

performed with simultaneous observation of individual liver lesions using ultrasonography. The VTTQ measurement of the right lobe may be superior to that of the left lobe for the diagnosis of liver fibrosis, and it may be valuable when assessing patients in whom taking a liver biopsy would be risky, such as those with liver cirrhosis.

Acknowledgments The authors wish to thank Siemens Medical Solutions USA, Inc., for the use of the Acuson S2000 device, Mochida Siemens Medical Systems Co., Ltd., in Japan for assisting in the setup of the device, and Ms. Natsumi Yamashita for her valuable advice with the statistical analysis. We have no financial interests linked to this work.

References

- Mazzagferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med*. 1996;334:693–9.
- National Institutes of Health. NIH consensus statement on management of hepatitis C. *Hepatology*. 2002;36:S3–20.
- Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med*. 2001;344:495–500.
- Maharaj B, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet*. 1986;1:523–5.
- Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol*. 2002;97:2614–8.
- Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology*. 2003;38:1449–57.
- Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet*. 2001;357:1069–75.
- Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology*. 2002;36:986–92.
- Guechot J, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronans and type III procollagen aminoterminal peptide serum assays as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem*. 1996;42:558–63.
- Nightingale KR, Zhai L, Dahl JJ, Frinkley KD, Palmeri ML. Shear wave velocity estimation using acoustic radiation force impulsive excitation in liver in vivo. In: Proceedings of institute of electrical and electronics engineers (IEEE) ultrasonics symposium, 2006. Vancouver: IEEE; 2006. p. 1156–60.
- Friedrich-Rust M, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, et al. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology*. 2009;252:595–604.
- Takahashi H, Ono N, Eguchi Y, Eguchi T, Kitajima Y, Kawaguchi Y, et al. Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. *Liver Int*. 2010;30:538–45.
- Palmeri ML, Frinkley KD, Zhai L, Gottfried M, Bentley RC, Ludwig K, et al. Acoustic radiation force impulse (ARFI) imaging of the gastrointestinal tract. *IEEE*. 2004;1:23–7.
- Dahl JJ, Pinton GF, Palmeri ML, Agrawal V, Nightingale KR, Trahey GE. A parallel tracking method for acoustic radiation force impulse imaging. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2007;54:301–12.
- Garra BS. Imaging and estimation of tissue elasticity by ultrasound. *Ultrasound Q*. 2007;23:255–68.
- Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, et al. Prospective comparison of transient elastography, fibrotest, APRI and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology*. 2005;128:343.
- Harada N, Soejima Y, Taketomi A, Yoshizumi T, Ikegami T, Yamashita Y, et al. Assessment of graft fibrosis by transient elastography in patients with recurrent hepatitis C after living donor liver transplantation. *Transplantation*. 2008;85:69–74.
- Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Ledinghen V, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology*. 2005;41:48.
- Foucher J, Chanteloup E, Vergniol J, Castera L, Le Bail B, Adhoute X, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut*. 2006;55:403–8.
- DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the area under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837–45.
- Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. *Ann Intern Med*. 1999;131:174–81.
- Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol*. 1998;28:930–8.
- Ingiliz P, Chhay KP, Munteanu M, Lebray P, Ngo Y, Roulot D, et al. Applicability and variability of liver stiffness measurements according to probe position. *World J Gastroenterol*. 2009;15:3398–404.
- Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol*. 2003;29:1705–13.

Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus

Takashi Kumada · Hidenori Toyoda · Seiki Kiriyaama · Makoto Tanikawa · Yasuhiro Hisanaga · Akira Kanamori · Toshifumi Tada · Junko Tanaka · Hiroshi Yoshizawa

Received: 9 August 2010 / Accepted: 22 October 2010 / Published online: 7 December 2010
© Springer 2010

Abstract

Background Increases in tumor markers are sometimes seen in patients with chronic liver disease without hepatocellular carcinoma (HCC). The aim of this study was to determine the relationship between the levels of three tumor markers [alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des- γ -carboxy prothrombin (DCP)] and hepatic carcinogenesis to identify hepatitis C virus (HCV) carriers at high risk for cancer development.

Methods A total of 623 consecutive HCV carriers with follow-up periods of >3 years were included. The average integration values were calculated from biochemical tests, and tumor markers, including AFP, AFP-L3%, and DCP, and factors associated with the cumulative incidence of HCC were analyzed.

Results HCC developed in 120 (19.3%) of the 623 patients. Age >65 years [adjusted relative risk, 2.303 (95% confidence interval, 1.551–3.418), $P < 0.001$], low platelet count [3.086 (1.997–4.768), $P < 0.001$], high aspartate aminotransferase value [3.001 (1.373–6.562), $P < 0.001$], high AFP level [≥ 10 , <20 ng/mL: 2.814 (1.686–4.697),

$P < 0.001$; ≥ 20 ng/mL: 3.405 (2.087–5.557), $P < 0.001$] compared to <10 ng/mL, and high AFP-L3% level [≥ 5 , <10%: 2.494 (1.291–4.816), $P = 0.007$; ≥ 10 %: 3.555 (1.609–7.858), $P < 0.001$] compared to <5% were significantly associated with an increased incidence of HCC on multivariate analysis.

Conclusions Increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥ 10 ng/mL AFP or patients with ≥ 5 % AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values.

