11 patients (1.8%) with SVR, 10 (3.8%) with BR and 75 (9.6%) with NR to IFN. Rates of hepatocarcinogenesis in patients with SVR, BR and NR were 0.7, 0.8 and 2.0% at the end of the 3rd year, 1.4, 2.0 and 3.8% at the 5th year, 1.6, 2.9 and 6.5% at the 7th year, 1.9, 3.6 and 9.6% at the 10th year and 1.9, 7.5 and 27.6% at the end of 15th year (fig. 2). Hepatocarcinogenesis was significantly less frequent in patients with SVR or BR than in patients with NR and those untreated (log-rank test, $p < 0.0001$).

Factors Influencing Hepatocarcinogenesis

Univariate analysis identified 9 factors significantly associated with carcinogenesis. They were fibrotic stage ($p < 0.001$), age ($p < 0.001$), α-fetoprotein ($p < 0.001$), aspartic aminotransferase ($p = 0.001$), retention of indocyanine green at 15 min ($p = 0.002$), total alcohol intake ($p = 0.002$), γ-GTP ($p = 0.005$) and HCV serotype ($p = 0.045$). IFN therapy ($p = 0.064$), histological activity of hepatitis ($p = 0.069$) and ALT ($p = 0.70$) were marginally associated with carcinogenesis.

In order to prove the role of IFN on carcinogenesis in patients with chronic hepatitis type C en masse, multivariate analysis was performed by non-time-dependent proportional hazard analysis. Fibrotic stage, γ-GTP, gender, IFN therapy, platelet count and age independently influenced the development of HCC in the cohort (table 2). Advanced liver fibrosis in F2/F3 stages imposed a higher risk for carcinogenesis with a hazard ratio of 8.68, 95% confidence interval (CI) 5.08–14.81, compared with the F1 stage. Similarly, higher γ-GTP levels (hazard ratio 2.64), male sex (2.38), low platelet count (2.22) and older age (1.90) posed higher carcinogenesis risks. After adjusting background clinical biases between treated and untreated patients for the 5 significant covariates identified in the multivariate analysis, IFN therapy significantly decreased the hepatocarcinogenesis rate in the entire patients with chronic hepatitis C with a hazard ratio of 0.42 (95% CI 0.29–0.61) in comparison with untreated patients.

Based on the multivariate analysis, curves of carcinogenesis rates were theoretically illustrated in treated and untreated patients with the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age (fig. 3).

Hazard of Hepatocarcinogenesis Stratified by the Response to IFN

Since the carcinogenesis rate in patients with SVR or BR was significantly lower than that of patients with NR or untreated patients by the product limit method, a mul-

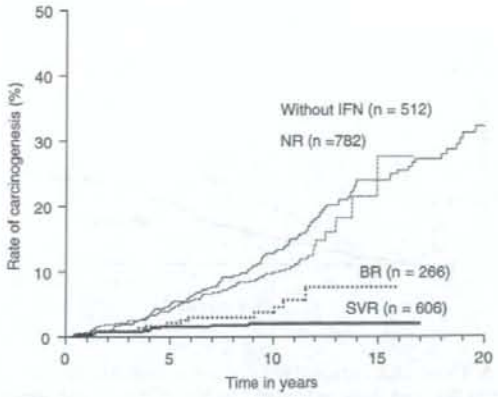

Fig. 2. Rates of hepatocarcinogenesis in patients with SVR, BR and NR to IFN. The rate in patients with NR (persistently elevated ALT or transiently normalized ALT for less than 6 months) was significantly higher than that in patients with SVR or BR.

Table 2. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	8.68	(5.08–14.81)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.64	(1.58–4.42)	<0.001
Gender			
Women	1		
Men	2.38	(1.56–3.70)	<0.001
IFN therapy			
No	1		
Yes	0.42	(0.29–0.61)	<0.001
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.22	(1.47–3.44)	<0.001
Age, years			
<50	1		
≥50	1.90	(1.27–2.85)	0.002

HR = Hazard ratio.
[a] Evaluated by the Cox proportional hazard analysis.

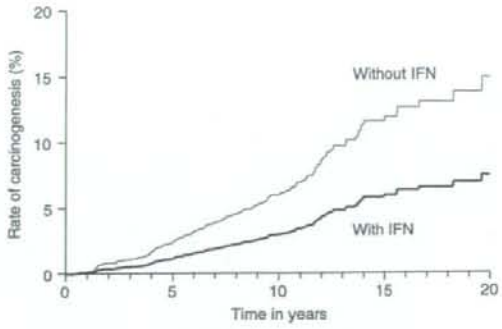

Fig. 3. Theoretical curves of hepatocarcinogenesis in patients treated with IFN and those untreated who have the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age. They are based on the analysis of 1,654 patients treated with IFN and 512 untreated patients.

Table 3. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C who had distinct responses to IFN therapy[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	9.90	(4.19–23.40)	<0.001
Gender			
Women	1		
Men	3.44	(1.89–6.25)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.68	(1.30–5.54)	0.008
Age, years			
<50	1		
≥50	2.56	(1.50–4.38)	0.001
AFP, ng/ml			
<20	1		
≥20	2.32	(1.34–4.02)	0.003
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.09	(1.14–3.75)	0.013
Response to IFN			
Without IFN	1		<0.001
NR	0.57	(0.13–2.56)	0.46
BR	0.12	(0.04–0.35)	<0.001
SVR	0.10	(0.03–0.30)	<0.001

HR = Hazard ratio; AFP = α-fetoprotein.
[a] Evaluated by the Cox proportional hazard analysis.

tivariate analysis was performed taking into account the response to IFN. Hazard ratios of patients with SVR and BR to IFN therapy were 0.10 (95% CI 0.03–0.30, $p < 0.001$) and 0.12 (95% CI 0.04–0.35, $p < 0.001$), respectively, in comparison with that of untreated patients, when the other 5 factors served as significant covariates (table 3). The hazard ratio of NR at 0.57 (95% CI 0.13–2.56) was less than 1, but fell short of making a significant difference against untreated patients.

Mortality and Causes of Death

During the observation period, 116 of the 2,166 (5.4%) patients died, including 52 of the 1,654 (3.1%) subjects treated with IFN and 64 of the 512 (12.5%) subjects without IFN. Estimated survival rates in the treated and untreated patients were 99.3 and 98.3% at 5 years, 97.8 and 96.0% at 10 years and 93.8 and 86.9% at 15 years, respectively. The survival rate of treated patients was significantly higher than that of untreated patients (log-rank test, $p < 0.0001$).

Discussion

Based on our epidemiological data obtained by long-term observations of patients with chronic hepatitis [2] and patients with cirrhosis [1], the life expectancy of patients with HCV-related chronic liver disease heavily depends on the development of HCC. The possibility of eventually developing HCC in patients with HCV infection and cirrhosis is staggeringly high at 75% [1]. Theoretically, the treatment of chronic HCV infection with IFN can prevent the development of HCC. From the ethical point of view, a prospective randomized trial with control untreated patients is not to be allowed at present when IFN has become the standard radical therapy for chronic hepatitis C; everyone can receive IFN, as expenses are being covered for by the medical insurance in Japan. Another difficulty involves the informed consent in prospective randomized studies. It requires at least 5 years in order that IFN can decrease the incidence of carcinogenesis in chronic hepatitis C, with a statistical difference in the carcinogenesis rate between treated and 'untreated' patients. Since any randomized studies are considered extremely difficult in the future, we attempted to carry out this retrospective study by the multivariate analysis with statistical adjustments for possible covariates.

