J Gastroenterol (2014) 49:555–563
DOI 10.1007/s00535-013-0883-1

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

High-sensitivity *Lens culinaris* agglutinin-reactive alpha-fetoprotein assay predicts early detection of hepatocellular carcinoma

Takashi Kumada · Hidenori Toyoda · Toshifumi Tada · Seiki Kiriyama · Makoto Tanikawa · Yasuhiro Hisanaga · Akira Kanamori · Junko Tanaka · Chiaki Kagebayashi · Shinji Satomura

Received: 19 May 2013 / Accepted: 5 September 2013 / Published online: 23 September 2013
© The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract
Background Prognosis of patients with hepatocellular carcinoma (HCC) remains poor because HCC is frequently diagnosed late. Therefore, regular surveillance has been recommended to detect HCC at the early stage when curative treatments can be applied. HCC biomarkers, including *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), are widely used for surveillance in Japan. A newly developed immunoassay system measures AFP-L3 % with high sensitivity. This retrospective study aimed to evaluate clinical utility of high-sensitivity AFP-L3 (hs-AFP-L3) as a predictor of early stage HCC in surveillance at a single site.
Methods Of consecutive 2830 patients in the surveillance between 2000 and 2009, 104 HCC-developed and 104 non-HCC patients were selected by eligibility criteria and propensity score matching. Samples were obtained from the HCC patients who had blood drawn annually for 3 years prior to HCC diagnosis.
Results In the present study, hs-AFP-L3 was elevated 1 year prior to diagnosis in 34.3 % of patients. The survival rate of patients with the hs-AFP-L3 $\geq$ 7 % at 1 year prior to diagnosis was significantly lower than that of patients with hs-AFP-L3 $<$ 7 %.
Conclusions Elevation of hs-AFP-L3 was early predictive of development of HCC even at low AFP levels and in absence of ultrasound findings of suspicious HCC. The hs-AFP-L3 should be added to surveillance programs with US because elevated hs-AFP-L3 may be a trigger to perform enhanced imaging modalities for confirmation of HCC.

Keywords Surveillance · A propensity score analysis · High-sensitivity AFP-L3 · DCP · HCC

Abbreviations

HCC	Hepatocellular carcinoma
AFP	Alpha-fetoprotein
AFP-L3	*Lens culinaris* agglutinin-reactive fraction of AFP
hs-AFP-L3	High-sensitivity AFP-L3
US	Ultrasound
DCP	Des-gamma-carboxy prothrombin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
ALT	Alanine aminotransferase
MRI	Magnetic resonance imaging

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide [1], and poor prognosis is reported because HCC is frequently diagnosed at late stages and is often untreatable. Therefore, surveillance for HCC has been advocated to detect HCC at

T. Kumada (✉) · H. Toyoda · T. Tada · S. Kiriyama · M. Tanikawa · Y. Hisanaga · A. Kanamori
Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, 4-86 Minaminokawa-cho, Ogaki, Gifu 503-8052, Japan
e-mail: hosp3@omh.ogaki.gifu.jp

J. Tanaka
Department of Epidemiology Infectious Disease Control and Prevention, Hiroshima University Institute of Biomedical and Health Sciences, Hiroshima, Japan

C. Kagebayashi · S. Satomura
Diagnostic Division, Wako Pure Chemical Industries Ltd., Osaka, Japan

early stages when curative treatments can be applied [2, 3]. Global liver associations, including the American Association for the Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), and the Asian Pacific Association for the Study of the Liver (APASL), recommend regular surveillance on patients at high risk for HCC [4–6]. The most common tests used for surveillance are alpha-fetoprotein (AFP) tests and ultrasound (US). EASL and APASL adopt AFP and US in their guidelines, while AASLD recommends only US. Interpretation of US can be challenging when routine screening and comparison to previous imaging results are impossible or when US are performed by different institutes or instruments, whereas HCC biomarker values can be used independently with appropriate cutoff values. The Japan Society of Hepatology (JSH) has recommended not only US but also assays of three biomarkers: AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) [7].