Keywords Alpha-fetoprotein (AFP) · *Lens culinaris* agglutinin-reactive fraction of AFP · Hepatic regeneration · Necroinflammatory activity · Hepatocarcinogenesis

Introduction

Serum alpha-fetoprotein (AFP) is a widely used marker for hepatocellular carcinoma (HCC) [1]. However, serum AFP levels are increased in patients with liver diseases other than HCC, including viral hepatitis [2–4], with a prevalence of 10–42% [2, 5–7]. Increases in AFP are a marker of hepatic regeneration following hepatocyte destruction in viral hepatitis [8]. However, the pathogenesis and clinical significance of this phenomenon remain unclear.

The *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) and des- γ -carboxy prothrombin (DCP) are also markers for HCC [9–12]. Available data suggest that these tumor markers are more highly specific for HCC than AFP alone [9]. However, there are no reports examining the prognostic value of these markers in hepatocarcinogenesis.

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

J. Tanaka · H. Yoshizawa
Department of Epidemiology,
Infectious Disease Control and Prevention,
Graduate School of Biomedical Sciences,
Hiroshima University, Hiroshima, Japan

Results of biochemical tests, including tumor markers, can fluctuate for a given patient and can vary between different patients, and repeated measurements over time may provide a more accurate picture of disease development or progression. The arithmetic mean value is often used to assess biochemical parameters over time, but this value can be greatly affected by the interval between measurements such that a short period of very high values can inappropriately skew the mean. We have previously argued that the average integration value is more meaningful than the arithmetic mean value for the purposes of monitoring disease progression [13, 14].

The aim of this study was to determine the relationship between three tumor markers (AFP, AFP-L3%, and DCP) to better identify hepatitis C virus (HCV) carriers at high risk for the development of HCC. Of note, we used the average integration values of these parameters in our analysis.

Patients, materials, and methods

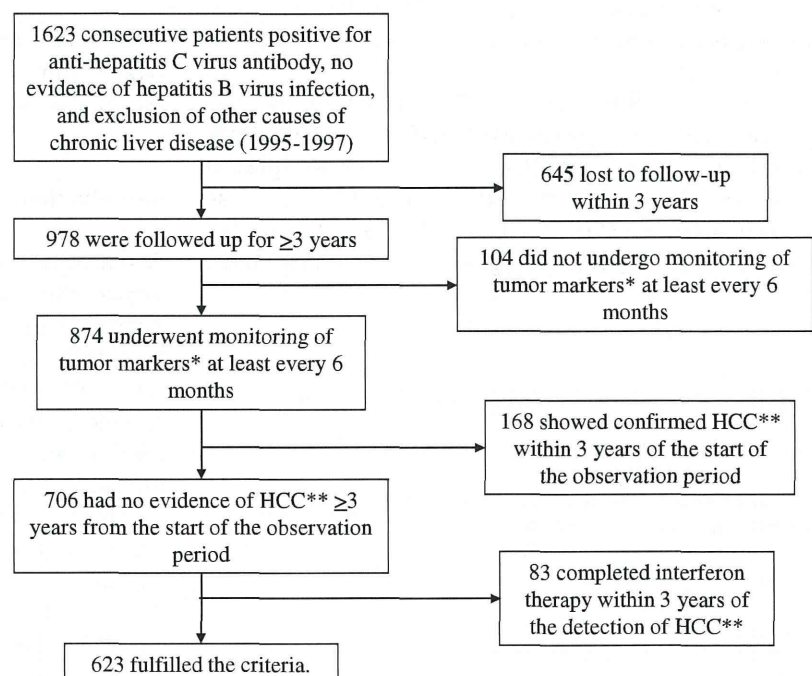
Patient selection

A total of 1623 consecutive patients positive for anti-HCV antibody visiting the Department of Gastroenterology at Ogaki Municipal Hospital during the period January 1995 to December 1997 were considered for enrollment. The present study cohort included the following criteria for enrollment: (1) positive for anti-HCV antibody by second-

or third-generation enzyme-linked immunosorbent assay and detectable HCV RNA for at least 6 months; (2) no evidence of positivity for hepatitis B surface antigen; (3) exclusion of other causes of chronic liver disease (i.e., alcohol consumption lower than 80 g/day, no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (4) follow-up period greater than 3 years; (5) measurement of AFP, AFP-L3%, and DCP at least every 6 months; (6) no evidence of HCC for at least 3 years from the start of the observation periods; and (7) interferon (IFN) therapy completed greater than 3 years before the detection of HCC in patients who received IFN therapy. A total of 623 patients fulfilled these criteria (Fig. 1).

Fibrosis was histologically evaluated in 187 of the 623 patients and staged according to Desmet et al. [15] as follows: F0, no fibrosis; F1, mild fibrosis; F2, moderate fibrosis; F3, severe fibrosis; and F4, cirrhosis. The remaining 436 patients were evaluated by ultrasound (US) findings and biochemical tests. The diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [16–18]. In this study patients who did not satisfy these criteria were classified as having chronic hepatitis. Four hundred and sixty-three patients were diagnosed with chronic hepatitis and 160 patients with cirrhosis.