In the product limit analysis, IFN significantly decreased the crude rate of hepatocarcinogenesis in the

entire cohort of 2,166 patients with chronic hepatitis C. Since there were some background differences between treated and untreated patients, we tried to correct for biases including stage of fibrosis, γ-GTP value, sex, platelet count and age, which significantly affect the carcinogenesis rate. Demographic, histological and biochemical factors having been adjusted, IFN is proven to bring about a significant decrease in the hazard of carcinogenesis in patients with chronic hepatitis C en masse (hazard ratio 0.42, $p < 0.001$ by the non-time-dependent model). Taking into consideration that a significant number of patients without IFN had received anti-inflammatory medicines, which might have contributed to suppression of hepatocarcinogenesis, the actual anticarcinogenic activity of IFN may be higher than the observed. Having published results of a similar study on a cohort of 1,643 patients with a median observation period of 5.4 years in 1999 [18], we could not establish the anticarcinogenic activity of IFN because of a low risk of carcinogenesis in untreated patients (1.2% per year). Nevertheless, we expected a significant statistical difference if we could extend the median observation period to longer than 7 or 10 years in our studied patients. This has been realized in the present study, in which 2,166 patients with and without IFN therapy were observed for a median of more than 10 years. As far as we are aware, it represents the first study that has demonstrated preventive effects of IFN on the carcinogenesis rate in a large cohort of patients in a single center, in correlation with distinct responses to it, such as SVR, BR and NR.

Treatment of patients with chronic HCV infection using IFN-α and ribavirin has led to sustained loss of serum HCV RNA in 40–50% of recipients with HCV genotype 1 and 75–80% with HCV genotype 2 or 3. However, to date, the combination therapy with IFN-α and ribavirin has not been evaluated for its impact on the risk of developing HCC. Monotherapy with IFN-α achieves sustained clearance of serum HCV RNA in only 20–30% of patients; the impact of IFN-α on the development of HCC has been evaluated only in patients who had received IFN-α without ribavirin [17–20, 25–27].

Multivariate analysis definitively demonstrated that IFN lessens the carcinogenesis risk in the patients whose ALT levels decreased after therapy. Furthermore, the anticarcinogenic capacity of IFN was demonstrated not only in the patients with persistent aminotransferase normalization, but also in those with transient normalization of ALT for at least 6 or 12 months. Many authors have already described that the activity of IFN to suppress the development of HCC in patients with HCV RNA clearance (SVR) is similar to that in patients with ALT normalization in the absence of eliminating HCV RNA (BR) [18, 25–27]. Based on these compelling lines of evidence, the anticarcinogenic activity of IFN is ascribed to the suppression of inflammatory and regenerative processes in hepatocytes. Moreno and Muriel [28] reported that IFN reverts liver fibrosis, and therefore, control of the necroinflammatory process can suppress the growth of HCC. Tarao et al. [29] reported that high aminotransferase levels increase the rate of HCC recurrence in patients with cirrhosis. Our results stand in favor of the view that the carcinogenic process in patients with chronic hepatitis C would be enhanced by fluctuating as well as persistently elevated levels of aminotransferases. It does seem that IFN exerts suppressive effects on HCC through reduction or complete remission of inflammatory activity. Recently, a few authors reported that even transient disappearance of HCV RNA during IFN therapy contributed to a low carcinogenesis rate in the clinical course of hepatitis [17, 27]. The significance of transient HCV in decreasing hepatocarcinogenesis should be further explored and confirmed by multicenter clinical studies with rigorous virological assessments.

HCC developed in a few patients with SVR 5 years after the HCV infection had been terminated by IFN, along with normalized ALT levels. These patients would have developed minute HCC in their livers already while receiving IFN which escaped the detection by imaging modalities or screening for serological tumor markers. This would indicate the limitation of IFN in preventing HCC. IFN will not be able to suppress HCC once it has developed, even when it succeeds in eliminating HCV and suppressing necroinflammatory processes in the liver.

With many difficulties in vaccine development, the recent progress in treatment of chronic HCV infection, from IFN monotherapy to combination therapy with ribavirin, is very auspicious. SVR and BR can be achieved in up to 56% of patients with combined IFN and ribavirin [30]. There is evidence that a sustained virological response can lead to decrease in fibrosis and even reversal of cirrhosis [31]. Because HCV-associated HCC occurs almost exclusively in patients with cirrhosis, successful treatment for SVR in patients without cirrhosis is likely to prevent future development of HCC [32]. However, once cirrhosis has been established, a preventive benefit of IFN monotherapy is restricted to the patients who can achieve SVR or BR. In their meta-analysis of 3 randomized and 11 nonrandomized controlled trials, Camma et

al. [33] have reported a low but statistically significant preventive effect.

In conclusion, IFN significantly decreases the rate of hepatocarcinogenesis in patients with chronic hepatitis C, irrespective of the response to it.

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Natural History of Compensated Cirrhosis in the Child-Pugh Class A Compared Between 490 Patients With Hepatitis C and 167 With B Virus Infections

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Natural histories of compensated cirrhosis in the Child-Pugh class A were compared between the 490 patients infected with hepatitis C virus (HCV) and 167 patients with hepatitis B virus (HBV) who were followed for more than 1 year up to 20 years without antiviral treatment. Patients with HCV were older (median age: 59 vs. 45 years), less predominantly male (59.0% vs. 76.0%), transfused more frequently (49.2% vs. 9.0%), and had higher aminotransferase as well as lower albumin levels and fewer platelets ($P<0.001$ for all). Death was commoner (55.1% vs. 35.9%, $P<0.001$) and hepatocellular carcinoma developed more often (53.9% vs. 28.7%, $P<0.001$) in patients with HCV than HBV. In multivariate analysis, low albumin levels (hazard ratio: 1.65), α-fetoprotein (1.55), alcohol consumption (1.49), age >55 years (1.47), and retention of indocyanine green (1.39) were independent risk factors for the survival in patients with HCV, while male gender (4.43), age >45 years (2.24), retention of indocyanine green (2.14), hepatitis B e antigen (2.11), and low platelet counts (1.91) were in those with HBV. Chances for survival was significantly different ($P<0.001$) among patients with HCV having low (number of factors: 0–1), medium (2–3), and high risks (4–5), as well as in those with HBV having low (0–1), medium (2–4), and high risks (5–6). In conclusion, survival and development of hepatocellular carcinoma, and factors for survival, are considerably different between patients with compensated cirrhosis infected with HCV and HBV, which would need to be taken into consideration in their management and planning treatment strategies. ***J. Med. Virol. 78:459–465, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: cirrhosis; hepatitis B virus; hepatitis C virus; hepatocellular carcinoma; natural history

INTRODUCTION

Deaths due to hepatocellular carcinoma are increasing and now rank the fourth over the world and in Japan. Up to 80% of hepatocellular carcinomas develop in patients with cirrhosis [Kew and Popper, 1984], most of whom have the end-stage liver disease induced by hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. The prognosis of cirrhotic patients, in terms of survival and the development of hepatocellular carcinoma or decompensation, depends on various host and viral factors [Ikeda et al., 1993; Kato et al., 1994; Fattovich et al., 1997; Niederau et al., 1998; Serfaty et al., 1998; Chiaramonte et al., 1999; Hu and Tong, 1999], and on treatment with interferon [Nishiguchi et al., 1995; Mazzella et al., 1996; Benvegnu et al., 1998].

Survival and development of hepatocellular carcinoma were compared between the 490 patients with compensated cirrhosis in the Child-Pugh class A who were infected with HCV and the 167 with HBV; they were followed for more than 1 year without receiving antiviral treatment. Risk factors for survival were evaluated in patients infected with HCV or HBV separately, and they were classified into groups with low, medium, and high risk, respectively. The prediction of survival in patients with compensated cirrhosis would help in planning strategies for therapeutic invention, including antiviral therapy and liver transplantation.