However, AFP levels are often elevated even in patients with benign liver diseases. The low specificity of AFP has been a cause of concern for use as a HCC marker [8–10]. In contrast, a rate of AFP-L3 in total AFP (AFP-L3 %) has been reported to be highly specific for HCC in many studies [11–13]; however, accurate measurements of AFP-L3 % have been limited to patients having AFP >20 ng/mL by insufficient analytical sensitivity on a conventional assay system that is a liquid-phase binding assay (LiBASys) [14]. Recently, a micro-total analysis system (μTAS) based lectin-affinity electrophoresis using microfluidics technology has enabled accurate measurements of AFP-L3 % even at low AFP [15]. The high-sensitivity AFP-L3 (hs-AFP-L3) assay has demonstrated improvement in clinical sensitivity and predicting of prognosis in HCC patients with AFP < 20 ng/mL [16–18]. The Liver Cancer Study Group of Japan has reported that 37 % of HCC patients had low AFP (<15 ng/mL) at the HCC diagnosis [19]. They also show that 34 % of patients had tumors with maximum diameter of <2 cm. Early HCC is a distinct clinical entity with a high rate of surgical cure and detection of early HCC results in long-term survival [20]. However, elevated AFP is not always observed in patients with such small tumors. Therefore, the hs-AFP-L3 assay which can measure serum levels at low AFP is expected to improve detection of HCC at the early stage. Moreover, lower cutoff values for hs-AFP-L3 has been considered to improve clinical sensitivity [16–18].

In this study, clinical utility in early prediction of development of HCC in our study cohort under surveillance using hs-AFP-L3 and analyzed retrospectively is reported.

Patients and methods

Patients

The study protocol was approved by the Institutional Ethics Committee of Ogaki Municipal Hospital in January 2009 and was in compliance with the Declaration of Helsinki. Written informed consent for use of stored serum samples for the study was obtained from the enrolled patients.

Between 2000 and 2009, a total of consecutive 2830 patients positive for hepatitis B surface antigen (HBsAg) or anti-hepatitis C virus (HCV) antibody who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital were prospectively enrolled in our HCC surveillance. Of the 2830, 1214 patients met eligibility criteria: HBsAg- or HCV RNA-positive for more than 6 months, follow-up period of >3 years before HCC diagnosis, availability of sera sampled at least twice at 12-month intervals, maximal tumor diameter <3 cm and 3 nodules or less at diagnosis, and no oral intake of warfarin which is a DCP-inducing agent.

Of these 1214 patients, 114 patients had HCC and 1100 patients had no evidence of HCC during follow-up period. To reduce the confounding effects of covariates between HCC and control patients, we selected patients using propensity score matching. Six covariates including age, gender, etiology (HBV or HCV), Child-Pugh classification, platelet number, and alanine aminotransferase (ALT) except tumor markers were used. We computed the propensity score by using logistic regression with the independent variable including age (<65 years or ≤65 years), sex (female or male), etiology (HBV or HCV), Child-Pugh classification (A, B, or C), platelet count (>150 × $10^3/m^3$ or ≤150 × $10^3/m^3$), and ALT activity (≤40 IU/mL or >40 IU/mL) as shown in previous reported cut-off values according to the previous reports [21, 22]. This model yielded a *c* statistic of 0.832 (95 % confidence interval [CI], 0.797–0.866), indicating a strong ability to differentiate between HCC and control patients. Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test [23]. The *P* value of the calculated propensity score was 0.647 based on the Hosmer–Lemeshow test and showed an absence of bias. We were able to match 104 HCC developed patients to 104 non-HCC developing patients. Table 1 shows demographics of HCC and non-HCC groups. The median of tumor size was 1.9 cm. The 69 % of HCC patients had single tumor and the 86 % of HCC patients were at TNM stage I and II.

Surveillance and diagnosis

According to Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [7], we performed US and three

Table 1 Demographics and propensity score matching

Characteristics		HCC ($n = 104$)	Non-HCC ($n = 104$)	P value
Age (years)	Median (range)	67 (37–81)	68 (14–84)	0.980
Gender	Male/female	58 (56 %)/46 (44 %)	58 (56 %)/46 (44 %)	0.889
Etiology	B/C/B + C	14 (13 %)/89 (86 %)/1 (1 %)	14 (13 %)/89 (86 %)/1 (1 %)	1.000
Child-Pugh classification	A/B/C	82 (79 %)/18 (17 %)/4 (4 %)	84 (81 %)/17 (16 %)/3 (3 %)	0.907
ALT (IU/L)	Median (range)	49 (7–361)	46 (12–321)	0.582
Platelet ($\times 10^4/mm^3$)	Median (range)	10.1 (3.2–34.0)	12.1 (2.1–41.4)	0.150
Tumor size (cm)	Median (25 %, 75 % quartile)	1.9 (1.5, 2.3)	NA	NA
Tumor number	Single/Multiple	72 (69 %)/32 (31 %)	NA	NA
TNM stage	I/II/III	49 (47 %)/41 (39 %)/14 (14 %)	NA	NA

biomarker studies (AFP, AFP-L3, and DCP) every 3–4 months and dynamic magnetic resonance imaging (MRI) every 12 months for cirrhosis patients under surveillance. For patients with chronic hepatitis, we performed US and three biomarker studies every 6 months. For diagnostic confirmation of HCC, patients had a dynamic MRI when US suggested progression in nodular lesion, change of echo pattern in nodules, or increased biomarkers: continuous elevation of AFP or increase to AFP 200 ng/mL or more, AFP-L3 15 % or more, or DCP 40 mAU/mL or more. The hs-AFP-L3 assay was not available for the surveillance of those days.