Fig. 1 Schematic flowchart of enrolled patients. *Serum alpha-fetoprotein (AFP), *Leish culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des- γ -carboxy prothrombin (DCP). **Hepatocellular carcinoma (HCC)



All patients were followed up at our hospital at least twice a year. During each follow-up examination, platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (γ -GTP), total bilirubin, cholinesterase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, total cholesterol, AFP, AFP-L3%, and DCP were measured. Platelet count and ALT, AST, γ -GTP, total bilirubin, cholinesterase, ALP, LDH, albumin, total cholesterol, AFP, AFP-L3%, and DCP values were expressed as average integration values [13, 14]. Briefly, using ALT as an example, the area of a trapezoid is calculated by multiplying the sum of two ALT values by one-half of the interval between the measurements. This value is then divided by the observation period to obtain the average integration value, and this technique provides a better representation of values over time when there are extremes of high and low values [14, 16]. In patients who developed HCC during the observation period, AFP, AFP-L3%, and DCP values obtained at least 1 year before the diagnosis of HCC were assessed. Serum AFP concentration was determined with a commercially available kit. AFP-L3% was measured by lectin-affinity electrophoresis and antibody-affinity blotting with the AFP Differentiation Kit L (Wako Pure Chemical Industries, Osaka, Japan) [10]. DCP was measured with a DCP reagent (Picolumi PIVKA-II; Eisai, Tokyo, Japan) [11]. Cutoff levels for AFP, AFP-L3%, and DCP were set at 20 ng/mL, 10%, and 40 mAU/mL, respectively, according to previous reports [10–12]. HCV genotype and quantification of HCV RNA (Amplicor 2; Roche Diagnostics, Tokyo, Japan) were determined in 513 cases. All patients underwent imaging modalities (US, computed tomography [CT], or magnetic resonance imaging [MRI]), every 3 months in patients with cirrhosis and every 6 months in patients with chronic hepatitis.

The diagnoses of HCC were confirmed by histologic examination of resected hepatic tumors or US-guided needle biopsy specimens. When biopsy of the tumor was contraindicated, the HCC diagnosis was made using clinical criteria and imaging findings obtained from B-mode US, CT angiography, or MRI [19, 20]. HCC was histologically diagnosed in 46 patients, and in the remaining 74 patients, the diagnosis was made based on clinical criteria [19, 20]. All tumors were 3 cm or less in maximum diameter, and there were 3 nodules or less on diagnosis.

One hundred eighty-nine patients received IFN therapy. Patients were classified into three groups according to the type of response to IFN therapy: sustained virologic response (SVR), defined as the absence of serum HCV RNA at 6 months after IFN therapy; the non-SVR group, defined as the presence of serum HCV RNA at 6 months after IFN therapy; and the no IFN therapy group.

Patients were classified into three groups for each of the tumor markers according to the average integration values of AFP, AFP-L3%, and DCP: A1, <10 ng/mL ($n = 452$); A2, ≥ 10 , <20 ng/mL ($n = 80$); and A3, ≥ 20 ng/mL ($n = 91$); L1, <5% ($n = 588$); L2, ≥ 5 , <10% ($n = 18$); and L3, $\geq 10\%$ ($n = 17$); and D1, <20 mAU/mL ($n = 379$); D2, ≥ 20 , <40 mAU/mL ($n = 170$); and D3, ≥ 40 mAU/mL ($n = 51$), respectively.

The present study ended on 31 December 2008 or the date of identification of HCC occurrence. The median follow-up period was 9.0 years (range 3.0–13.0 years). The total number of blood examinations was 25,721, and the median number of blood examinations was 23 (range 6–105) per subject.

Statistical analysis

Statistical analysis was performed with the Statistical Program for Social Science (SPSS ver.17.0 for Windows; SPSS Japan, Tokyo, Japan). Continuous variables are shown as medians (ranges). The Mann–Whitney *U*-test was used for continuous variables, and Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed by the Kaplan–Meier method, and differences were tested by the log-rank test. The Bonferroni correction was performed for multiple comparisons. The Cox proportional hazards model and forward selection method were used to estimate the relative risk of HCC development associated with age (≤ 65 or > 65 years), sex (female or male), body mass index (BMI ≤ 25.0 or > 25.0 kg/m²), HCV genotype (type 1 or type 2), viral concentration (≤ 100 or > 100 KIU/mL), platelet count ($< 12.0 \times 10^4/\text{mm}^3$ or $\geq 12.0 \times 10^4/\text{mm}^3$), ALT (≤ 35 or > 35 IU/mL), AST (≤ 40 or > 40 IU/mL), total bilirubin (≤ 1.2 or > 1.2 mg/dL), γ -GTP (≤ 56 or > 56 IU/mL), ALP (≤ 338 or > 338 IU/mL), cholinesterase (< 431 or ≥ 431 IU/mL), LDH (≤ 250 or > 250 IU/mL), albumin (< 3.5 or ≥ 3.5 g/dL), total cholesterol (< 130 or ≥ 130 mg/dL), cirrhosis (presence or absence), and IFN treatment (no therapy, non-SVR, or SVR) for univariate and multivariate analyses. We used the lower or upper limit of the reference values at our institute as cutoff values for platelet count, ALT, AST, total bilirubin, γ -GTP, ALP, cholinesterase, LDH, albumin, and total cholesterol levels. Statistical significance was set at $P < 0.05$.

The study protocol was approved by the Ethics Committee at Ogaki Municipal Hospital in January 2009 and the study was performed in compliance with the Helsinki Declaration. Informed consent was obtained from each patient for analyzing patient records and images.