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MATERIALS AND METHODS

Cirrhotic Patients Infected With HCV or HBV

During 22 years from 1980 to 2001, 1,321 adult patients were diagnosed with liver cirrhosis at the Department of Hepatology, Toranomon Hospital in the Metropolitan Tokyo. Eight hundred two (60.7%) were infected persistently with HCV, 362 (27.4%) with HBV, 26 (2.0%) with both, and 131 (9.9%) with neither. They were aged at a median of 55 years (range: 19–86 years) including 913 (69.1%) men, and 398 of these patients (30.1%) had received antiviral treatment with interferon and/or lamivudine.

Criteria for inclusion in the present study were: (1) histological diagnosis of cirrhosis in Child-Pugh class A [Pugh et al., 1973]; (2) absence of signs for decompensation (ascites, encephalopathy, or gastrointestinal bleeding) or hepatocellular carcinoma at entry; (3) no evidence for coexisting liver disease such as autoimmune hepatitis, alcohol-related liver disease, hemochromatosis, and Wilson's disease (patients with idiopathic portal hypertension, Budd-Chiari syndrome, subacute hepatitis, or chronic aggressive hepatitis accompanied by severe bridging necrosis were also excluded); (4) ongoing infection with either HBV or HCV (patients co-infected with both were excluded); (5) no serological markers for concurrent infection with hepatitis A virus, hepatitis D virus or the human immunodeficiency type-1 virus; (6) no history of antiviral treatment including interferon; and (7) follow-up for at least 1 year after diagnosis of cirrhosis. These inclusion criteria were fulfilled by 490 patients with HCV and 167 with HBV infection (Table I).

Peripheral blood counts, serum biochemistry including liver function tests, α-fetoprotein (AFP), and the percent retention of indocyanine green at 15 min (ICG R_{15}), as well as genotypes of HCV and HBV, were determined on diagnosis of compensated viral cirrhosis in Child-Pugh class A. Patients were examined at regular intervals for liver function, HCV and HBV markers, as well as the development of hepatocellular carcinoma by means of serum AFP, ultrasonography, and computed tomography. They were screened for varices by endoscopy at regular intervals, and received treatment when there was an imminent risk of bleeding. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethic Committee of the institution. Every patient gave an informed consent for this study.

Diagnosis of Hepatitis Virus Infections

Infection with HBV was diagnosed by the detection of hepatitis B surface antigen (HBsAg) with enzyme-linked immunosorbent assay (ELISA) using commercial assay kits (ELISA, F-HBsAg; Sysmex, Kobe, Japan), and that with HCV by ELISA for antibody to HCV (anti-HCV) of the second generation (Ortho HCV2.0 ELISA; Ortho Diagnostic Systems, Raritan, New Jersey). Persistent HCV infection was confirmed by the detection of HCV RNA in serum by the polymerase chain reaction. Hepatitis B e antigen (HBeAg) was determined by ELISA (ELISA, F-HBe; Sysmex).

Genotypes of Hepatitis Viruses

Genotypes of HCV were determined by the polymerase chain reaction with type-specific primers deduced from the 5′-non-structural region by the method reported previously [Chayama et al., 1993]. The six major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan). The method employs the combination of epitopes on preS2-region products that is specific for each genotype [Usuda et al., 1999, 2000]. Genotype G was determined by the preS2 serotype for genotype D and HBsAg subtype *adw*, and H was recognized by that for genotype C and subtype *adw*, respectively; these combinations were specific for genotypes G and H [Kato et al., 2001, 2004]. Genotyping was possible in all the 490 patients with HCV, while it was feasible in 137 (82.0%) patients with HBV.

Statistical Analyses

Differences in categorical variables were evaluated by the chi-squared test or Fisher's exact test, and those in

TABLE I. Baseline Characteristics of Patients With Cirrhosis Infected With HCV or HBV

Features	HCV (n = 490)	HBV (n = 167)	Differences (*P*-value)
Age (years)	59 (25–82)	45 (20–71)	<0.001
Male	289 (59.0%)	127 (76.0%)	<0.001
Transfusion	241 (49.2%)	15 (9.0%)	<0.001
Total alcohol >500 kg	86 (17.6%)	28 (16.8%)	NS[b]
AST (U/L)	64 (16–1,313)	34 (8–307)	<0.001
ALT (U/L)	58 (9–315)	30 (8–510)	<0.001
Zinc turbidity test	11.9 (0.7–23.5)	8.2 (1.8–22.7)	<0.001
Albumin (g/dl)	3.8 (3.0–5.1)	4.1 (3.2–5.2)	<0.001
Bilirubin (mg/dl)	1.1 (0.4–3.0)	1.0 (0.3–2.6)	<0.001
α-fetoprotein (ng/ml)	14 (2–748)	8 (1–1,520)	<0.001
Platelets ($100 \times 10^3/mm^3$)	9.6 (1.7–39.8)	12.8 (4.8–24.9)	<0.001
ICG R_{15} (%)[a]	28 (2–76)	18 (4–41)	<0.001

[a]Retention of inodcyanine green at 15 min in percent.
[b]Not significant.

continuous variables by the Mann–Whitney's *U*-test. Survival and development of hepatocellular carcinoma were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log-rank test. Independent risk factors associated with the progression to hepatocellular carcinoma were evaluated by the stepwise Cox regression analysis. Data analysis was performed with use of SPSS statistical software version 10 (SPSS, Inc., Chicago, Illinois). A *P*-value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics of Patients With Compensated Cirrhosis in the Child-Pugh Class A Who Were Infected With HCV or HBV

Table I compares demographic and laboratory features between the 490 patients persistently infected with HCV and the 167 with HBV when they were diagnosed with Child-Pugh class-A cirrhosis. There were substantial differences between them. Cirrhotic patients with HCV were significantly older, less often male, had received transfusions more frequently, and had liver function worse than those with HBV. In addition, albumin levels and platelet counts were lower, and the percent retention of indocyanine green at 15 min (ICG R_{15}) was higher in cirrhotic patients infected with HCV than with HBV.

Survival and Development of Hepatocellular Carcinoma in Patients Infected With HCV or HBV

Outcomes were compared between the 490 cirrhotic patients infected with HCV and the 167 with HBV who were followed-up without antiviral therapies for longer than 1 year. There were no differences in the duration of follow-up between them with the median of 8.2 years (range: 1.0–24.0) for HCV and 9.2 years (1.2–23.7) for HBV. During the follow-up ranging to 20 years, death occurred more frequently (55.1% vs. 35.9%, $P < 0.001$) and hepatocellular carcinoma developed more often (53.9% vs. 28.7%, $P < 0.001$) in patients with HCV than HBV. Table II compares the causes of death in patients with HCV and HBV. Hepatocellular carcinoma was the leading cause of death in both, with a significant difference in the development between patients with HCV and HBV (74.1% vs. 58.3%, $P = 0.018$). Causes of death in cirrhotic patients without hepatocellular carcinoma were principally decompensation; they tended to occur less often in patients with HCV than HBV infection (17.8% vs. 28.3%, $P = 0.073$). In surviving patients, hepatocellular carcinoma developed more frequently in those with HCV than HBV (17.8% vs. 28.3%, $P = 0.001$). Death and hepatocellular carcinoma occurred at annual incidence rates of 6.3% and 8.3% in patients with HCV, respectively, and 3.6% and 3.3% in those with HBV.