Forty-five patients were diagnosed as HCC histologically (surgical specimen, 39 patients; US-guided needle biopsy specimens, 6 patients). The remaining 59 patients were diagnosed as HCC as typical findings of dynamic MRI including hypervascular in the arterial phase with washout in the portal venous or delayed phase [4].

Treatments

Individual decisions for a primary treatment were generally made on the basis of the guidelines for HCC in Japan [7]. Patients were initially assessed for eligibility for resection. When patients declined or were deemed ineligible for resection, they underwent locoregional ablative therapy (LAT) as a second option or transcatheter arterial chemoembolization (TACE) as a third one. Of the enrolled 104 patients, 99 patients underwent resection ($n = 39$), LAT ($n = 23$), or TACE ($n = 37$: including patients with both LAT and TACE). Five patients did not receive any treatment for HCC. No patient underwent liver transplantation.

Imaging modalities

B-mode US was performed with an Aplio XV or XG ultrasound system (Toshiba Medical System, Tokyo, Japan) equipped with a convex probe (PUT-375BT). MR imaging was performed using a superconducting scanner operating at 1.5 T (Signa Twin Speed; General Electric Medical Systems, Milwaukee, WI). MR images were obtained in the axial plane with a phased–array multicoil for the body. To scan whole livers, the section thickness was 8–10 mm with 2- and 3-mm intersectional gaps, depending on liver size. Breath-hold T1-weighted in-phase and out-of-phase fast spoiled gradient-recalled echo (SPGR, 200/dual echo [4.3/2.1] [TR/TE], 80° flip angle, one signal averaged) MR images were obtained with a field of view of 36–42 cm and a 256 × 192 matrix during a 22-s acquisition time. T2-weighted fat suppression fast spin-echo (2000/85 [TR/TE], two signal averaged) MR images with respiratory synchronization were obtained with a field of view of 36–42 cm and a 352 × 256 matrix. Breath-hold double arterial dynamic fast SPGR images (115/1.2 [TR/TE], 70° flip angle, one signal averaged) were obtained with a field of view of 36–42 cm and 512 × 192 matrix during a 12-s acquisition time. Dynamic MR images were obtained before and after an antecubital intravenous bolus injection of 0.1 mmol/kg of gadopentetate dimeglumine (Magnevist; Bayer in Japan, Tokyo, Japan) followed by 15–20 ml of a sterile normal saline flush. The optimum timing of start of scanning was decided for each case after 1 ml test injection of gadopentetate dimeglumine. The scan times were about 25, 40, and 60 s, and 2–2.5 min after initiation of the contrast injection, representing the early hepatic artery, late hepatic artery, portal vein, and equilibrium phase, respectively. All MR images except T2-weight MR images were obtained using array spatial sensitivity encoding technique (ASSET).

Assays of hs-AFP-L3, AFP, and DCP

For this retrospective study, the measurements of hs-AFP-L3, AFP, and DCP were achieved by using a microchip capillary electrophoresis and liquid-phase binding assay on μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd.) [16]. Analytical sensitivity of the μTAS is 0.3 ng/mL AFP, and percentage of AFP-L3 can be

measured when AFP-L3 is over 0.3 ng/mL. Analytical sensitivity of LiBASys is 0.8 ng/mL AFP, but AFP-L3 % can not be calculated at AFP < 10 ng/mL.

Samples were obtained from 104 HCC patients who had blood drawn annually for 3 years prior to the HCC diagnosis and stored at −80 °C until the measurements. In the HCC patients, stored serum samples at −3 years (over 30 months before, $n = 94$), −2 years (from 18 to 30 months before, $n = 97$), −1 year (from 6 to 18 months before, $n = 103$), and 0 year ($n = 104$) at the time of the HCC diagnosis were measured. In the non-HCC patients, similarly, stored serum samples at −3 years ($n = 99$), −2 years ($n = 104$), and −1 year ($n = 102$), and 0 year ($n = 104$) from the end of follow-up were measured.

