Fucosylated Fraction of Alpha-fetoprotein as a Serological Marker of Early Hepatocellular Carcinoma

SATOSHI MORIYA[1], MANABU MORIMOTO[1], KAZUSHI NUMATA[1], AKITO NOZAKI[1], YU SHIMOYAMA[1], MASAAKI KONDO[1], MASAYUKI NAKANO[2], SHIN MAEDA[3] and KATSUAKI TANAKA[1]

[1]*Gastroenterological Center, Yokohama City University Medical Center, Minami-ku, Yokohama, Japan;*
[2]*Department of Diagnostic Pathology, Ofuna Chuo Hospital, Kamakura, Japan;*
[3]*Division of Gastroenterology, Yokohama City University Graduate School of Medicine, Kanazawa-ku, Yokohama, Japan*

Abstract. *Aim: This study aimed to evaluate the fucosylated fraction of alpha-fetoprotein (AFP-L3) as a marker of early hepatocellular carcinoma (HCC). Patients and Methods: We diagnosed early HCC in 15 patients (15 tumors) by gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid-enhanced magnetic resonance imaging and confirmed the diagnoses using new criteria of the International Consensus Group for Hepatocellular Neoplasia. We measured the AFP-L3%, simultaneously, using a liquid-phase binding assay–electrokinetic analyte transport assay. We compared the AFP-L3% levels between patients with early HCC and a control cohort with benign liver disease. Results: The AFP-L3% levels were higher in patients with early HCC than in the controls (4.1%±4.0% vs. 2.0%±3.5%, p=0.024). The sensitivity and specificity with AFP-L3% were 33.3% and 78.7% at a cut-off value of 5%, and 20.0% and 88.0% at a cut-off value of 7%, respectively. Conclusion: AFP-L3% is a suitable serological marker for evaluating early HCC.*

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world and the third most common cause of cancer-related death (1). The 5-year survival rate of patients with untreated HCC is less than 5% (2), and despite many advances in multidisciplinary treatment, complete curative treatment of early-stage HCC is the only treatment option for long-term patient survival (3). Therefore, the ability to detect early-stage HCC is crucial for reducing the mortality associated with this neoplasm.

The International Consensus Group for Hepatocellular Neoplasia (ICGHN) has proposed the following new histopathological criteria in the multistep development of HCC from a dysplastic nodule, to early HCC, and then advanced HCC (4). Because early HCC does not always show a typical vascular pattern on contrast-enhanced dynamic images, the sensitivity of imaging techniques for the diagnosis of early HCC is lower than that for advanced HCC (5). Recently, a new-generation contrast-enhanced medium in magnetic resonance imaging (MRI) has been proposed for the detection of liver tumors: gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid (Gd-EOB-DTPA). The lesions characterized by reduced Gd-EOB-DTPA uptake on MRI in patients with chronic liver disease were confirmed as early HCC after histopathological confirmation, using resected samples or fine-needle biopsies (6, 7).

Alpha-fetoprotein (AFP) is used as a serological marker for diagnosing HCC (8, 9), and it has been recommended as a surveillance tool according to the guidelines of the European Association for the Study of the Liver (10), the Asian Pacific Association for the Study of the Liver (11), and the Japanese Society of Hepatology (12). However, increases in AFP are noted not only in patients with HCC but also in patients with chronic hepatitis and cirrhosis (13). In contrast, the fucosylated fraction of AFP (AFP-L3) has been reported to be a specific marker for HCC (14). AFP-L3% measurement has been suggested for the early diagnosis of HCC (15, 16), however, AFP-L3% determined using a conventional assay was not a useful diagnostic indicator in patients with an AFP concentration of <20 ng/ml, including those with early-stage HCC (17). Recent technical improvements have increased the clinical utility of a highly sensitive assay for AFP-L3%, namely the liquid-phase binding assay–electrokinetic analyte transport assay (LBA–EATA) (18), enabling for the measurement of AFP-L3% in patients with low AFP concentrations.

