino Y: Evaluation of sorafenib for hepatocellular carcinoma by contrast-enhanced ultrasonography: A pilot study. World J Gastroenterol 18: 5753-5758, 2012.
15. Sugimoto K, Moriyasu F, Kamiyama N, Metoki R, Yamada M, Imai Y and Iijima H: Analysis of morphological vascular changes of hepatocellular carcinoma by microflow imaging using contrast-enhanced sonography. Hepatol Res 38: 790-799, 2008.
16. Yang H, Liu GJ, Lu MD, Xu HX and Xie XY: Evaluation of the vascular archirecture of hepatocellular carcinoma by micro flow imaging: Pathologic correlation. J Ultrasound Med 26: 461-467, 2007.
17. Linden RA, Trabulsi EJ, Forsberg F, Gittens PR, Gomella LG and Halpern EJ: Contrast enhanced ultrasound flash replenishment method for directed prostate biopsies. J Urol 178: 2354-2358, 2007.
18. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
19. Kudo M: Hepatocellular carcinoma 2009 and beyond: From the surveillance to molecular targeted therapy. Oncology 75 (Suppl 1): 1-12, 2008.
20. Zhao H, Yao JL, Wang Y and Zhou KR: Detection of small hepatocellular carcinoma: Comparison of dynamic enhancement magnetic resonance imaging and multiphase multirow-detector helical CT scanning. World J Gastroenterol 13: 1252-1256, 2007.
21. Bruix J, Sala M and Llovet JM: Chemoembolization for hepatocellular carcinoma. Gastroenterology 127 (Suppl 1): S179-S188, 2004.
22. Moschouris H, Malagari K, Gkoutzios P, Kalokairinou M, Stamatiou K, Chatzimichail K, Kornezos I, Karagiannis E, Kiltenis M and Papadaki MG: Intermediate and advanced hepatocellular carcinoma treated with the antiangiogenic agent sorafenib. Evaluation with unenhanced and contrast-enhanced ultrasonography. Med Ultrason 14: 87-94, 2012.
23. Tanaka H, Iijima H, Higashiura A, Yoh K, Ishii A, Takashima T, Sakai Y, Aizawa N, Iwata K, Ikeda N, *et al*: New malignant grading system for hepatocellular carcinoma using the Sonazoid contrast agent for ultrasonography. J Gastroenterol 49: 755-763, 2014.
24. Sato K, Tanaka S, Mitsunori Y, Mogushi K, Yasen M, Aihara A, Ban D, Ochiai T, Irie T, Kudo A, *et al*: Contrast-enhanced intraoperative ultrasonography for vascular imaging of hepatocellular carcinoma: Clinical and biological significance. Hepatology 57: 1436-1447, 2013.
25. Jain RK: Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science 307: 58-62, 2005.
26. Matsui O, Kadoya M, Kameyama T, Yoshikawa J, Takashima T, Nakanuma Y, Unoura M, Kobayashi K, Izumi R and Ida M: Benign and malignant nodules in cirrhotic livers: Distinction based on blood supply. Radiology 178: 493-497, 1991.
27. Sakamoto M, Hirohashi S and Shimosato Y: Early stages of multistep hepatocarcinogenesis: Adenomatous hyperplasia and early hepatocellular carcinoma. Hum Pathol 22: 172-178, 1991.