Statistical analysis

To evaluate the diagnostic accuracy and predictive values of AFP, hs-AFP-L3, and DCP, sensitivity and specificity were calculated with cutoff values in the guidelines [7]. Furthermore, cutoff values of 5, 7, and 10 % for hs-AFP-L3 were used for this retrospective study according to previous reports [13, 16]. Serial changes of three biomarkers before the diagnosis of HCC were analyzed by Wilcoxon matched pair signed rank test. For the evaluation of prognosis, the long-term survival of patients with HCC was determined by the Kaplan–Meier method and the log-rank test was used to compare the survival rates. The values were considered significant when P value was <0.05. The analyses were performed using JMP10 statistical software (SAS Institute Japan, Japan).

The propensity score matching was performed with SPSS, version 18.0 for Windows (International Business Machines Corporation, Tokyo, Japan).

Results

Dynamic changes of biomarkers

The dynamic changes of hs-AFP-L3, AFP, and DCP in HCC patients at −3, −2, −1, and 0 year before diagnosis are shown in Fig. 1a, b, and c. The levels of hs-AFP-L3 at −1 year were significantly elevated from the levels at −2 years ($P = 0.0001$). The levels of hs-AFP-L3 at −0 year were significantly elevated from the levels at −1 year ($P = 0.0003$, Table 2). AFP and DCP were significantly elevated between −1 and 0 year ($P = 0.0315$

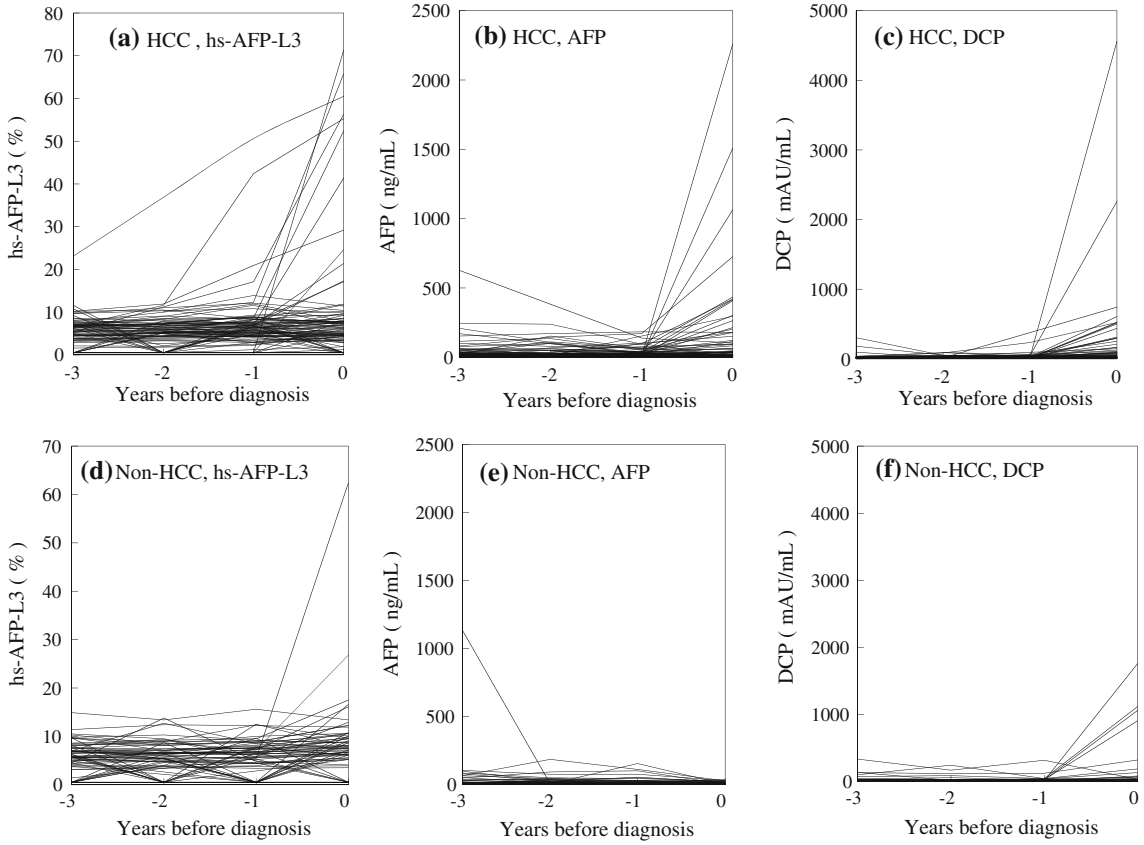

Fig. 1 Dynamic changes of biomarkers: **a** hs-AFP-L3, **b** AFP, and **c** DCP in each HCC patient ($n = 104$), and **d** hs-AFP-L3, **e** AFP, and **f** DCP in each non-HCC patient ($n = 104$)