Fig. 2 Overall cumulative incidence rate of HCC

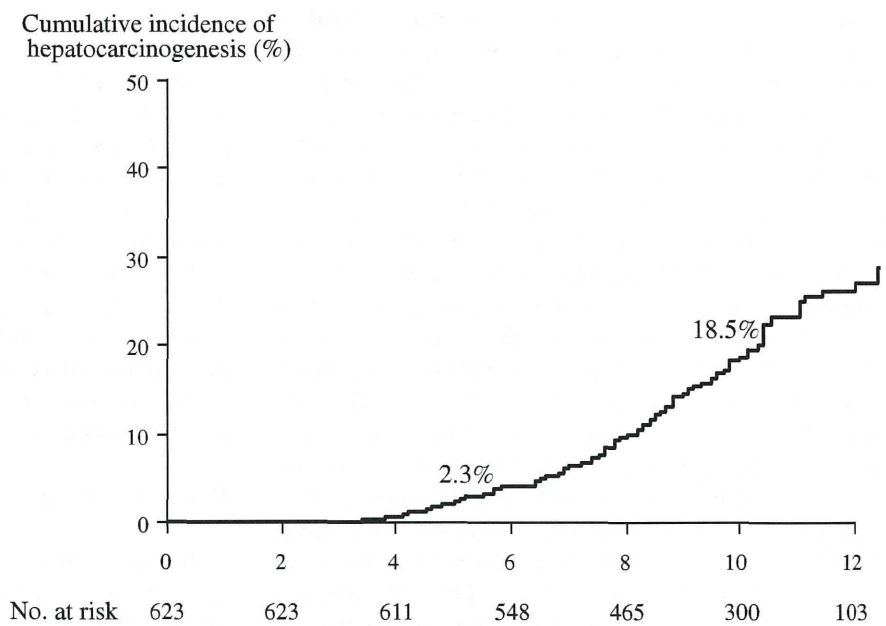


Table 1 Patient characteristics

Age (years)	61 (26–84)
Sex (F/M)	265/358
BMI (kg/m ²)	22.5 (12.0–34.9)
HCV genotype (type 1/type 2)	356/157
Viral concentration (KIU/mL)	270 (0.5–6300)
AFP (ng/mL)	4.8 (0.8–341.5)
AFP-L3 (%)	0.1 (0.0–32.5)
DCP (mAU/mL)	18.1 (8.5–99.6)
Platelets (×10 ⁴ /mm ³)	14.8 (3.0–33.9)
ALT (IU/L)	46.4 (10.1–340.4)
AST (IU/L)	48.5 (13.3–168.9)
γ-GTP (IU/L)	37.6 (9.9–2207)
Total bilirubin (mg/dL)	0.6 (0.2–2.7)
ALP (IU/L)	276.4 (86.8–845.5)
Cholinesterase (IU/L)	242.9 (38.8–545.30)
LDH (IU/L)	196.4 (118.4–650.1)
Albumin (g/dL)	4.0 (2.4–4.9)
Total cholesterol (mg/dL)	155.8 (77.9–264.1)
Fibrosis (F0/F1/F2/F3/F4) ^a	32/73/56/24/2
Cirrhosis (present/absent)	160/463
IFN therapy (none/non-SVR/SVR)	434/146/43

Continuous variables are quoted as medians (ranges)

BMI body mass index, HCV hepatitis C virus, AFP alpha-fetoprotein, AFP-L3 *Lens culinaris* agglutinin-reactive fraction of AFP, DCP des-γ-carboxy prothrombin, ALT alanine aminotransferase, AST aspartate aminotransferase, GTP gamma glutamyl transpeptidase, ALP alkaline phosphatase, LDH lactate dehydrogenase, IFN interferon, SVR sustained virologic response

^a Staging of chronic hepatitis according to Desmet et al. [15]

Results

HCC developed in 120 (19.3%) of the 623 patients. The 5- and 10-year cumulative incidences of HCC were 2.3 and 18.5%, respectively (Fig. 2). Demographic and medical data for the 623 patients are summarized in Table 1.

Factors associated with the incidence of hepatic carcinogenesis on univariate analysis

Factors associated with the incidence of HCC are listed in Table 2. Age ≥65 years, high AFP level, high AFP-L3% level, high DCP level, low platelet count, high ALT level, high AST level, high LDH level, high ALP level, low cholinesterase level, low albumin level, presence of cirrhosis, and response to IFN therapy were significantly associated with the development of HCC on univariate analysis.

The 5-, 7-, and 10-year cumulative incidences of HCC were 1.1, 2.1, and 7.5% in group A1; 2.6, 9.6, and 42.1% in group A2; and 6.6, 18.3, and 50.0% in group A3, respectively, and the cumulative incidence of HCC differed significantly between groups A1 and A2 and groups A1 and A3 (Fig. 3). The 5-, 7-, and 10-year cumulative incidences of HCC were 1.4, 4.6, and 15.6% in group L1; 19.6, 39.7, and 73.6% in group L2; and 12.5, 25.0, and 56.7% in group L3, respectively, and the cumulative incidence of HCC differed significantly between groups L1 and L2 and groups L1 and L3 (Fig. 4). The 5-, 7-, and 10-year cumulative incidences of HCC were 0.5, 4.6, and

Table 2 Factors associated with hepatocarcinogenesis (univariate analysis)

	Crude hazard ratio (95% CI)	P
Age (years)		
≤65	1	
>65	2.318 (1.580–3.400)	<0.001
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	6.061 (3.768–9.750)	<0.001
A3; ≥20	8.985 (5.874–13.744)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	8.032 (4.388–14.700)	<0.001
L3; ≥10	3.781 (1.838–7.778)	<0.001
DCP (mAU/mL)		
D1; <20	1	
D2; ≥20, <40	1.209 (0.788–1.855)	0.385
D3; ≥40	4.535 (2.840–7.241)	<0.001
Platelets ($\times 10^4/\text{mm}^3$)		
≥12.0	1	
<12.0	5.887 (3.982–8.702)	<0.001
ALT (IU/L)		
≤35	1	
>35	2.632 (1.574–4.400)	<0.001
AST (IU/L)		
≤40	1	
>40	8.120 (4.115–16.024)	<0.001
LDH (IU/L)		
≤250	1	
>250	1.970 (1.249–3.106)	<0.001
ALP (IU/L)		
≤338	1	
>338	2.509 (1.724–3.650)	<0.001
Cholinesterase (IU/L)		
>431	1	
≤431	3.288 (2.209–4.893)	<0.001
Albumin (g/dL)		
≥3.5	1	
<3.5	3.948 (2.635–5.917)	<0.001
Cirrhosis		
Absent	1	
Present	3.474 (2.413–5.002)	<0.001
IFN therapy		
No therapy	1	
Non-SVR	0.312 (0.180–0.539)	<0.001
SVR	0.215 (0.075–0.620)	0.004