Correspondence to: Manabu Morimoto, MD, Gastroenterological Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan. Tel: +81 452615656, Fax: +81 452619492, e-mail: morimoto@urahp.yokohama-cu.ac.jp

Key Words: Alpha-fetoprotein, fucosylated fraction of alpha-fetoprotein, early hepatocellular carcinoma, Gd-EOB-DTPA-enhanced MRI.

Table I. *Patients' characteristics*.

	Early HCC n=15	Control n=183	*p*-Value
Gender, n (%)			
Male/female	9 (60)/6 (40)	97 (53)/86 (47)	0.602
Age, years	67.5±6.5	62.6±13.4	0.018
Etiology, n (%)			
HBV/HCV/none	2 (13)/13 (87)/0	39 (21)/114 (62)/30 (16)	0.244
ALT, IU/l	50.6±30.3	53.8±52.8	0.816
Albumin, g/dl	4.0±0.6	4.2±0.5	0.163
Platelet, $\times 10^4/mm^3$	11.5±6.0	14.8±6.6	0.067
Tumor size, mm	13.5±3.2		
AFP, ng/ml	12.0±8.7	9.6±35.8	0.796
AFP-L3, %	4.1±4.0	2.0±3.5	0.024

The data represent the mean±standard deviation or number of patients (percentage). HCC, Hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; AFP-L3, fucosylated fraction of alpha-fetoprotein.

We screened for early HCC characterized by reduced Gd-EOB-DTPA uptake on MRI in patients with cirrhosis or chronic hepatitis. Subsequently, early HCC was histopathologically confirmed according to the new criteria using fine-needle biopsy, while simultaneously measuring serum levels of AFP-L3%. The aim of this study was to evaluate the clinical usefulness of the highly sensitive assay of AFP-L3% as a marker for the early diagnosis of HCC.

Patients and Methods

Patients. Between April 2011 and March 2012, we screened 300 patients with chronic liver disease for HCC lesions using Gd-EOB-DTPA-enhanced MRI. Reduced uptake of Gd-EOB-DTPA on T1-weighted hepatobiliary phase images 20 min after contrast medium injection indicated the presence of novel tumors in 15 patients. The diagnosis was histopathologically-confirmed on biopsies from the 15 tumors, with concurrent measurement of serum levels of AFP and AFP-L3%.

We obtained frozen blood samples from a control cohort (n=183) of patients with chronic hepatitis or cirrhosis caused by hepatitis B or C virus between January 2010 and August 2010. None of the patients in the control cohort demonstrated HCC during the 2-year follow-up period.

All patients provided written informed consent to participate in this study and the study was approved by the Institutional Review Board.

Imaging diagnosis. MRI was performed using a 1.5-T whole-body imager (Avant; Siemens Medical System, Erlangen, Germany). Immediately after an intravenous bolus injection of 0.1 μmol/kg of Gd-EOB-DTPA (Primovist®; Bayer Schering Pharma AG, Berlin, Germany) flushed with 10 ml of sterile saline solution from the antecubital vein, dynamic T1-weighted 2-dimensional gradient-echo sequence without fat suppression was performed during arterial dominant (20-35 sec), portal venous (70-90 sec) and delayed (4-5 min) phases. The hepatobiliary phase was reached 20 min after contrast injection, and the images were obtained using T1-weighted fat-suppressed volumetric interpolated breath-hold examination sequences and fast low-angle shot sequences. In addition, T2-weighted respiratory-triggered turbo spin-echo pace sequences and diffusion-weighted echo planar imaging sequences were also obtained.

AFP and AFP-L3% measurements. We measured the serum levels of AFP and AFP-L3% using a commercially available automatic measurement system based on a combined LBA–EATA (Wako Pure Chemical Industries Ltd., Osaka, Japan) of blood samples collected at the time of imaging (18, 19). The lower limit of quantitation for the AFP and AFP-L3 assay was 0.3 ng/ml.

Histopathological examination. We performed a sonography-guided or fusion image-guided (20) percutaneous fine-needle biopsy on the newly-detected tumors. Blinded histopathological diagnoses were performed according to the new histological criteria defined by the ICGHN in 2009 with the consensus of two pathologists specializing in liver lesions (4). Tumors were diagnosed as early HCC if they had the following characteristics: increased cell density with little cell atypia, architectural alterations of a thin trabecular structure and acinus in some areas, and stromal invasion.