Table 2 Serial changes of three biomarkers in HCC patients (Wilcoxon matched pair signed rank test)

Analyte	*P* value		
	At −3 year and −2 year	At −2 year and −1 year	At −1 year and 0 year
hs-AFP-L3	0.2935	0.0001	0.0003
AFP	0.4278	0.5359	0.0315
DCP	0.0926	0.6302	<0.0001

and $P < 0.0001$, respectively, Table 2). In non-HCC patients, no significant differences were observed for any markers (Fig. 1d–f). Only hs-AFP-L3 in HCC patients were significantly elevated 1 year prior to HCC diagnosis.

Sensitivity and specificity at diagnosis

Diagnostic sensitivity and specificity were evaluated for the hs-AFP-L3, AFP, DCP, and the combination of biomarkers (Table 3). The sensitivity was calculated by using HCC patient samples at diagnosis ($n = 104$) and the specificity was calculated by using non-HCC patient samples at −3 years ($n = 100$) to ensure that none had developed HCC for the following 3 years. Of the 104 HCC patients, 43 patients (41.3 %) had AFP < 10 ng/mL at which the conventional assay was not able to calculate AFP-L3 %. The sensitivity and specificity for hs-AFP-L3 were 11.5 and 100.0 %, respectively at a cutoff value of 15 %. A cutoff value of 7 % improved the sensitivity to 39.4 %. A combination assay with hs-AFP-L3, AFP, and DCP resulted in sensitivity of 60.6 % at diagnosis.

Sensitivity and specificity for 3 years before diagnosis

We calculated sensitivities using HCC samples at 3, 2, and 1 years prior to diagnosis. Similarly, specificities were calculated by using non-HCC samples (Table 4). The sensitivity and specificity for hs-AFP-L3 at −1 year were 34.3 and 74.7 %, respectively. The sensitivities at −1 year for AFP and DCP were 35.0 and 12.1 %, respectively. In HCC patients, hs-AFP-L3 turned positive at 34 patients (33.3 %) and stayed in positive at 27 patients (26.2 %) for two years till the diagnosis of HCC. In contrast, hs-AFP-L3 turned positive at 25 patients (24.3 %) and stayed in positive at 22 patients (21.4 %) for 2 years till the end of follow-up in non-HCC patients.

Table 3 Sensitivity and specificity at diagnosis

Analyte	Cutoff	Sensitivity (%)	Specificity (%)
hs-AFP-L3	5 %	50.9	51.0
	7 %	39.4	77.0
	10 %	16.3	96.0
	15 %	11.5	100.0
AFP	20 ng/mL	41.4	90.4
	200 ng/mL	12.5	99.0
DCP	40 mAU/mL	34.6	94.0
All biomarkers	7 % + 200 ng/mL + 40 mAU/mL	60.6	76.0

Comparison of tumor characteristics and survival rates

Comparing tumor characteristics at detection of HCC by a level of hs-AFP-L3 at −1 year, the tumor size, the number of tumors, and TNM stage between patients with hs-AFP-L3 ≥ 7 % and <7 % ($P = 0.064$, 0.821, and 0.504, respectively) were not statistically significant. The number of patients receiving curative treatments such as resection and LAT was significantly higher in patients with hs-AFP-L3 < 7 % ($P = 0.020$) (data not shown).

During the follow-up period after the diagnosis that was ranged from 4 to 110 months (median of 39 months), the survival rate of patients with hs-AFP-L3 ≥ 7 % was significantly lower than that of patients with hs-AFP-L3 < 7 % by using values at −1 year ($P = 0.039$) (Fig. 2). There was no statistical significance between patients with DCP ≥ 40 mAU/mL and patients with DCP < 40 mAU/mL ($P = 0.831$). No patients had AFP > 200 ng/mL at −1 year. The survival rate of patients with hs-AFP-L3 ≥ 7 % had a lower tendency than that of patients with hs-AFP-L3 < 7 % at HCC diagnosis ($P = 0.1501$).

