Results

Nodule characteristics

Of the 49 nodules that were examined repeatedly with Gd-EOB-DTPA-enhanced MRI, 13 (26.5%) showed hyperattenuation of nodules in the arterial phase of dynamic MRI during follow-up period. Table 1 shows the characteristics of the nodules that were vascularized and those that were not. At the start of follow-up, there were no differences between nodules with and without vascularization in T1-weighted MR images, T2-weighted MR images, hepatobiliary phase images, CTHA images, presence of typical HCC at other sites, frequency of MRI examination, or observation periods. On the other hand, the nodules with vascularization were significantly larger than those without vascularization ($P = 0.0260$). The changes of nodule size with vascularization were significantly larger than those without vascularization during follow-up period ($P = 0.0153$). The signal intensities of T1-weighted (hypo/iso/hyper) and T2-weighted MR images (hypo/iso/hyper) at the end of follow-up were 2/10/1 and 0/10/3 in nodules with vascularization, and 3/23/10 and 1/33/2 in those without vascularization, respectively.

Cumulative incidence of vascularization

The overall 6-month and 12-month cumulative incidence of vascularization

was 27.6% and 43.5%, respectively. The cutoff point of tumor size was determined using Cox proportional hazards model. The optimal cutoff point was 15 mm as shown in Table2. The 6-month and 12-month cumulative incidence of vascularized nodules with maximum diameters >= 15 mm were 43.3% and 77.3%, respectively. In contrast, the 6-month and 12-month cumulative incidence of vascularized nodules with maximum diameters < 15 mm were 16.9% and 16.9%, respectively. The difference between these incidences was significant ($P = 0.0147$, log-rank test, Figure 1). Figures 2-5 show two cases that progressed to hypervascular HCCs.

Discussion

The multistep process is thought to be the main route of hepatocarcinogenesis. Correlations between imaging results and the development of hypervascular HCC, however, have not been sufficiently elucidated. In this study, 13 of 49 nodules (26.5%) showed hypervascular spots during follow-up period and the overall 1-year cumulative incidence of vascularization was 43.5%. Hypointense hepatocellular nodules observed at the hepatobiliary phase may show precancerous lesions, referred to as dysplastic nodules or early HCCs in the multistep carcinogenesis process. It is thought that the frequency of vascularization among these nodules is high.

We also evaluated the signal intensity of MRI at the start of the follow-up period. According to previous reports, high-grade dysplastic nodules and early HCCs occasionally are hypointense relative to the surrounding cirrhotic liver on T2-weighted MR images and hyperintense on T1-weighted MR images[9, 18]. On the contrary, almost all moderately differentiated HCCs are hyperintense and some early HCCs are isointense on T2-weighted MR images. In this study, however, there were no differences in the signal intensities on T1- and T2-weighted images between nodules that progressed to classical HCCs and those that did not. It is still unclear why dysplastic nodules and early HCCs are hypointense on T2-weighted MR images and

hyperintense on T1-weighted MR images, and further investigations are necessary. The nodule size at the start of follow-up was significantly associated with whether hypointense hepatocellular nodules progressed to hypervascular HCCs. Kojiro has previously shown that tumors that grow to a diameter of approximately 1.5 cm begin to proliferate more actively due to dedifferentiation[19]. In addition, the development of unpaired arteries is insufficient until tumors reach this size[20]. A tumor diameter of 1.5 cm is thought to be a critical threshold for the vascularization of hypointense hepatocellular nodules.

Gd-EOB-DTPA is transported into hepatocytes via organic anion transporters (OATPs) and excreted into bile canaliculi via a multidrug resistance-associated protein (MRP2)[21]. OATP1B3, a sodium-independent organic anion transporter that is expressed in the basolateral membrane of hepatocytes, is critical for the transport of agents such as Gd-EOB-DTPA, and the uptake of endogenous substances and xenobiotics into hepatocytes[12, 22-24]. Narita et al. reported that OATP1B3 expression was not observed in most HCCs that also did not take up Gd-EOB-DTPA[25]. In addition, the authors reported that uptake of Gd-EOB-DTPA during the hepatobiliary phase was observed only in moderately differentiated HCCs, and that three-well differentiated HCCs did not accumulate Gd-EOB-DTPA. Tsuda et al. used a rat model of hyperplastic

nodules, which corresponded to human low- or high-grade dysplastic nodules and well-differentiated HCCs, to show that oatp1 activity decreased in comparison with that observed in control samples[26]. In the current study, hepatocellular nodules that were hypointense only during the hepatobiliary phase but were not enhanced during the arterial phase of dynamic MRI were thought to represent dysplastic nodules or early (well-differentiated) HCCs.