Causes of death other than hepatocellular carcinoma and decompensation in patients with HCV and HBV were non-hepatic cancers in 4.4% and 3.3%, respectively, and other causes in 3.7% and 10.0%.

Figure 1a compares the survival between cirrhotic patients infected with HCV and HBV. The survival was not different during the initial 6 years. Later on, however, patients infected with HCV fared increasingly worse than those with HBV ($P < 0.001$) with respective survival rates: 52% (n = 172) versus 65% (n = 74) at 10 years; 30% (n = 54) versus 53% (n = 44) at 15 years; and 16% (n = 12) versus 42% (n = 13) at 20 years.

The development of hepatocellular carcinoma is compared between patients with HCV and HBV in Figure 1b. There were no differences in the development of hepatocellular carcinoma during the initial 3 years. Thereafter, however, hepatocellular carcinoma developed increasingly more often in patients with HCV than HBV ($P < 0.001$); their respective frequencies were: 32% (n = 263) versus 22% (n = 98) at 5 years; 60% (n = 98) versus 33% (n = 63) at 10 years; 75% (n = 24) versus 42% (n = 35) at 15 years; and 77% (n = 6) versus 47% (n = 11) at 20 years. The yearly incidence of hepatocellular carcinoma during the first 10 years was 6.0% in patients with HCV and 3.3% in those with HBV.

Factors Influencing the Survival in Cirrhotic Patients With HCV Infection

Variables associated with the survival were evaluated by univariate analysis in cirrhotic patients infected with HCV (Table III). At the diagnosis of cirrhosis, age was significantly higher and total alcohol intake >500 kg less frequent in alive than deceased patients. Albumin,

TABLE II. Causes of Death in Patients With HCV or HBV and Occurrence After the Diagnosis of Compensated Cirrhosis in Child-Pugh Class A

Causes	HCV (n = 270)	HBV (n = 60)	Differences (*P*-value)
Hepatocellular carcinoma	200 (74.1%)	35 (58.3%)	0.018
Decompensation	48 (17.8%)	17 (28.3%)	NS[b] (0.073)
Liver failure	25 (52%)[a]	9 (53%)[a]	
Bleeding	6 (13%)[a]	3 (18%)[a]	
Infection	17 (35%)[a]	5 (29%)[a]	
Non-hepatic cancer	12 (4.4%)	2 (3.3%)	NS
Others	10 (3.7%)	6 (10.0%)	NS

[a]Percentage of deaths by decompensation is shown.
[b]Not significant.

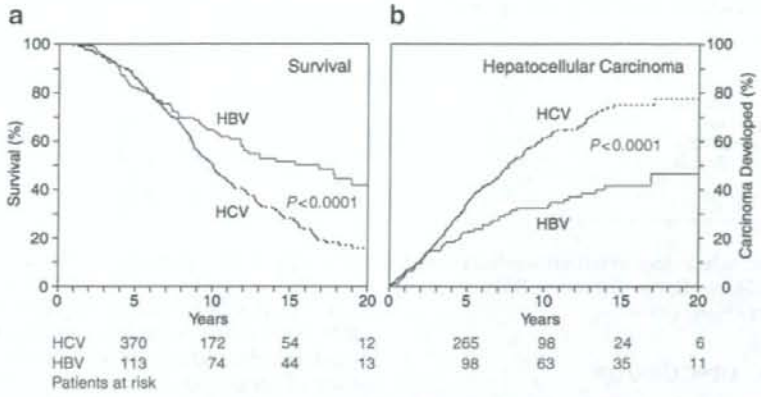

Fig. 1. Kaplan–Meier life tables for survival (a) and development of hepatocellular carcinoma (b) in patients with compensated cirrhosis in Child-Pugh Class A who were infected with HCV or HBV.

bilirubin levels, AFP, platelet counts, and ICG R_{15} were worse in dead than surviving patients. Five of the seven variables associated with death kept significance in multivariate analysis (Table IV). They were albumin levels <4.0 g/dl (hazard ratio: 1.65), AFP >20 ng/ml (1.55), alcohol intake >500 kg (1.49), age >55 years (1.47), and ICG R_{15} >30% (1.39).

Factors Influencing the Survival in Cirrhotic Patients With HBV Infection

Likewise, variables were evaluated for an association with the survival by univariate analysis in cirrhotic patients infected with HBV (Table V). Age >45 years at the diagnosis of cirrhosis, male gender and HBeAg were significantly more frequent in dead than surviving patients. The other factors associated significantly with death in HBV patients overlapped with those in HCV patients, except for the zinc turbidity test and bilirubin (Tables III and V). Only five of the nine variables associated with death retained significance in multivariate analysis (Table VI). Of them, male gender posed the highest risk (hazard ratio, 4.43) followed by age >45 years (2.24), ICG R_{15} >30% (2.14), HBeAg (2.11), and platelet counts $<100 \times 10^3/mm^3$ (1.91).

Survival of Cirrhotic Patients With Distinct Levels of Risk

Survival was compared among patients with different risk levels (Fig. 2). High, medium, and low risks for patients with HCV were defined by the sum of risk factors (shown in Table IV) at 0–1, 2–3, and 4–5, respectively, while those for patients with HBV (Table VI) were determined by that at 0–1, 2–4, and 5–6; the male gender was scored 2 due to its hazard ratio twice as high as the other factors (Table VI). There were significant differences in the survival among patients having distinct risk levels who were infected with either HCV or HBV.

Influence of HCV and HBV Genotypes on the Survival

The survival was not different between patients infected with HCV of genotypes 1b and non-1. Although

TABLE III. Univariate Analysis for Factors Influencing the Survival of Patients With HCV-Associated Cirrhosis

Factors	Category	Alive (n = 220)	Dead (n = 270)	Differences (*P*-value)
Age	>55 years	156 (70.9%)	174 (64.4%)	0.004
Gender	Male	116 (52.7%)	172 (63.7%)	NS
Transfusion	Received	104 (47.3%)	137 (50.7%)	NS
Total alcohol	>500 kg	27 (12.3%)	60 (22.2%)	0.027
AST (U/L)	>77 U/L	71 (32.3%)	102 (37.8%)	NS
ALT (U/L)	>100 U/L	43 (19.5%)	45 (16.7%)	NS
Zinc turbidity test	>12	105 (47.7%)	131 (48.5%)	NS
Albumin	>4.0 g/dl	83 (37.7%)	114 (42.2%)	<0.001
Bilirubin	>1.5 mg/dl	44 (20.0%)	56 (20.7%)	0.004
α-fetoprotein	>20 ng/ml	72 (32.7%)	115 (42.6%)	0.001
Platelets	$<100 \times 10^3/mm^3$	118 (53.6%)	150 (55.6%)	0.001
ICG R_{15}	>30%	87 (39.5%)	120 (44.4%)	0.001

TABLE IV. Multivariate Analysis for Factors Influencing the Survival of Patients With HCV-Associated Cirrhosis

Factors	Category	Hazard ratio	95% Confidence interval	*P*-value
Albumin	<4.0 g/dl	1.65	1.25–1.65	<0.001
α-fetoprotein	>20 ng/ml	1.55	1.19–1.55	<0.001
Total alcohol	>500 kg	1.49	1.10–1.49	0.009
Age	>55 years	1.47	1.12–1.47	0.006
ICG R_{15}	>30%	1.39	1.07–1.39	0.016

the survival tended to be better in patients infected with HBV of genotype C than B, the difference fell just short of being significant ($P = 0.08$).