Triggers to perform MRI for suspicious HCC and positivity rates for hs-AFP-L3

In this study population, US was performed median of 4 times between −1 year and diagnosis day. The 104 HCC

Table 4 Sensitivity and specificity for three years before diagnosis

Analyte	Year	Sensitivity (%)	Specificity (%)
hs-AFP-L3 ≥7 %	−1	34.3	74.7
	−2	25.3	80.6
	−3	24.5	77.0
AFP ≥20 ng/mL	−1	35.0	86.4
	−2	31.0	83.0
	−3	33.0	86.0
DCP ≥40 mAU/mL	−1	12.1	93.9
	−2	8.4	94.9
	−3	4.3	94.0

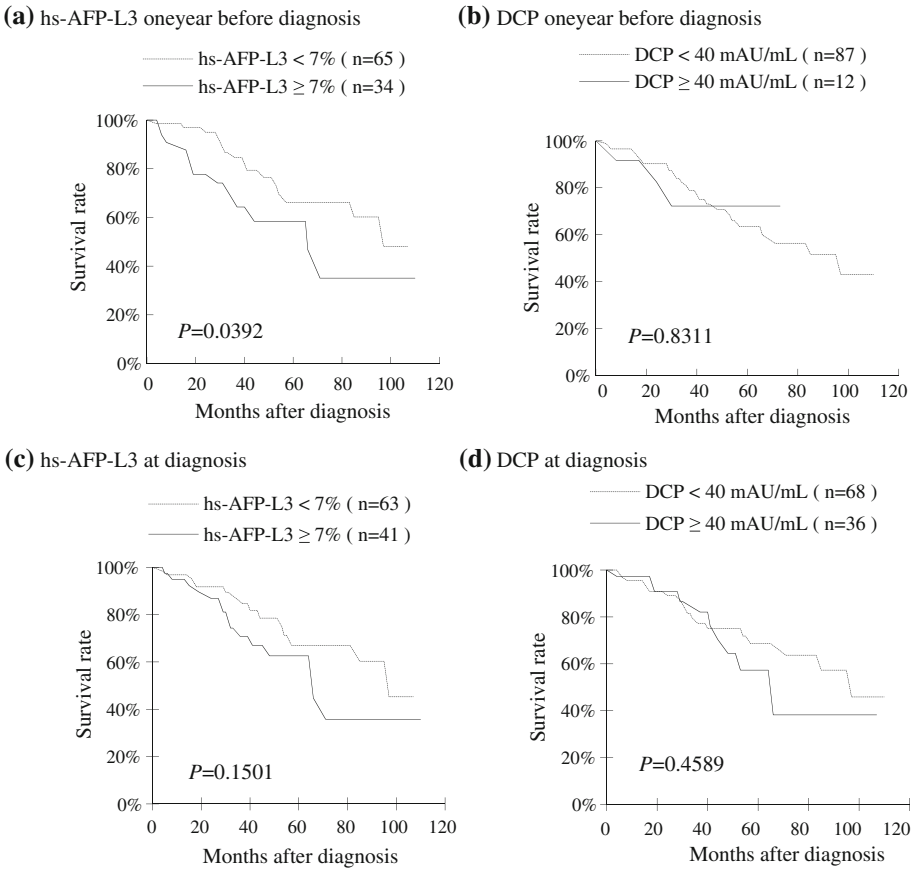

Fig. 2 Survival rates by levels of biomarkers: **a** hs-AFP-L3 and **b** DCP 1 year before, **c** hs-AFP-L3 and **d** DCP at diagnosis

patients were classified into three groups by a trigger to perform MRI for diagnostic confirmation (Table 5). US findings triggered MRI for 86 patients. The 86 patients were classified further by US findings: increase of the tumor number (51/86), increase of the tumor size (18/86), or change of the echo pattern in nodules (17/86). Five patients were monitored by MRI as results of elevated biomarkers. The remaining 13 patients were screened by MRI instead of US because interpretation of US was difficult in patients who were obese or had severe liver atrophy.

In the present retrospective study for hs-AFP-L3, 29.6 % of patients who were diagnosed with HCC by the trigger of US had hs-AFP-L3 $\geq$ 7 % 1 year prior to the diagnosis day. In the patients who had changes of the echo pattern in nodules, the positivity rate for hs-AFP-L3 at -1 year was 50.0 % and relatively higher compared to the other groups by US.

Table 5 Triggers to perform MRI for suspicious HCC and positivity rates for hs-AFP-L3

Triggers to perform MRI	*n*	hs-AFP-L3 >7 % At -1 year (%)	hs-AFP-L3 >7 % At diagnosis (%)
(a) Ultrasound	86	29.6	36.0
Increase of the tumor number	51	27.7	39.2
Increase of the tumor size	18	16.7	11.1
Change of the echo pattern in nodules	17	50.0	52.9
(b) Biomarkers	5	80.0	60.0
(c) Others	13	46.2	53.8

Discussion

Most studies on HCC biomarkers have focused on the accuracy at the time of diagnosis and the prediction of prognosis. So far there are a few studies which have evaluated early prediction of development of HCC in patients at high risk for HCC by biomarkers.