Continuous variables are quoted as medians (ranges)

CI confidence interval, AFP alpha-fetoprotein, AFP-L3 *Lens culinaris* agglutinin-reactive fraction of AFP, DCP des- γ -carboxy prothrombin, ALT alanine aminotransferase, AST aspartate aminotransferase, LDH lactate dehydrogenase, ALP alkaline phosphatase, IFN interferon, SVR sustained virologic response

14.8% in group D1; 1.8, 4.3, and 16.3% in group D2; and 10.0, 25.0, and 48.2% in group D3, respectively, and the cumulative incidence of HCC differed significantly

between groups D1 and D3 and groups D2 and D3 (Fig. 5).

Factors associated with the incidence of hepatic carcinogenesis on multivariate analysis

Factors associated with the incidence of HCC as analyzed by the Cox proportional hazards model and the forward selection method are listed in Table 3. Age >65 years, low platelet count, high AST level, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC. Factors associated with the incidence of HCC were analyzed in patients with chronic hepatitis and cirrhosis (Table 4). High age, low platelet count, high AST level, and high AFP level were significantly associated with the incidence of HCC in chronic hepatitis, and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in cirrhosis. Factors associated with the incidence of HCC were analyzed in patients with and without IFN treatment (Table 5). Male sex, low platelet count, low cholinesterase level, and high AFP level were significantly associated with the incidence of HCC in patients with IFN therapy and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in patients without IFN therapy.

Discussion

Advances in US, CT, and MRI have allowed for the more frequent and earlier detection of small HCC tumors less than 2 cm in diameter during the routine follow-up of patients with chronic liver disease [21–23]. However, the performance and resolution of the imaging device, the skills of individual operators, and the diagnostic acumen of the interpreting radiologist all affect the early detection of HCC. AFP, AFP-L3%, and DCP levels have been used as prognostic markers rather than diagnostic markers for HCC [9]. However, the detection rate of small HCC tumors with these markers is low; AFP-L3% and DCP have low sensitivity, and AFP has low specificity. Sassa et al. [12] reported detection rates of 22.6 and 48.4% for AFP-L3% and DCP, respectively, in patients with small HCC tumors. It is currently thought that serum markers are useful for follow-up after HCC therapy in patients with high tumor marker levels before treatment [24].

We have previously reported that the average integration value of ALT correlates with the cumulative incidence of hepatocarcinogenesis, even within the normal range [13, 14]. In the present study, the average integration value of AFP was not selected as a factor associated with the

Fig. 3 Incidence of HCC according to the average integration value of AFP. The cumulative incidence of HCC differed significantly between groups A1 (<10 ng/mL) and A2 (≥10, <20 ng/mL) and groups A1 and A3 (≥20 ng/L)

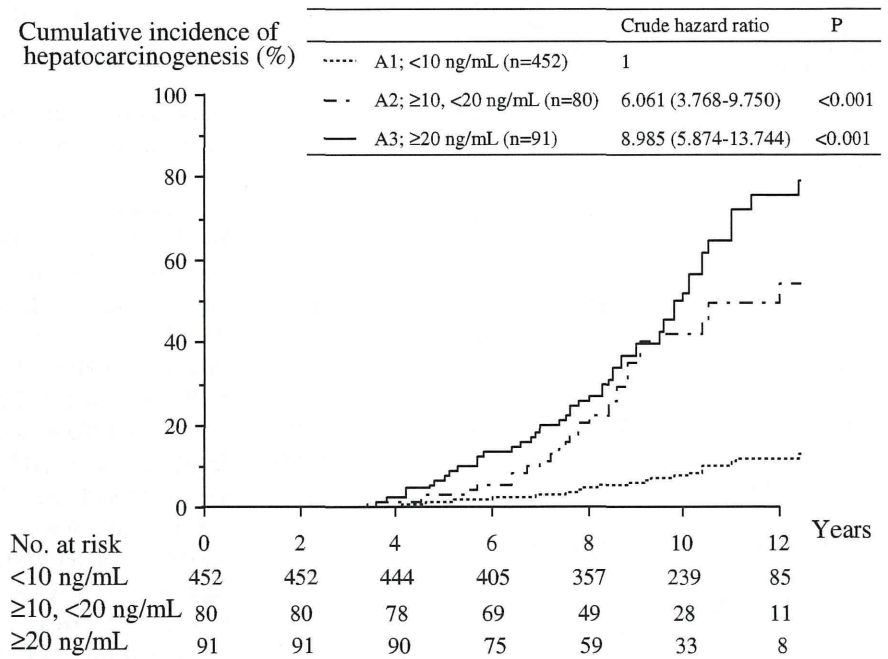
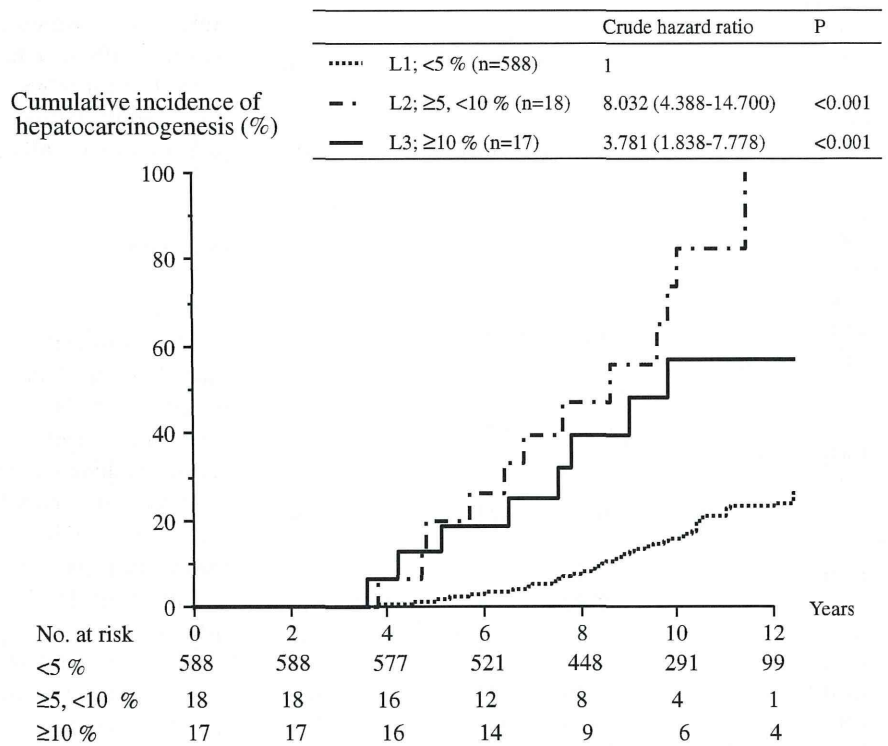