Statistical analysis. Results are expressed as the mean±standard deviation and were analyzed using PASW Statistics 17.0 (IBM SPSS, Inc., Chicago, IL, USA). We evaluated the optimal cut-off values for AFP and AFP-L3% based on receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, positive predictive value, and negative predictive value of AFP and AFP-L3% for the optimal cut-off values were calculated. We used the Mann-Whitney *U*-test and χ^2-test for evaluating the statistical significance of the data, and the values were considered significant when the *p*-value was <0.05.

Results

The demographic characteristics of the patients are summarized in Table I. No significant differences were observed between the two cohorts, except for age. Early HCC diagnosis was histopathologically confirmed in all 15 tumors characterized by

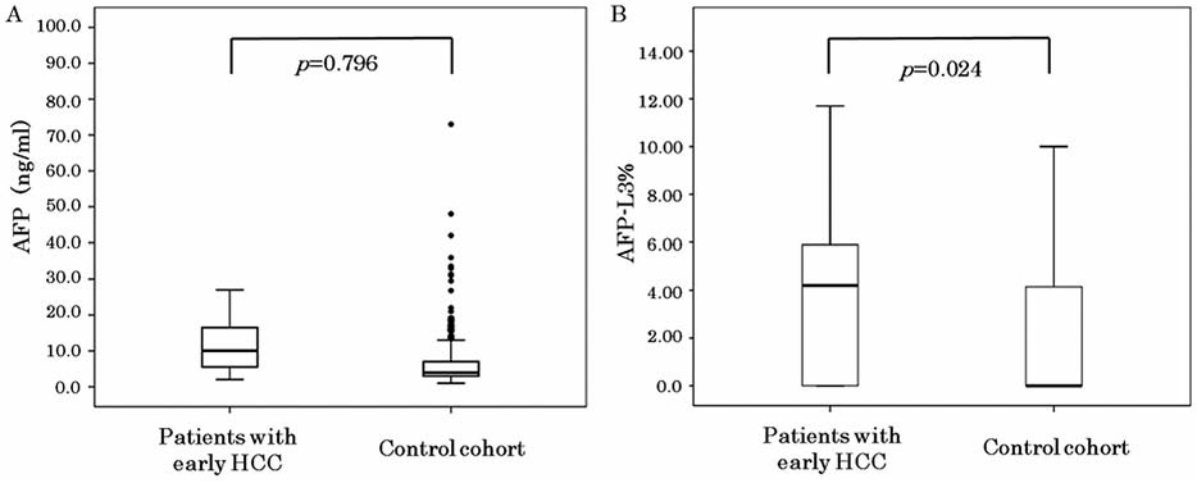

Figure 1. *Alpha-fetoprotein (AFP) (A) and fucosylated fraction of alpha-fetoprotein (AFP-L3) (B) in patients with early hepatocellular carcinoma and patients in the control cohort. No significant differences were seen in mean AFP levels between the two groups (12.0 ng/ml vs. 9.6 ng/ml, p=0.796, χ^2-test); however AFP-L3% was significantly higher in the early HCC group than in the control group (4.1% vs. 2.0%, p=0.024, χ^2-test). Boxes refer to the 25th and 75th percentile values, with a line indicating median levels, whereas the interquartile range extends outside the boxes. Points outside the interquartile range are outliers.*

reduced uptake of Gd-EOB-DTPA on MRI. The mean serum levels of AFP and AFP-L3% were 12.0 ng/ml and 4.1%, respectively, in patients with early HCC; and 9.6 ng/ml, and 2.0%, respectively, in the control patients. AFP-L3% was significantly higher in patients with early HCC than in the control patients (p=0.024) (Figure 1). ROC evaluations of early HCC are shown in Figure 2. The areas under the curve for AFP and AFP-L3% as markers of early HCC were 0.729 (95% confidence interval, CI=0.597-0.861) and 0.667 (95% CI=0.527-0.808), respectively. Sensitivities for AFP as a marker of early HCC were 93.3%, 80.0%, 66.7%, 53.3%, and 26.7% at cut-off values of 3, 5, 7, 10, and 20 ng/ml, respectively (Table II). Sensitivities for AFP-L3% as a marker of early HCC were 53.3%, 33.3%, 20.0%, and 13.3% at cut-off values of 3, 5, 7, and 10%, respectively (Table II).