Taketa et al. [24] have reported that AFP-L3 values elevated above the cutoff value of 15 % with an average of 4.0 $\pm$ 4.9 months before the detection of HCC by imaging techniques. Sato et al. [25] also have demonstrated that lectin-reactive AFP elevated 3–18 months before the detection. However, only samples with AFP levels higher than 30 ng/mL were measured in their study. Recent data

indicated that the elevated AFP is not typical at HCC diagnosis for patients under in surveillance in Japan. Therefore, hs-AFP-L3 is expected to be more useful at low levels of AFP. Even though there were some differences in AFP concentration among the studies, they reported that elevation of AFP-L3 prior to diagnosis was associated with development of HCC.

Shiraki et al. [26] detected the small tumor <2 cm in maximum diameter in more than half of the patients. In the study population, they demonstrated clinical utility of lectin-reactive AFP as an early indicator while low AFP was reported limiting of the early recognition of HCC. Shimauchi et al. [27] demonstrated that AFP-L3 and DCP values showed elevated in about half of the patients at 6 months before the recognition of HCC by imaging techniques. These two markers were mutually complementary. In our study, DCP was not significantly elevated 1 year prior to diagnosis.

Lok et al. [28] have reported in a retrospective study of AFP and DCP values in patients in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis Trial who had blood drawn every 3 months for 12 months prior to HCC diagnosis. They have concluded that the biomarkers are needed to complement ultrasound in the detection of early HCC but neither DCP nor AFP is optimal. For the study, early stage HCC was defined as a single tumor nodule <3 cm in diameter with no evidence of vascular invasion or metastasis, and only 61.5 % of patients presented with early stage HCC. In our study, median of tumor size was 1.9 cm and all patients with <3 cm. Tumor volume doubling time is reported to be 90–132 days [29] and it may take a half year or 1 year for a nodule to develop from <2 cm to >3 cm. Therefore, HCC patients in our study were diagnosed 1 year earlier than the patients in Lok's study. Clinically the tumor size between <2 cm and 3 cm is one of the factor for making decisions of treatments, and it has been reported that survival rate of patients with tumor size <2 cm is higher [20]. Therefore, HCC should be diagnosed at the earlier stage with tumor <2 cm in order to achieve better outcome.

It is well known that AFP-L3 concentration correlates well with AFP; however, AFP-L3 % is not correlated with AFP [24, 30]. AFP-L3 % is a marker that is independent of AFP. Therefore, we have used AFP-L3 % for analysis.

In the present study, hs-AFP-L3 was significantly elevated 1 year prior to HCC diagnosis in 34.3 % of patients at a cutoff value of 7 %. Tamura et al. [16] reported that a cutoff value of 7 % is most appropriate for discriminating HCC from benign liver disease using this assay. Therefore, patients with elevated hs-AFP-L3 value under surveillance should be followed up closely. The specificity of 80 % or less before diagnosis may actually mislead because the non-HCC patients selected by matching with the HCC patients were potentially higher risk group for HCC and would likely develop HCC later.

In previous studies, elevated AFP-L3 has been reported to be correlated to a shorter doubling time of tumor volume, increased hepatic arterial supply, and pathologic features such as infiltrative tumor growth pattern, capsule infiltration, vascular invasion, and intrahepatic metastasis [31, 32]. These findings are often difficult to diagnose by various imaging modalities in small HCCs. Such blood supply changes typically result in change of echo pattern in nodules. In this study, therefore, high positivity rates for hs-AFP-L3 at −1 year in the patients who had such changes of echo pattern may be associated with developing HCC. The survival rate of patients with hs-AFP-L3 > 7 % at −1 year was significantly poorer compared to patients with hs-AFP-L3 < 7 %. However, differences of the detected tumor size and number were not statistically significant between patients with hs-AFP-L3 ≥ 7 % and <7 %. AFP-L3-positive HCC nodules may be aggressive and have high malignancy potential even though the tumor size is small. Therefore, it may be useful in early detection of the aggressive tumor to perform enhanced imaging techniques such as MRI for patients with elevated hs-AFP-L3. Survival rate of patients with the hs-AFP-L3 elevation at HCC diagnosis showed a poorer tendency; however, there were no statistical differences. HCC treatments were done just after the HCC diagnosis. Therefore, HCC tumors in patients with the hs-AFP-L3 elevation 1 year before HCC diagnosis might have 1 year to grow. This 1 year may reflect the difference of survival of two groups. DCP is a good marker for poor prognosis of HCC. However, the difference of overall survival between patients with DCP ≥40 and <40 mAU/mL was not observed due to the early stage (small) HCC without obvious vascular invasion.