This study has some limitations. First, there was no pathologic evidence for the identities of the evaluated hepatocellular nodules. One of the major purposes of this study, however, was to define standard criteria to allow the prognosis of borderline lesions—low- or high-grade dysplastic nodules and early HCCs—to be predicted using imaging rather than biopsy. Second, the interval between follow-up examinations varied among and within patients. Prospective studies using Gd-EOB-DTPA–enhanced MRI performed at constant intervals are required to resolve this limitation. Most of the MRI examinations in this study, however, were performed at intervals between 3 and 6 months, which is consistent with usual practices for follow-up in patients at high-risk for HCC. Third, there was no control group without hypointense nodules in the hepatobiliary phase in this study. The study comparing groups between with and without hypointense nodules in the hepatobiliary phase are recommended to obtain the

precise incidence of vascularization in future.

In conclusion, hypointense nodules that were characterized by a maximum diameter of at least 15 mm at the hepatobiliary phase, but were not enhanced at the arterial phase using dynamic MRI, often progress to hypervascular HCC. Therefore, patients with hypointense nodules characterized by a maximum diameter $\geq$ 15 mm should be followed-up carefully because of the high incidence of vascularizartion

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Figure 1. Cumulative incidences of vascularized nodules based on the maximum diameter.

The 6-month and 12-month cumulative incidence of vascularized nodules with a maximum diameter $\geq$ 15 mm was significantly higher than that of vascularized nodules with a maximum diameter < 15 mm.

Figure 2. Images of hepatitis C-related cirrhosis in a 75-year-old woman at the beginning of follow-up.

a) A CT during arterial portography (CTAP) image shows no nodules.

b) A CT during hepatic arteriography (CTHA) image shows no nodules.

c) The arterial phase of dynamic gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)–enhanced MRI shows no nodules.

d) The hepatobiliary phase of Gd-EOB-DTP–enhanced MRI shows a markedly hypointense nodule (arrow).

Figure 3. Images of a 75-year-old woman with hepatitis C-related cirrhosis 9 months after the start of follow-up (the same case as that in Figure 2).

a) A CTAP image shows a hypoattenuating nodule (arrow).

b) A CTHA image shows hyperattenuating foci in the nodule (arrow).

c) The arterial phase of dynamic Gd-EOB-DTP–enhanced MRI shows a

hyperattenuating nodule (arrow).

d) The hepatobiliary phase of Gd-EOB-DTP–enhanced MRI shows a slightly hypointense nodule (arrow).

Figure 4. MR images of a 75-year-old man with hepatitis C-related cirrhosis during a 15-month follow-up period.

a) The arterial phase of dynamic Gd-EOB-DTP–enhanced MRI at the start of follow-up shows no nodules.

b) The hepatobiliary phase of Gd-EOB-DTP–enhanced MRI at the start of follow-up shows a slightly hypointense nodule (arrow).

c) The arterial phase of dynamic Gd-EOB-DTP–enhanced MRI 9 months after the start of follow-up shows no nodules.

d) The hepatobiliary phase of Gd-EOB-DTP–enhanced MRI 9 months after the start of follow-up shows a markedly hypointense nodule (arrow).

e) The arterial phase of dynamic Gd-EOB-DTP–enhanced MRI 15 months after the start of follow-up shows hyperattenuating foci in the nodule (arrow).

f) The hepatobiliary phase of Gd-EOB-DTP–enhanced MRI 15 months after the start of follow-up shows a markedly hypointense nodule (arrow).