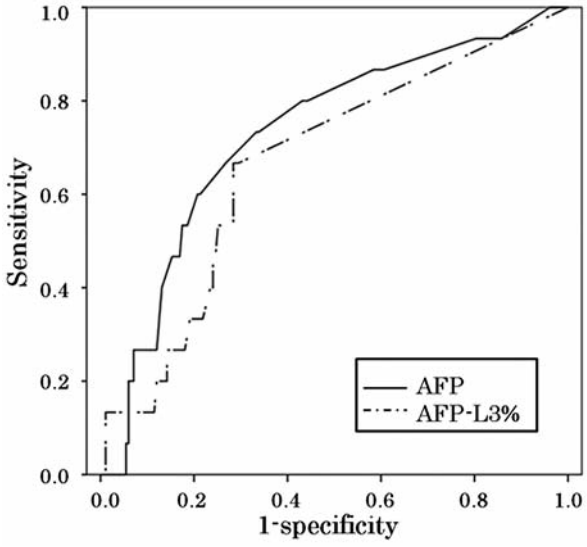

Figure 2. *Receiver operating characteristic curves for alpha-fetoprotein (AFP) and fucosylated fraction of alpha-fetoprotein (AFP-L3). The areas under the curves for AFP and AFP-L3% were 0.729 and 0.667, respectively.*

Discussion

We measured AFP-L3% in early HCC, defined by new histological criteria, and demonstrated that AFP-L3% is a potential serological marker for early HCC.

AFP has been used for many years as a serum marker for the diagnosis and screening of HCC (8, 9). In patients at high risk for HCC, the Japanese treatment guidelines recommend the measurement of tumor markers, including AFP and des-gamma-carboxy prothrombin, concurrently with computed tomography (CT)/ultrasonography (12). According to the 17th Nationwide Follow-up Survey of Primary HCC in Japan, 36.4% of HCC patients were AFP-positive when the cut-off value was set at 15 ng/ml (21). Hepatologists, however, have noted a non-specific increase in serum AFP concentration in chronic liver inflammation (13). In contrast, AFP-L3% has been reported to be a specific marker for diagnosis (14), recurrence (22), biological behavior (23), and prognosis (24) in patients with HCC with chronic liver disease.

Recent advances in diagnostic imaging facilitate the detection of small tumors, *i.e.* early-stage HCC (25-27).

Table II. *Diagnostic accuracy measurements according to receiver operating characteristic curve analysis for alpha-fetoprotein (AFP) and fucosylated fraction of alpha-fetoprotein (AFP-L3).*

	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
AFP	3 ng/ml	93.3	19.7	27.1	8.7	97.3
	5 ng/ml	80.0	56.8	55.1	13.2	97.2
	7 ng/ml	66.7	73.2	72.7	16.9	96.4
	10 ng/ml	53.3	82.5	80.3	20.0	95.6
	20 ng/ml	26.7	92.9	87.9	23.5	93.9
	100 ng/ml	0.0	99.5	91.9	0.0	92.4
AFP-L3%	3%	53.3	71.6	70.2	13.3	94.9
	5%	33.3	78.7	75.3	11.4	93.5
	7%	20.0	88.0	82.8	12.0	93.1
	10%	13.3	98.4	91.9	40.0	93.3
	15%	0.0	98.9	91.4	0.0	92.3

PPV, Positive predictive value; NPV, negative predictive value.

Furthermore, the establishment of surveillance programs for HCC for high-risk patients has contributed to the diagnosis of early-stage HCC (21). The usefulness of AFP-L3% measured using a conventional assay system was limited in samples with total AFP <20 ng/ml (15, 16). Conventional AFP assays may not diagnose small HCCs during the early stage because many patients with small tumors often have low AFP concentrations (28-30). To address this shortcoming, we measured AFP and AFP-L3% in blood samples of patients with early HCC using a highly sensitive assay method (18, 19).