DISCUSSION

There are an estimated 170 million people infected persistently with HCV [Cohen, 1999] and 350 million with HBV [Lee, 1997] in the world. In Japan alone, approximately one million patients are presumed to be infected with persistent HCV or HBV by surveys on first-time blood donors [Tanaka et al., 2004]. As on April 2002, a national program was launched for identifying persistent HCV and HBV infections in Japanese older than 40 years in a 5-year project. Since viral hepatitis progresses insidiously, cirrhosis will be diagnosed in many HCV and HBV carriers who are recognized during the survey. Hence, there is a pressing need to foretell the prognosis of cirrhotic patients in special terms of survival and development of hepatocellular carcinoma in them. The present study for long-term prognosis of cirrhotic patients was planned and conducted for such purposes. It is an extension to a previous study in 1993 in which 795 cirrhotic patients were evaluated for factors influencing the development of hepatocellular carcinoma [Ikeda et al., 1993].

Differences between HCV and HBV infections in terms of survival as well as development of hepatocellular carcinoma having emerged in the previous study [Ikeda et al., 1993] is confirmed and extended in the present study with new insights. To exclude any influence of antiviral treatment, only cirrhotic patients in the Child-Pugh class A who had not received antiviral treatment entered this study; they all were followed-up for longer than 1 year after they were diagnosed with compensated cirrhosis. Overall incidence rates of yearly deaths in the 490 patients with HCV and the 167 with HBV were 6.3% and 3.6%, respectively, and those of hepatocellular carcinoma were 8.3% and 3.3% during observation periods ranging up to 20 years. When the yearly incidence of hepatocellular carcinoma was estimated by the Kaplan–Meier life-table method during the first 10 years, it was 6.0% for patients with HCV and 3.3% for those with HBV. The yearly incidence of hepatocellular carcinoma in cirrhotic patients with HCV at 8.3% in this study is compared with 7.0% in Taiwan [Tsai et al., 1997] as well as 5.9% in Spain [Planas et al., 2004], while it is much higher than 3% in France [Serfaty et al., 1998]. The incidence of hepatocellular carcinoma in those with HBV at 3.3%, however, is lower than 6.6% in Taiwan [Tsai et al., 1997] but much higher than 1.5–1.8% in European patients [Fattovich et al., 1995; Benvegnu et al., 2004].

The influence of viral genotypes on the survival of patients with compensated cirrhosis was evaluated both in HCV and HBV infections. There were no appreciable differences in the survival between patients with HCV genotype 1 and non-1, in agreement with previous reports [Zhou et al., 1996; Serfaty et al., 1998]. It is surprising, however, that the survival tended to be better in patients with HBV genotype C than B ($P = 0.08$), despite overwhelming evidence for HBV genotype C inducing more severe liver disease than HBV genotype B [Orito et al., 2001; Kao, 2002; Miyakawa and Mizokami, 2003]. This may have reflected a referral bias towards patients with advanced liver disease who would visit hospitals [Sumi et al., 2003]. Most patients infected with HBV genotype B

TABLE V. Univariate Analysis for Factors Influencing the Survival of Patients With HBV-Associated Cirrhosis

Factors	Category	Alive (n = 107)	Dead (n = 60)	Differences (*P*-value)
Age	>45 years	44 (41.1%)	34 (56.7%)	0.045
Gender	Male	74 (69.2%)	51 (85.0%)	0.003
HBeAg	Positive	45 (42.1%)	34 (56.7%)	0.004
Transfusion	Received	10 (9.3%)	5 (8.3%)	NS
Total alcohol	>500 kg	13 (12.1%)	13 (21.7%)	0.048
AST (U/L)	>77 U/L	20 (18.7%)	11 (18.3%)	NS
ALT (U/L)	>100 U/L	16 (15.0%)	10 (16.7%)	NS
Zinc turbidity test	>12	20 (18.7%)	18 (30.0%)	0.014
Albumin	>4.0 g/dl	78 (72.9%)	40 (66.7%)	0.009
Bilirubin	>1.5 mg/dl	11 (10.3%)	7 (11.7%)	NS
α-fetoprotein	>20 ng/ml	34 (31.8%)	24 (40.0%)	0.044
Platelets	$<100 \times 10^3/mm^3$	25 (23.4%)	27 (45.0%)	<0.001
ICG R_{15}	>30%	12 (11.2%)	15 (25.0%)	<0.001

TABLE VI. Multivariate Analysis for Factors Influencing the Survival of Patients With HBV-Associated Cirrhosis

Factors	Category	Hazard ratio	95% Confidence interval	*P*-value
Gender	Male	4.43	1.72–11.44	0.002
Age	>45 years	2.24	1.27–3.95	0.005
ICG_{15}	>30%	2.14	1.10–4.17	0.025
HBeAg	Positive	2.11	1.21–3.69	0.009
Platelets	$<100 \times 10^3/mm^3$	1.91	1.08–3.36	0.026

resolve hepatitis spontaneously, while only a fraction develop serious disease.

There have been only a few studies that compare the natural history of compensated cirrhosis between patients with HCV and HBV infections in substantial numbers [Chiaramonte et al., 1999; Fattovich et al., 2002]. The results of the present study are in accord with their finding in some aspects, but not in others. Remarkably, there is a substantial discrepancy in the cause of death in cirrhotic patients between Western countries and Japan. Most patients with cirrhosis in Western countries die of hepatic decompensation, while hepatocellular carcinoma is the leading cause of death in Japan. The role of hepatocellular carcinoma in death may differ between cirrhotic patients with HCV and HBV infections. During follow-up ranging to 20 years, hepatocellular carcinoma caused death more frequently in cirrhotic patients infected with HCV than HBV (74.1% [200/270] vs. 58.3% [35/60], $P = 0.018$). Conversely, decompensation was somewhat lower as a cause of death in cirrhotic patients with HCV than HBV infections (17.8% vs. 28.3%, $P = 0.073$).

In an international multicenter study conducted in Europe, 297 patients with cirrhosis had been followed-up for 6.5 years since they developed cirrhosis without receiving antiviral treatment [Fattovich et al., 2002]. Hepatocellular carcinoma developed in 23 of the 136 (17%) patients with HCV, compared with 22 of 161 (14%) with HBV. Likewise, in a multicenter study carried out in Italy [Chiaramonte et al., 1999], the development of hepatocellular carcinoma during 5.4 years was not significantly different between patients infected with HCV and HBV, although it tended to be more frequent in those with HCV (20.5%) than HBV (9.1%). In the European study [Fattovich et al., 2002], decompensation was the cause of death or transplantation in 18 of 35 (51%) patients with HCV and 21 of 35 (60%) with HBV, in contrast to 17.8% and 28.3%, respectively, in the present study.

There are differences in factors influencing the natural history of patients with compensated cirrhosis, who are infected with HCV or HCV, between European and Asian countries. Unlike in European countries where HBV is transmitted during the adulthood by sexual contacts or intravenous drug use, HBV in Japan has been transmitted perinatally or during the infancy until 1986 when the national immunoprophylaxis in high-risk babies was initiated [Koyama et al., 2003; Noto et al., 2003]. Hence, the duration of HBV infection is much different between Europe and Japan, with possible exception of Italy and Greece where perinatal transmission once prevailed. Moreover, patients with cirrhosis die of decompensation less frequently in Japan than in Europe. The reason could be attributed partly to routine screening of varices and prophylaxis of bleeding that has become routine in Japan.