AFP is a good marker to distinguish high-risk group for HCC development in the future [22]; however, AFP was not elevated 1 year prior to HCC development. AFP-L3 was elevated 1 year prior to diagnosis of small HCC in 34.3 % of patients.

Interpretation of US can be challenging without comparison to previous imaging results and performance of US can be limited in patients who are obese or have severe background liver cirrhosis. In the present study, sensitivity of the combined three biomarkers was 60.6 % at diagnosis, and measurements of biomarkers are expected to complement to US in surveillance.

In conclusion, elevation of hs-AFP-L3 was early predictive of development of HCC even at low AFP levels and in absence of US findings of suspicious HCC. Prognosis of patients with elevated hs-AFP-L3 was significantly poorer. HCC may be diagnosed earlier to receive curative treatments by the elevated hs-AFP-L3 as a trigger of enhanced imaging techniques. Additional prospective studies are

expected to demonstrate whether routine measurements of hs-AFP-L3 in HCC surveillance can improve overall patient survival.

Acknowledgments This work was supported by Health and Labour Sciences Research Grants (Research on Hepatitis) from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest All authors declare that the authors report no conflicts of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893–917.
2. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130:417–22.
3. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology. 2000;32:842–6.
4. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–2.
5. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. J Hepatol. 2001;35:421–30.
6. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian pacific association for the study of the liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010;4:439–74.
7. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res. 2008;38:37–51.
8. Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer. 1989;64:2117–20.
9. Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein changes in the course of chronic hepatitis: relation to bridging hepatic necrosis and hepatocellular carcinoma. Liver. 1986;6:133–7.
10. Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, et al. Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol. 2001;32:240–4.
11. Aoyagi Y, Isemura M, Suzuki Y, Sekine C, Soga K, Ozaki T, et al. Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. Lancet. 1985;2:1353–4.
12. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y, et al. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. Gastroenterology. 1990;99:508–18.
13. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of *Lens culinaris* agglutinin-reactive alpha-fetoprotein. J Gastroenterol Hepatol. 2001;16:1378–83.
14. Yamagata Y, Katoh H, Nakamura K, Tanaka T, Satomura S, Matsuura S. Determination of alpha-fetoprotein concentration based on liquid-phase binding assay using anion exchange chromatography and sulfated peptide introduced antibody. J Immunol Methods. 1998;212:161–8.
15. Kagebayashi C, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, et al. Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem. 2009;388:306–11.
16. Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. Dig Dis Sci. 2010;55:2095–101.
17. Hanaoka T, Sato S, Tobita H, Miyake T, Ishihara S, Akagi S, et al. Clinical significance of the highly sensitive fucosylated fraction of alpha-fetoprotein in patients with chronic liver disease. J Gastroenterol Hepatol. 2011;26:739–44.
18. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, et al. Clinical utility of highly sensitive *Lens culinaris* agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. Cancer Sci. 2011;102:1025–31.
19. Ikai I, Kudo M, Arii S, Omata M, Kojiro M, Sakamoto M, et al. Report of the 18th follow-up survey of primary liver cancer in Japan. Hepatol Res. 2010;40:1043–59.
20. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, et al. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. Hepatology. 1998;28:1241–6.
21. Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol. 2009;50:729–35.
22. Kumada T, Toyoda H, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, et al. Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus. J Gastroenterol. 2011;46:536–44.
23. Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley; 2000.
24. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive a-fetoproteins in early detection of hepatocellular carcinoma. Cancer Res. 1993;53:5419–23.
25. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med. 1993;328:1802–6.
26. Shiraki K, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. Hepatology. 1995;22:802–7 Oncol Rep. 2000; 7:249–256.
27. Shimauchi Y, Tanaka M, Kuromatsu R, Ogata R, Tateishi Y, Itano S, et al. A simultaneous monitoring of *Lens culinaris* agglutinin A-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. Oncol Rep. 2000;7:249–56.
28. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. Gastroenterology. 2010;138:493–502.
29. Okazaki N, Yoshino M, Yoshida T, Suzuki M, Moriyama N, Takayasu K, et al. Evaluation of the prognosis for small hepatocellular carcinoma based on tumor volume doubling time. A preliminary report. Cancer. 1989;63:2207–10.

30. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology. 1990;12:1420–32.
31. Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, et al. Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. J Hepatol. 1999;30:125–30.
32. Tada T, Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, et al. Relationship between Lens culinaris agglutinin-reactive alpha-fetoprotein and pathologic features of hepatocellular carcinoma. Liver Int. 2005;25:848–53.