A recent phase III trial demonstrated the diagnostic superiority of using Gd-EOB-DTPA-enhanced MRI compared with un-enhanced MRI and spiral CT for the detection of small lesions (≤20 mm) (31). Reduced Gd-EOB-DTPA uptake was speculated to be an early event in hepatocarcinogenesis, presenting the possibility of diagnosing early HCC that does not show a typical vascular pattern (6, 7, 32). Gd-EOB-DTPA-enhanced MRI is expected to be recommended as a key surveillance modality for early HCC in future guidelines.

Our study has some limitations, including a small sample size, and a non-randomized study design, which creates potential bias. In addition, the pathological diagnosis of early HCC was based on new criteria using needle-biopsied specimens. This could have caused sampling errors, with the potential of underestimating histological grades. Despite these limitations, to our knowledge, our study is the first to indicate the clinical usefulness of a highly sensitive AFP-L3% assay for the evaluation of early HCC, confirmed using the new histological criteria. Additional large-scale prospective studies are warranted to confirm our findings.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Acknowledgements

This study was partially supported by a Grant-in-Aid for Cancer Research (Grant No. 21-1) from the Ministry of Health, Labour, and Welfare of Japan.

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90, 2011.
2 Llovet JM, Burroughs A and Bruix J: Hepatocellular carcinoma. Lancet *362*: 1907-1917, 2003.
3 Forner A, Reig ME, de Lope CR and Bruix J: Current strategy for staging and treatment: The BCLC update and future prospects. Semin Liver Dis *30*: 61-74, 2010.
4 International Consensus Group for Hepatocellular Neoplasia: Pathologic diagnosis of early hepatocellular carcinoma: A report of the International Consensus Group for Hepatocellular Neoplasia. Hepatology *49*: 658-664, 2009.
5 Bruix J and Sherman M: Management of hepatocellular carcinoma. Hepatology *42*: 1208-1236, 2005.
6 Rhee H, Kim MJ, Park YN, Choi JS and Kim KS: Gadoxetic acid-enhanced MRI findings of early hepatocellular carcinoma as defined by new histologic criteria. J Magn Reson Imaging *35*: 393-398, 2012.
7 Sano K, Ichikawa T, Motosugi U, Sou H, Muhi AM, Matsuda M, Nakano M, Sakamoto M, Nakazawa T, Asakawa M, Fujii H, Kitamura T, Enomoto N and Araki T: Imaging study of early hepatocellular carcinoma: Usefulness of gadoxetic acid-enhanced MR imaging. Radiology *261*: 834-844, 2011.
8 Di Bisceglie AM and Hoofnagle JH: Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer *64*: 2117-2120, 1989.
9 Oka H, Tamori A, Kuroki T, Kobayashi K and Yamamoto S: Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology *19*: 61-66, 1994.
10 Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M and Rodes J: Clinical management of hepatocellular carcinoma.