It would be important to determine factors for long-term survival in the management of cirrhotic patients. Factors influencing survival were evaluated in multivariate analysis for classifying cirrhotic patients into three groups with respective low, medium, and high risks for survival, respectively, in HCV or HBV infection (Fig. 2). These protocols would be useful in taking care of patients in accordance with their different risks, for

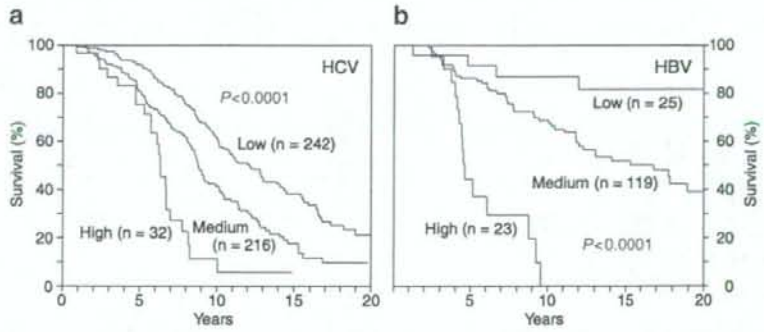

Fig. 2. Kaplan–Meier life tables for survival of patients with compensated cirrhosis who were infected with HCV (**a**) or HBV (**b**) and had low, medium, or high risk. High, medium, and low risk were defined by the sum of risk factors at 0–1, 2–3, and 4–5, respectively, in patients with HCV and that at 0–1, 2–4, and 5–6 in those with HBV; the male gender was scored 2 due to its hazard ratio twice as high as the other factors.

providing them with timely treatment towards an extended survival. In addition, they would help in selecting patients for antiviral treatment [Nishiguchi et al., 1995; Mazzella et al., 1996; Benvegnu et al., 1998; Kasahara et al., 1998; Poynard and Opolon, 1998; Reddy et al., 1999]. Furthermore, the patients who need liver transplantation would be identified well beforehand by these systems, and better still, the need for liver transplantation may be delayed or prevented by timely medical interventions.

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Dysplastic Nodules Frequently Develop into Hepatocellular Carcinoma in Patients with Chronic Viral Hepatitis and Cirrhosis

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BACKGROUND. Advances in imaging technology have enhanced the detection of small nodular lesions during the course of chronic liver disease.

METHODS. Between 1995 and 2002, the authors examined 154 consecutive patients with small hepatic nodules without hepatocellular carcinoma (HCC) over a median duration of 2.8 years. The median size of these nodules was 14 mm (range, 7–40 mm). The initial histopathologic diagnosis included high-grade dysplastic nodule (HGDN) ($n = 13$), low-grade dysplastic nodule (LGDN) ($n = 42$), and regenerative nodule (RN) ($n = 99$).

RESULTS. A total of 29 (18.8%) nodules developed into HCC during the observation period. Cumulative HCC development rates at the first, third, and fifth year were 46.2%, 61.5%, and 80.8% for HGDN; 2.6%, 30.2%, and 36.6% for LGDN; and 3.3%, 9.7%, and 12.4% for RN, respectively. The rate of HCC development was significantly higher in the HGDN group than for other types ($P < 0.001$). Multivariate analysis disclosed that histopathologic diagnosis ($P < 0.001$) and findings on computed tomographic arterial portography (CT-AP) ($P = 0.004$) were significantly associated with future HCC development. The hazard ratios of HGDN and LGDN were 16.8 (95% confidence interval [CI], 6.19–45.6) and 2.96 (95% CI, 1.20–7.31), respectively. A decrease in portal blood flow also showed a significantly high hazard ratio of 3.04 (95% CI, 1.42–6.50). Approximate annual development rate to HCC was 20% in patients with HGDN and 10% in LGDN.

CONCLUSION. HGDN should be considered a precancerous lesion when it appears during follow-up of chronic viral hepatitis or cirrhosis. Reduced portal blood flow in the nodule on computed tomography-AP is also an important predictor for development of hepatocellular carcinoma. ***Cancer* 2006;106:636–47.**

KEYWORDS: hepatocellular carcinoma, dysplastic nodule, multistep carcinogenesis.

With advances in imaging diagnosis, hepatic nodular lesions are now frequently found during the course of chronic liver disease.[1–6] According to a previous study, hepatocellular carcinoma (HCC) develops at a rate of about 3–10% per year in patients with chronic viral hepatitis and cirrhosis.[7–10]

If an intrahepatic nodular lesion is found, it is always necessary to consider development of HCC. Screening for HCC is mostly performed by ultrasonography (US). If hepatic nodular lesions are found on US, further imaging diagnosis such as computed tomography (CT), magnetic resonance imaging (MRI), and hepatic angiography are performed to decide if the nodules represent HCC. Fine needle aspiration biopsies are considered only when HCC is not diagnosed by

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such imaging techniques, because careless needle insertion into HCC may lead to tumor seeding to the surrounding liver tissue.[11,12]

Some hepatic nodules are diagnosed as HCC by both imaging and biopsy examination and can be treated with appropriate modalities, whereas others are diagnosed as nonmalignant. During the observation of such "nonmalignant" hepatic nodules, some progress to HCC while others disappear or stay unchanged for long periods.

It is generally considered that there are two different pathways to the development of HCC.[13,14] One is a "multistep" carcinogenesis process similar to the process described for colorectal cancer,[15,16] and the other is called "de novo" carcinogenesis. With the former type, it is important to determine which types of hepatic nodules are precancerous. In other words, identification of risk factors for development of HCC is necessary for follow-up of such hepatic nodules.

The aim of the present study was to elucidate outcomes and factors associated with development of HCC during long-term follow-up of small hepatic nodules that were initially diagnosed as nonmalignant lesions.

MATERIALS AND METHODS

Patients

Medical records of patients who were hospitalized for evaluation of hepatic nodular lesions at Toranomon Hospital between 1995 and 2002 were reviewed retrospectively. A total of 1425 patients were hospitalized during this period, and 1171 (82.2%) of the 1425 patients were already diagnosed as HCC by detailed image analysis. Fine needle aspiration biopsies were carried out in the remaining 254 (17.8%) patients and 88 (34.6%) were histologically diagnosed as HCC. After exclusion of hepatic hemangiomas ($n = 4$), focal nodular hyperplasia ($n = 3$), and alcoholic hyperplastic nodules ($n = 5$), the remaining 154 patients who had a histologic diagnosis, but were not diagnosed as HCC, were enrolled in the current study. If two or more hepatic nodules were observed in the same patient, then the largest nodule was included in the current study. Clinical backgrounds of these patients are listed in Table 1. The background of all 154 patients included chronic liver disease as 108 (70.1%) patients were positive for hepatitis C virus (HCV), 32 (20.8%) patients were positive for hepatitis B virus (HBV), and 2 (1.3%) patients were positive for both HCV and HBV. Twelve (7.8%) of 154 were negative for both HCV and HBV. Among these 12 patients, only 4 (33.3%) had a history of habitual heavy alcohol intake of more than 80 g per day. In addition, cirrhosis was evident in 124 (80.5%) patients. In our institution, informed consent is not required for reviewing patient records, including images, and, therefore, no such consent was obtained. However, at the time of the study, each patient provided informed consent for conducting imaging studies, including dye injection and needle biopsy.

TABLE 1
Clinical Background of 154 Patients with Small Hepatic Nodular Lesions

Characteristic	Median (range)
Age in yrs	63 (33–81)
Gender, male:female[a]	101:53
HBV:HCV:HBV+HCV:others[a]	32:108:2:12
Previous history of HCC, yes:no[a]	40:114
Presence of cirrhosis, yes:no[a]	124:30
Diameter of nodule, mm	14 (7–40)
Albumin, g/dL	3.7 (2.6–4.7)
Bilirubin, mg/dL	1.0 (0.4–3.1)
ICG R15, %	27 (4–79)
Platelet, $\times 10^4/mm^3$	10.2 (3.4–38.0)
Prothrombin time, %	88 (50–100)
AFP, ng/mL	13 (1–1070)
DCP, AU/mL	13 (<10–182)
Observation periods, yrs	2.8 (0.1–8.9)

HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma. ICG R15: indocyanine green retention rate at 15 minutes; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin.
[a] Data are expressed as ratios, not median and range.