Figure 1

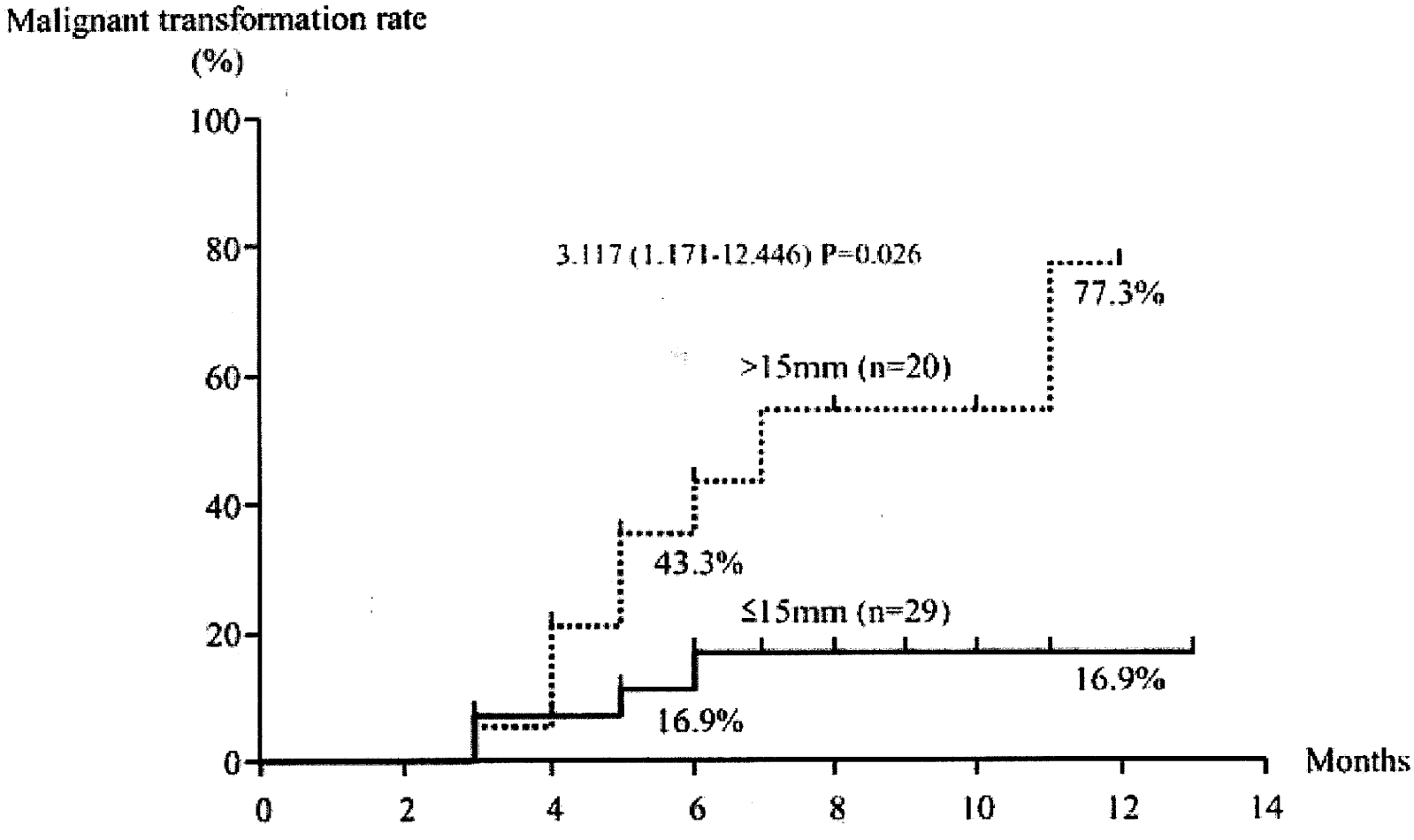

Figure1

Figure 2

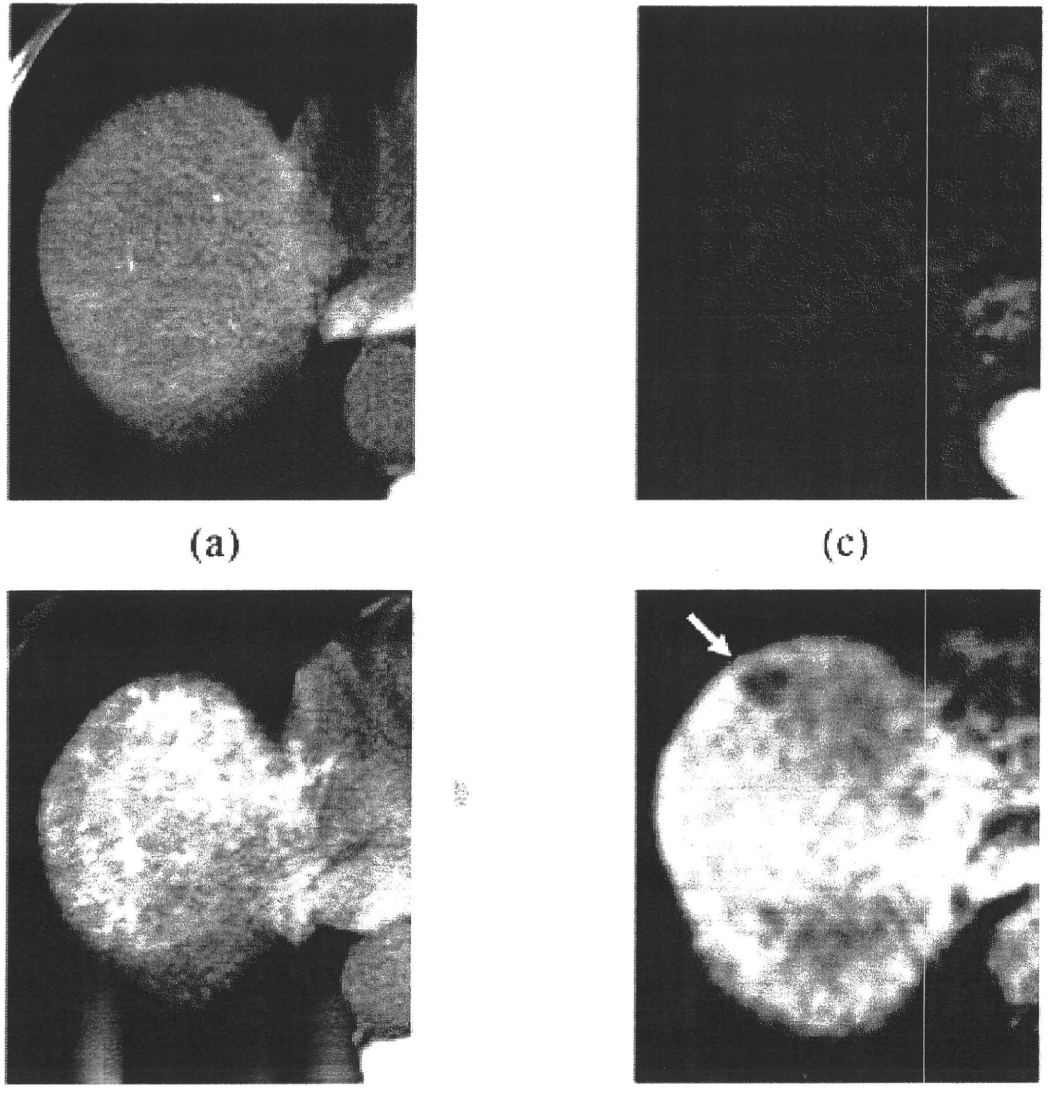

Figure2

Figure 3

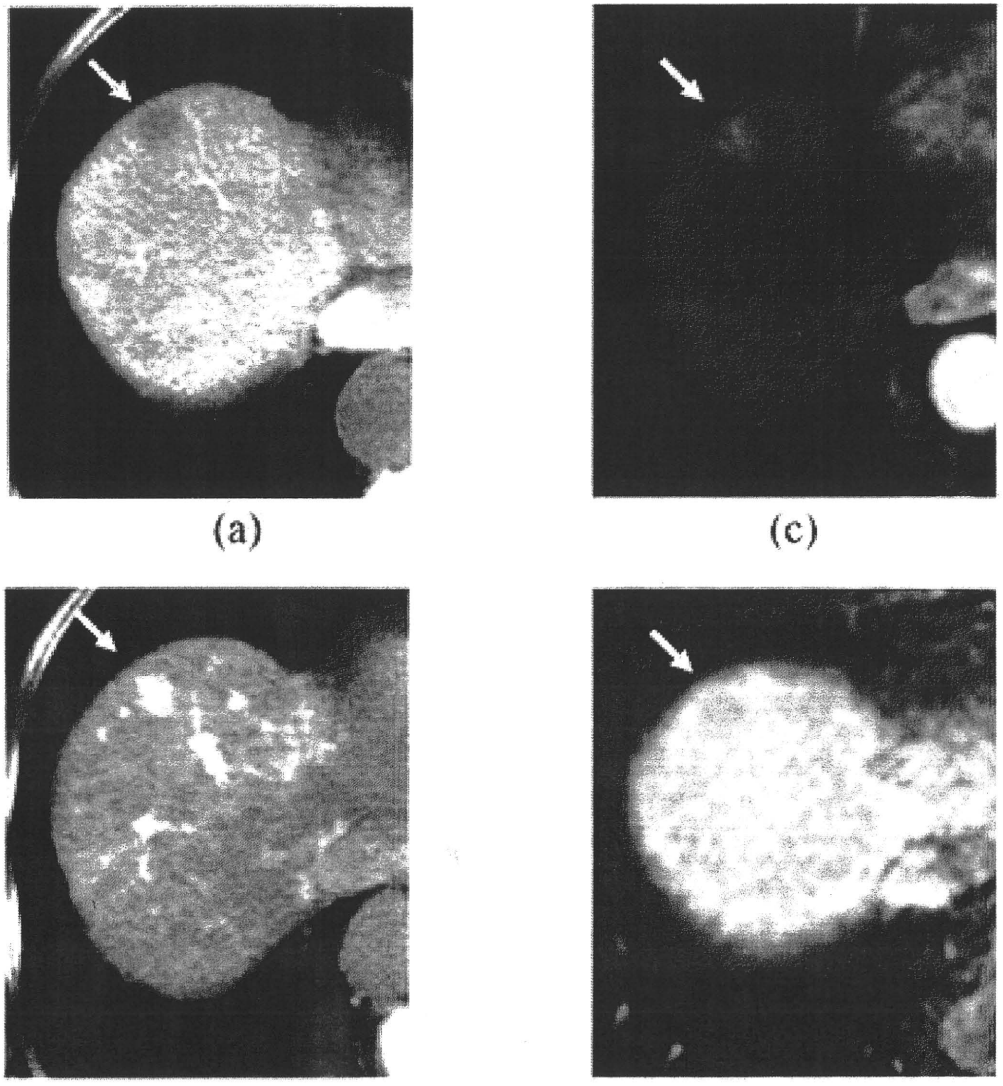

Figure3

Figure 4

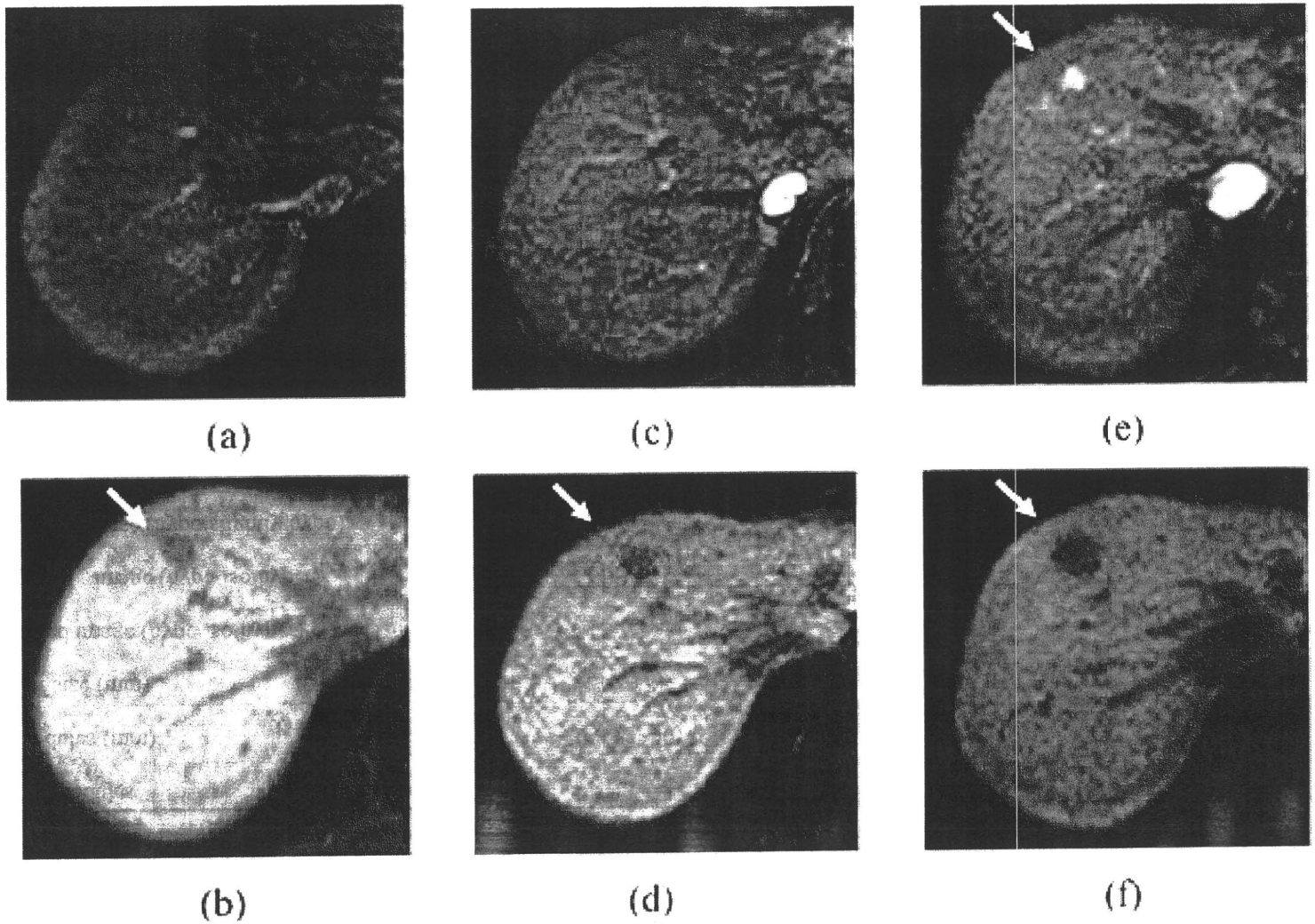

Figure4

Table

Table 1, Nodules' characteristics

	Malignant transformation (+)	Malignant transformation (-)	P
Number of nodules	13	36	
Size of nodules (mm)	20 (12 ~ 40)	14 (8 ~ 40))	0.0260
Change of size (mm)	4 (0 ~ 8)	1 (-8 ~ 13)	0.0153