Fig. 4 Incidence of HCC according to the average integration value of AFP-L3%. The cumulative incidence of HCC differed significantly between groups L1 (<5%) and L2 (≥5, <10%) and groups L1 and L3 (≥10%)



incidence of HCC on multivariate analysis. AFP production is thought to be increased in response to injury, possibly due to increased hepatocyte turnover, in patients with HCV who do not have HCC [25]. In contrast, increased ALT levels are correlated with hepatocellular necrosis but not with hepatocyte proliferation. This difference may at

least partially explain the absence of correlation between ALT and AFP levels.

The multivariate analysis in our series was carried out to minimize the influence of confounding factors, and 5 factors were selected by the forward selection method. Age >65 years, low platelet count, high AST value, high AFP

Fig. 5 Incidence of HCC according to the average integration value of DCP. The cumulative incidence of HCC differed significantly between groups D1 (<20 mAU/mL) and D3 (≥40 mAU/mL) and groups D2 (≥20, <40 mAU/mL) and D3

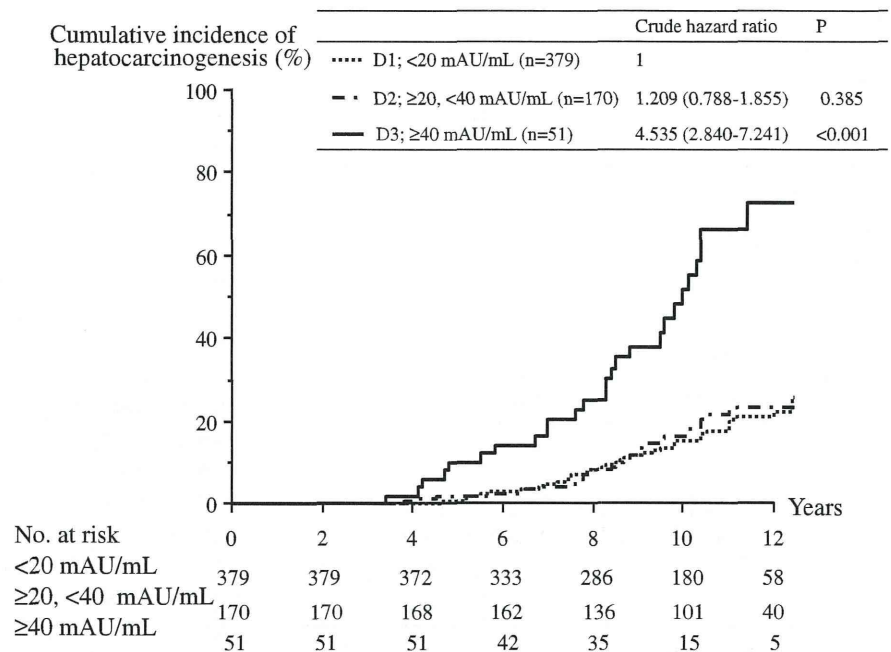


Table 3 Factors associated with hepatocarcinogenesis (multivariate analysis)

	Adjusted hazard ratio (95% CI)	P
Age (years)		
≤65	1	
>65	2.303 (1.551–3.418)	<0.001
Platelets (×10 ⁴ /mm ³)		
≥12.0	1	
<12.0	3.086 (1.997–4.768)	<0.001
AST (IU/L)		
≤40	1	
>40	3.001 (1.373–6.562)	0.006
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	2.814 (1.686–4.697)	<0.001
A3; ≥20	3.405 (2.087–5.557)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	2.494 (1.291–4.816)	0.007
L3; ≥10	3.555 (1.609–7.858)	0.002