Conclusions of the Barcelona-2000 EASL conference. J Hepatol *35*: 421-430, 2001.
11 Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, Kudo M, Lee JM, Choi BI, Poon RT, Shiina S, Cheng AL, Jia JD, Obi S, Han KH, Jafri W, Chow P, Lim SG, Chowla YK, Budihusodo U, Gani RA, Lesmana CR, Putranto TA, Liaw YF and Sarin SK: Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int *4*: 439-474, 2010.
12 Makuuchi M and Kokudo N: Surveillance algorithm and diagnostic algorithm for hepatocellular carcinoma. Hepatol Res *40*: 6-7, 2010.
13 Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ and Bock T: Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol *99*: 860-865, 2004.
14 Aoyagi Y, Isemura M, Suzuki Y, Sekine C, Soga K, Ozaki T and Ichida F: Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. Lancet *2*: 1353-1354, 1985.
15 Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, Satomura S, Matuura S, Kawai T and Hirai H: A collaborative study for the evaluation of lectin-reactive alpha-fetoprotein in early detection of hepatocellular carcinoma. Cancer Res *53*: 5419-5423, 1993.
16 Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R and Tomita K: Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology *62*: S57-S63, 2002.
17 Nakamura K, Imajo N, Yamagata Y, Katoh H, Fujio K, Tanaka T, Satomura S, Matsuura S: Liquid-phase binding assay of alpha-fetoprotein using a sulfated antibody for bound/free separation. Anal Chem *70*: 954-957, 1998.
18 Kagebayashi C, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, Kurosawa T, Watanabe M, Kawabata T, Chang W, Li C, Bousse L, Wada HG and Satomura S: Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem *388*: 306-311, 2009.
19 Kawabata T, Wada HG, Watanabe M and Satomura S: Electrokinetic analyte transport assay for alpha-fetoprotein immuoassay integrates mixing, reaction and separation on-chip. Electrophoresis *29*: 1399-1406, 2008.
20 Kunishi Y, Numata K, Morimoto M, Okada M, Kaneko T, Maeda S and Tanaka K: Efficacy of fusion imaging combining sonography and hepatobiliary phase MRI with Gd-EOB-DTPA to detect small hepatocellular carcinoma. Am J Roentgenol *198*: 106-114, 2012.
21 Ikai I, Arii S, Okazaki M, Okita M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M and Kudo M: Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. Hepatol Res *37*: 676-691, 2007.
22 Hayashi K, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, Sone Y, Miyata A, Shimizu H and Satomura S: Usefulness of measurement of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and recurrence of small hepatocellular carcinoma. Am J Gastroenterol *94*: 3028-3033, 1999.
23 Khien VV, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K and Satomura S: Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology proven hepatocellular carcinoma. Int J Biol Markers *16*: 105-111, 2001.
24 Yamashita F, Tanaka M, Satomura S and Tanikawa K: Prognostic significance of *Lens culinaris* agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinomas. Gastroenterology *111*: 996-1001, 1996.
25 Takayasu K, Moriyama N, Muramatsu Y, Makuuchi M, Hasegawa H, Okazaki N and Hirohashi S: The diagnosis of small hepatocellular carcinomas: Efficacy of various imaging procedures in 100 patients. Am J Roentgenol *155*: 49-54, 1990.
26 Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, Piscaglia F, Gramantieri L, Zanetti M and Sherman M: Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. Gut *48*: 251-259, 2001.
27 Shimizu A, Ito K, Koike S, Fujita T, Shimizu K and Matsunaga N: Cirrhosis or chronic hepatitis: Evaluation of small (≤2cm) early-enhancing hepatic lesions with serial contrast-enhanced dynamic MR imaging. Radiology *226*: 550-555, 2003.
28 Alpert E and Feller ER: Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology *74*: 856-858, 1978.
29 Kubo Y, Okuda K, Musha H and Nakashima T: Detection of hepatocellular carcinoma during a clinical follow-up of chronic liver disease. Gastroenterology *74*: 578-582, 1978.
30 Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, Katoh H, Endoh T, Sassa T and Satomura S: Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: Special reference to imaging diagnosis. J Hepatol *30*: 125-130, 1999.
31 Ichikawa T, Saito K, Yoshioka N, Tanimoto A, Gokan T, Takehara Y, Kamura T, Murakami T, Ito K, Hirohashi S, Nishie A, Saito Y, Onaya H, Kuwatsuru R, Morimoto A, Ueda K, Kurauchi M and Breuer J: Detection and characterization of focal liver lesions: A Japanese phase III, multicenter comparison between gadoxetic acid disodium-enhanced magnetic resonance imaging and contrast-enhanced computed tomography predominantly in patients with hepatocellular carcinoma and chronic liver disease. Invest Radiol *45*: 133-141, 2010.
32 Kogita S, Imai Y, Okada M, Kim T, Onishi H, Takamura M, Fukuda K, Igura T, Sawai Y, Morimoto O, Hori M, Nagano H, Wakasa K, Hayashi N and Murakami T: Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma: Correlation with histological grading and portal blood flow. Eur Radiol *20*: 2405-2413, 2010.

Received December 12, 2012
Revised January 29, 2013
Accepted January 31, 2013