T1-weighted image (hypo/iso/hyper)	5/7/2	3/24/9	0.6592
T2-weighted image (hypo/iso/hyper)	1/9/3	4/31/1	0.1033
Hepatobiliary phase (hypo/slightly hypo)	9/4	32/4	0.1834
CTHA (isoattenuating/hypoattenuating)	3/10	4/32	0.3629
Presence of typical HCC at other site (yes/no)	3/10	13/23	0.5018
Frequency of MRI examination	4 (2 ~ 8)	4 (2 ~ 9)	0.6608
Observation periods (months)	5 (3 ~ 11)	6 (3 ~ 13)	0.0971

Continuous variables were expressed as median (range). Abbreviations: hypo, hypointensity; iso, isointensity; hyper, hyperintensity; CTHA, CT during hepatic arteriography HCC; hepatocellular carcinoma; MRI, magnetic resonance imaging,

Table 2, The hazard ratios of various cutoff points.

Cutoff point	Malignant transformation (+)	P
10 mm	26.748 (0.048-14895.852)	0.308
13 mm	2.228 (0.610-8.138)	0.225
15 mm	3.117 (1.171-12.446)	0.026
18 mm	3.135 (1.022-9.612)	0.046
20 mm	1.768 (0.576-5.425)	0

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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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.

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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.

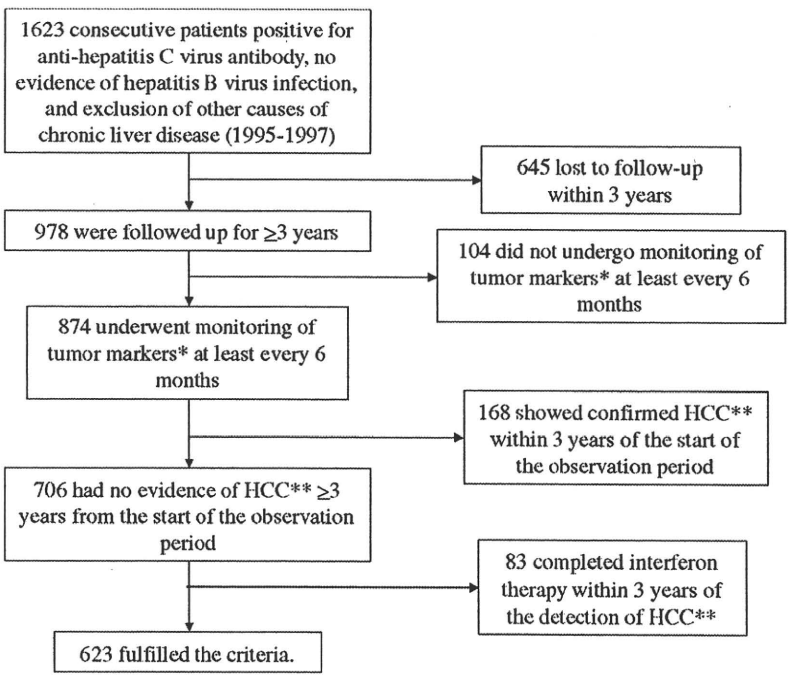

Fig. 1 Schematic flowchart of enrolled patients. *Serum alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP). **Hepatocellular carcinoma (*HCC*)