AST aspartate aminotransferase, AFP alpha-fetoprotein, AFP-L3 *Lens culinaris* agglutinin-reactive fraction of AFP

level, and high AFP-L3% level were significantly associated with hepatic carcinogenesis in our multivariate analysis, but serum ALT level was not a risk factor for developing HCC. Ikeda et al. [26] reported that the cumulative incidence of HCC increased significantly in cirrhotic patients with an AFP level ≥10 ng/mL compared to those with an AFP level

<10 ng/mL, and the adjusted risk ratio was 15.788 in HCV patients. They speculated that AFP is a marker of disease activity or severity and cellular regeneration, and it acts as a better predictor of HCC with viral etiology of cirrhosis. As an index of hepatic regeneration, the AFP level better represents the risk of hepatic carcinogenesis than an index of liver injury (e.g., ALT level). In addition to AFP, AFP-L3% was identified as a factor predicting the development of HCC, and this is a specific marker for the existence of HCC. Therefore, elevations in AFP-L3% may reflect an occult cancer that is undetectable with current imaging modalities. More intensive surveillance is needed for patients such as those who fulfill the criteria of groups L2 and L3 in our series, although these groups were very small in size. However, similar to other laboratory values, as high AFP-L3% values may be associated with severe liver damage, it is necessary to interpret these values carefully. DCP is well known to be also a specific marker of HCC. DCP is more closely related to tumor size than AFP and AFP-L3% [27]. Therefore, it is thought that these were the reasons that DCP was not selected as a predictive marker for HCC in our multivariate analysis.

Among the other risk factors we identified for the development of HCC, a low platelet count stands out. The platelet count is a useful marker for the diagnosis of cirrhosis [28], and cirrhosis is an established risk factor for HCC in HCV carriers [26, 28–30]. Taken together with our other findings, the low platelet count suggests that HCC develops in patients with progressive or advanced liver disease. We additionally used ultrasound (US) to distinguish cirrhotic patients from non-cirrhotic patients [16–18]. The presence of cirrhosis on US was strongly associated with an increased

Table 4 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with chronic hepatitis and cirrhosis

	Chronic hepatitis (n = 463)	Cirrhosis (n = 160)
Age (years): ≤65 vs. >65	<0.001	0.008
Gender: female vs. male		<0.001
Platelets ($\times 10^4/\text{mm}^3$): ≥ 12.0 vs. <12	0.001	0.007
AST (IU/L): ≤ 40 vs. >40	0.043	
AFP (ng/mL): <10 vs. ≥ 10 , <20 vs. ≥ 20	<0.001	0.003
AFP-L3 (%): <5 vs. ≥ 5 , <10 vs. ≥ 10		0.017

AST aspartate aminotransferase, AFP alpha-fetoprotein, AFP-L3 *Lens culinaris* agglutinin-reactive fraction of AFP

Table 5 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with and without IFN treatment

	With IFN (n = 189)	Without IFN (n = 434)
Age (years): ≤65 vs. >65		0.001
Gender: female vs. male	0.005	<0.001
Platelets ($\times 10^4/\text{mm}^3$): ≥ 12.0 vs. <12.0	0.047	<0.001
Cholinesterase (IU/L): ≥ 431 vs. <431	0.007	
AFP (ng/mL): <10 vs. ≥ 10 , <20 vs. ≥ 20	<0.001	<0.001
AFP-L3 (%): <5 vs. ≥ 5 , <10 vs. ≥ 10		<0.001

IFN interferon, AFP alpha-fetoprotein, AFP-L3 *Lens culinaris* agglutinin-reactive fraction of AFP

incidence of HCC on univariate analysis, but US-determined cirrhosis was not identified as a risk factor on multivariate analysis. Histologic assessment of fibrosis and cirrhosis was obtained in only 187 patients (30.0%), and patients with F4 fibrosis had a higher incidence of HCC in our univariate analysis. However, the population of patients with material available for histologic review was only one-third the size of the entire study population, and this small number may have negatively affected our ability to detect the predictive nature of fibrosis at all levels of severity. In contrast to serum ALT, serum AST levels were significantly associated with the incidence of HCC. AST levels are often abnormal in patients with cirrhosis when ALT values are in the normal range, and the AST/ALT ratio is frequently greater than 1 in cirrhotic patients [31]. Elevated AST activity is a surrogate marker for cirrhosis. Aging is associated with a number of events at the molecular, cellular, and physiological levels that influence carcinogenesis and subsequent cancer growth [32]. It has been hypothesized that an age-associated decrease in DNA repair [33] contributes to the development of HCC.

Recent reports have shown that AFP levels fall following the administration of IFN with or without ribavirin [34, 35]. IFN has been shown to have antiviral, anti-inflammatory, and anticancer activities [36]. One study demonstrated an

anticancer effect of IFN when this agent was given following intrahepatic recurrence after HCC resection [37], and in our study, previous treatment with IFN was a factor associated with a reduced incidence of HCC on univariate analysis. The median ages of our patients with and without IFN treatment were 53 years (range 28–71) and 65 years (range 26–84), respectively; the age in those receiving IFN was significantly lower than the age in the group without IFN ($P < 0.0001$). It is thought that age and IFN therapy are confounding factors because IFN therapy has better results in younger patients. Although IFN was not identified as a predictive factor on multivariate analysis, the possibility cannot be denied that IFN may play an important role in modulating AFP levels prior to the onset of HCC.

In conclusion, increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥ 10 ng/mL AFP or patients with $\geq 5\%$ AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values. Intensive imaging modalities including US, CT, and MRI are recommended every 3–6 months for these patients.

Acknowledgments This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest There is no conflict of interest to disclose.