Image Analysis

US or helical dynamic CT was carried out every 3 months for follow-up and examined for a change in imaging findings. US examination was generally performed with B-mode fundamental and harmonic imaging. Contrast-enhanced US using Levovist (Schering, Berlin, Germany) was performed in some cases. Dynamic CT scans were performed using a single-detector helical CT scanner (Hi-Speed advantage SG, GE Yokogawa Medical Systems, Tokyo, Japan). Furthermore, all CT scans taken throughout the study were performed using this scanner. In these studies, 95 mL of 350 mg I/mL Iomeprol (Iomeron 350, Eisai, Tokyo), as the contrast medium, was rapidly injected intravenously at 0.06 mL/kg body weight/sec. Phase-1, -2, and -3 imaging were performed at 25, 60, and 180 seconds (slice thickness: 10 mm, 5 mm, and 10 mm, respectively) after the start of injection, respectively.

The radiologic study was conducted by intraarterial digital subtraction angiography, including celiac and mesenteric angiography and selective angiography of the common hepatic artery. Computed tomographic arterial portography (CT-AP) and computed tomographic hepatic angiography (CT-HA) were carried out in the CT room after completion of hepatic

angiography using 5-French catheter. CT-AP scans were carried out with slip-ring technology, 5-mm thick sections, and 5-mm collimation. Overlapping reconstructions were obtained every 2.5 mm. Data acquisition was started 25 seconds after initiation of a transcatheter injection into the superior mesenteric artery of 90 mL of nonionic contrast material containing 120 mg I/mL at 3 mL/sec, by using an automated power injector. The duration of scanning was around 25–35 seconds, depending on liver size, during a single breath hold. CT-HA scans were also obtained with 5-mm thick sections, 5-mm collimation, 2.5-mm reconstruction intervals. Data acquisition was started 10 seconds after initiation of a transcatheter hepatic artery injection (using the same automated power injector) of 20–30 mL of nonionic contrast material that contained 70 mg I/mL at 1.0 mL/sec.

Both CT-AP and CT-HA were qualitatively analyzed retrospectively, and the final diagnosis was established by consensus between two experienced radiologists who were blinded to the histologic diagnosis and clinical outcome. The CT-AP findings in hepatic nodules were classified as isoattenuating or low-attenuating, compared with the surrounding liver parenchyma. If only part of the nodule was hypodense, then we defined such nodule as low-attenuating. Likewise, CT-HA findings were classified as isoattenuating or high-attenuating compared with surrounding liver parenchyma. Because we were interested in increased intranodular arterial blood flow in the current study, if the nodule was hypodense on CT-HA, it was regarded as isoattenuating.

Follow-Up Protocol

On the basis of the above-mentioned strategies, when the tumor diameter enlarged or there was a change in US pattern or change of enhancement features, reexamination, including imaging and tumor biopsy, was considered. When a typical hypervascular staining pattern was obtained on angiography or a hyperattenuating nodule was detected on the arterial phase of the dynamic CT, the nodule was diagnosed as HCC without histologic examination.

Histopathologic Examination

All specimens were obtained by percutaneous fine needle aspiration biopsy (FNAB) by using a 21-gauge Mashima needle under US guidance. Tissue samples were collected not only from tumor tissue but also from nontumor tissue to compare architectural and nuclear differences. To avoid misdiagnosis due to sampling error or variation, sampling was carried out at least twice from different areas of the nodule. Furthermore, the US image was recorded on a video recorder to confirm that tumor samples were correctly obtained. Sections of 3–6-μm in thickness were cut after formalin-fixation and paraffin-embedding of specimens. Sections were stained with hematoxylin and eosin (H & E) and silver for reticulin fibers.

Tissue samples obtained by tumor biopsy were classified into HCC, high-grade dysplastic nodule (HGDN), low-grade dysplastic nodule (LGDN), or regenerative nodule (RN), according to criteria proposed by an international working party.[17] In brief, LGDN was characterized by a slight increase (< 1.5 times) of cell density and nuclear-cytoplasmic ratio compared with the surrounding liver tissue, an absence of structural dysplasia, and sometimes showed a large or small change. HGDN also had features of LGDN, and, in addition, there was an increase in cell density of between 1.5 to 2 times, high nuclear-cytoplasmic ratio, cytoplasmic basophilia, and irregular nuclear contour. If stromal or portal tract invasion of the tumor was seen in the specimen, the nodule was considered well differentiated HCC.[18]

Statistical Analysis

Differences in background features and laboratory data among the three groups were analyzed by the chi-square test and Kruskal–Wallis test. The time between first biopsy and development of HCC was analyzed by using the Kaplan–Meier technique, and differences in curves were tested by using the log-rank test. Independent risk factors associated with HCC progression rate were studied using stepwise Cox regression analysis.[19] Potential risk factors for malignant transformation that were assessed included the following 18 variables: age, gender, etiology of background liver disease, previous history of HCC, presence of cirrhosis, albumin, bilirubin, indocyanine green retention rate at 15 minutes (ICG R15), platelet count, prothrombin time, alpha fetoprotein (α-fetoprotein (AFP)), des-gamma-carboxy prothrombin (DCP), diameter of the nodule, US pattern, conventional CT findings, CT-AP findings, and CT-HA findings. A probability of < 0.05 was considered significant. Data analysis was performed using SPSS statistical software version 10 (SPSS Inc., Chicago, Illinois).

RESULTS

Image Diagnosis and Malignant Transformation of Hepatic Nodular Lesions

Each imaging technique and lesion feature were examined. Because all tissue samples in the current study were obtained under US guidance, the detection rate on US was 100%. Of hepatic lesions examined, 85 (55.2%) nodules were low-echoic whereas the remain-

ing 69 (44.8%) were high-echoic. Furthermore, 35 (22.7%) nodules were observed on helical dynamic CT. Among these, 26 were isoattenuating at arterial phase and low-attenuating at portal venous phase and/or the equilibrium phase. The other nine showed low attenuation throughout the scanning. None of the nodules was high-attenuating at the arterial phase on CT.

CT-AP was carried out in 144 patients and 49 (34.0%) nodules were detected. Among these, 30 nodules were slightly low attenuating, whereas the remaining 19 were markedly low attenuating relative to surrounding liver parenchyma. CT-HA was performed in 142 patients and 40 (28.2%) nodules were detected. Only 10 nodules were high attenuating, whereas 30 nodules were low attenuating on CT-HA.

The cumulative HCC development rate was evaluated by Kaplan–Meier method for each imaging diagnosis. The HCC development rates for high echoic nodules were 4.8% at 1 year, 13.7% at 3 years, and 22.5 % at 5 years. These rates were 9.8%, 25.7%, and 25.7 % for low-echoic nodules, respectively, and the rates were not significantly different between the high- and low-echoic groups. The HCC development rate of CT-detected and -undetected nodules were 9.7% and 4.3% at 1 year, 32.2% and 16.8% at 3 years, and 38.3% and 20.6% at 5 years, respectively. CT-detected nodules were more likely to transform to HCC than CT-undetected nodules (log-rank test, $P = 0.039$) (Fig. 1A). HCC developed from CT-AP low-attenuating nodules at a rate of 13.8%, 35.3%, and 39.7% at 1, 3, and 5 years, respectively. These rates were 4.7%, 14.5%, and 19.4%, respectively, for nodules that did not show low attenuation. The CT-AP low-attenuation nodules often transformed to HCC (log-rank test, $P = 0.005$) (Fig. 1B). Similarly, HCC developed from CT-HA high-attenuation nodules at a rate of 9.2%, 20.0%, and 30.5% at 1, 3, and 5 years, respectively. These rates were 5.7%, 18.3%, and 23.4%, respectively, for high-attenuation nodules, and the rates were not significantly different between the two groups.