References

- Colombo M, de Franchis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med*. 1991;325:675–80.
- Kew MC, Purves LR, Bersohn I. Serum alpha-fetoprotein levels in acute viral hepatitis. *Gut*. 1973;14:939–42.
- Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. *Gastroenterology*. 1978;74:856–8.
- Eleftheriou N, Heathcote J, Thomas HC, Sherlock S. Serum alpha-fetoprotein levels in patients with acute and chronic liver disease. Relation to hepatocellular regeneration and development of primary liver cell carcinoma. *J Clin Pathol*. 1977;30:704–8.
- Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med*. 1995;332:1463–6.
- Bayati N, Silverman AL, Gordon SC. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. *Am J Gastroenterol*. 1998;93:2452–6.
- Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. *Am J Gastroenterol*. 2004;99:860–5.
- 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.
9. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. *Hepatology*. 1990;12:1420–32.
 10. Shimizu K, Katoh H, Yamashita F, Tanaka M, Tanikawa K, Taketa K, et al. Comparison of carbohydrate structures of serum α -fetoprotein by sequential glycosidase digestion and lectin affinity electrophoresis. *Clin Chim Acta*. 1996;254:23–40.
 11. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer*. 1998;82:1643–8.
 12. Sassa T, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-gamma-carboxy prothrombin and *Levis culinaris* agglutinin A-reactive alpha-fetoprotein in patients with small hepatocellular carcinoma. *Eur J Gastroenterol Hepatol*. 1999;11:1387–92.
 13. Kumada T, Toyoda H, Kiriyaama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. *Gut*. 2007;56:738–9.
 14. Kumada T, Toyoda H, Kiriyaama 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.
 15. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology*. 1994;19:1513–20.
 16. Shen L, Li JQ, Zeng MD, Lu LG, Fan ST, Bao H. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. *World J Gastroenterol*. 2006;28:1292–5.
 17. Iacobellis A, Fusilli S, Mangia A, Clemente R, Festa V, Giacobbe A, et al. Ultrasonographic and biochemical parameters in the non-invasive evaluation of liver fibrosis in hepatitis C virus chronic hepatitis. *Aliment Pharmacol Ther*. 2005;22:769–74.
 18. Caturelli E, Castellano L, Fusilli S, Palmentieri B, Niro GA, del Vecchio-Blanco C, et al. Coarse nodular US pattern in hepatic cirrhosis: risk for hepatocellular carcinoma. *Radiology*. 2003;226:691–7.
 19. Kudo M. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. *Semin Liver Dis*. 1999;19:297–309.
 20. Torzilli G, Minagawa M, Takayama T, Inoue K, Hui AM, Kubota K, et al. Accurate preoperative evaluation of liver mass lesions without fine-needle biopsy. *Hepatology*. 1999;30:889–93.
 21. Tanaka S, Kitamura T, Nakanishi K, Okuda S, Yamazaki H, Hiyama T, et al. Effectiveness of periodic checkup by ultrasonography for the early diagnosis of hepatocellular carcinoma. *Cancer*. 1990;66:210–4.
 22. Takayasu K, Furukawa H, Wakao F, Muramatsu Y, Abe H, Terauchi T, et al. CT diagnosis of early hepatocellular carcinoma: sensitivity, findings, and CT-pathologic correlation. *AJR Am J Roentgenol*. 1995;164:885–90.
 23. Ebara M, Ohto M, Watanabe Y, Kimura K, Saisho H, Tsuchiya Y, et al. Diagnosis of small hepatocellular carcinoma: correlation of MR imaging and tumor histologic studies. *Radiology*. 1986;159:371–7.
 24. Toyoda H, Kumada T, Kiriyaama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. *Clin Gastroenterol Hepatol*. 2006;4:111–7.
 25. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol*. 2005;43:434–41.
 26. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology*. 1993;18:47–53.
 27. Nakamura S, Noura S, Sakaguchi K, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol*. 2006;101:2038–43.
 28. Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, Tung HD, et al. Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. *Cancer*. 2006;107:2212–22.
 29. Kaneko S, Unoura M, Takeuchi M, Terasaki S, Ogino H, Matsushita E, et al. The role of hepatitis C virus in hepatocellular carcinoma in Japan. *Intervirol*. 1994;37:108–13.
 30. Tarao K, Shimizu A, Ohkawa S, Harada M, Ito Y, Tamai S, et al. Development of hepatocellular carcinoma associated with increases in DNA synthesis in the surrounding cirrhosis. *Gastroenterology*. 1992;103:595–600.
 31. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clin Chem*. 2000;46:2050–68.
 32. Anisimov VN. Biology of aging and cancer. *Cancer Control*. 2007;14:23–31.
 33. Goukassian D, Gad F, Yaar M, Eller MS, Nehal US, Gilchrist BA. Mechanisms and implications of the age-associated decrease in DNA repair capacity. *FASEB J*. 2000;14:1325–34.
 34. Murashima S, Tanaka M, Haramaki M, Yutani S, Nakashima Y, Harada K, et al. A decrease in AFP level related to administration of interferon in patients with chronic hepatitis C and a high level of AFP. *Dig Dis Sci*. 2006;51:808–12.
 35. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. *J Med Virol*. 2007;79:1485–90.
 36. Hisaka T, Yano H, Ogasawara S, Momosaki S, Nishida N, Takemoto Y, et al. Interferon-alphaCon1 suppresses proliferation of liver cancer cell lines in vitro and in vivo. *J Hepatol*. 2004;41:782–9.
 37. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor—a prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology*. 2000;32:228–32.