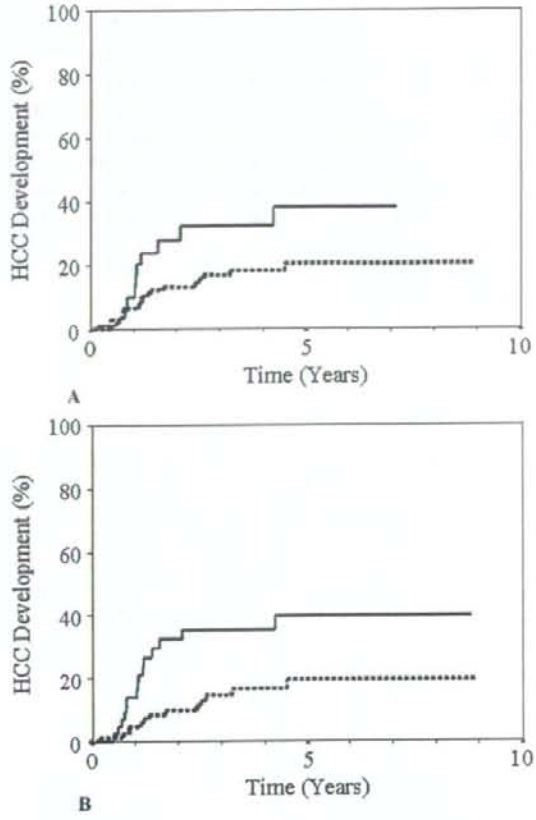

FIGURE 1. Cumulative HCC development rate of hepatic nodular lesions based on **(A)** detection by computed tomography (CT). Solid line: HCC developed from CT-detected nodules, dashed line: HCC from CT-undetected nodules (log-rank test: $P = 0.039$), and **(B)** CT-AP findings. Solid line: HCC from low-attenuating nodules, dashed line: HCC from no attenuation lesions (log-rank test: $P = 0.005$).

Histologic Diagnosis and Malignant Transformation of Hepatic Nodular Lesions

Histologic diagnosis of 154 nodules based on examination of initial biopsies was as follows: HGDN, $n = 13$; LGDN, $n = 42$; whereas the remaining 99 nodules did not show any abnormal histologic features and were, therefore, considered RN. The clinical backgrounds of the three groups classified by histologic features are summarized in Table 2. There were no differences in these parameters among the three groups. Tumor diameter, US pattern, detection of the nodule on dynamic CT, and attenuation pattern on CT-AP and CT-HA were also compared (Table 3), but there were no significant differences among the three groups.

Twenty-nine (18.8%) patients of 154 with nodules progressed to HCC during the median follow-up of 2.8 years. The diagnosis of HCC was made histologically ($n = 24$) or by imaging modalities ($n = 5$). The 24 HCC that were histologically diagnosed comprised well differentiated HCC ($n = 16$) and moderately differentiated HCC ($n = 8$). The cumulative HCC progression rate was 7.0% at 1 year, 16.1% at 2 years, 19.9% at 3 years, and 24.4% at 5 years. The first histologic diagnosis and the final outcome of the 154 nodules at the end of the observation period are shown in Figure 2. A total of 9 (69.2%) of 13 nodules transformed to HCC

TABLE 2
Comparison of Background of Patients with Small Hepatic Nodules with Regard to Initial Histologic Diagnosis

Parameter	HGDN (n = 13)	LGDN (n = 42)	LRN (n = 99)	Significance
Age, yrs[a]	64 (34–70)	64 (39–76)	61 (33–81)	NS
Gender, male:female	9:4	24:18	68:31	NS
HBV:HCV:HBV+HCV:others	3:10:0:0	10:31:0:1	19:67:2:11	NS
Previous history of HCC, yes:no	10:3	33:9	71:28	NS
Presence of cirrhosis, yes:no	10:3	36:6	78:21	NS
Albumin, g/dL[a]	3.4 (3.1–4.0)	3.6 (2.7–4.4)	3.7 (2.6–4.7)	NS
Bilirubin, mg/dL[a]	1.1 (0.7–1.8)	1.1 (0.4–2.6)	1.0 (0.4–3.1)	NS
ICG R15, %[a]	33 (20–46)	28 (4–56)	24 (7–79)	NS
Platelet, $\times 10^4/mm^3$[a]	9.3 (5.0–15.8)	9.8 (3.5–38.0)	10.4 (3.4–38.0)	NS
Prothrombin time, %[a]	86 (45–100)	86 (54–100)	88 (52–100)	NS
AFP, ng/mL[a]	13 (2–244)	16 (1–403)	11 (2–1070)	NS
DCP, AU/mL[a]	12 (<10–31)	13 (<10–182)	13 (<10–178)	NS

HGDN: high-grade dysplastic nodule; LGDN: low-grade dysplastic nodule; LRN: large regenerative nodule; HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; ICG R15: indocyanine green retention rate at 15 minutes; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin.
[a] Data are expressed as median (range).

TABLE 3
Tumor Diameter and Image Characteristics of HGDN, LGDN, and RN

Image characteristics	HGDN (n = 13)	LGDN (n = 42)	LRN (n = 99)	Significance
Diameter of nodule[a]	15 (10-25)	14 (7-23)	14 (7-40)	NS
Ultrasonography, high echo: low echo	3:10	18:24	48:51	NS
Detection on dynamic CT, yes:no	2:11	9:33	24:75	NS
Low attenuating area on CT-AP, yes:no	6:7	14:25	29:63	NS
High attenuating area on CT-HA, yes:no	1:12	4:34	5:86	NS

HGDN: high-grade dysplastic nodule; LGDN: low-grade dysplastic nodule; LRN: large regenerative nodule; CT-AP: computed tomographic-arterial portography; CT-HA: computed tomographic-hepatic angiography; NS: not significant.
[a] Data are expressed as median (range).

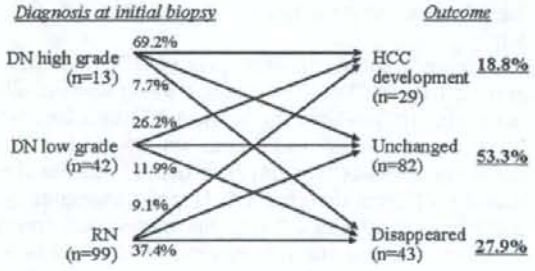

FIGURE 2. Initial histologic diagnoses and outcomes of 154 patients with small hepatic nodular lesions.

from HGDN. Only one nodule disappeared during the follow-up period. Similarly, 11 (26.2%) nodules of 42 progressed to HCC from LGDN, and 5 (11.9%) nodules disappeared. A total of 9 (9.1%) HCC arose from RNs at first biopsy, and 37 (37.4%) disappeared.

The cumulative HCC development rate calculated by the Kaplan–Meier method was 7.0% at 1 year, 19.9% at 3 years, and 24.4% at 5 years. The development of HCC occurred within the first 5 years, and no patient has subsequently developed HCC to date. Figure 3 shows HCC development rate according to each tissue diagnosis. The HCC transformation rate from HGDN was 46.2% at 1 year, 61.5% at 2 and 3 years, and 80.8% at 5 years. The rate of development of HCC from LGDN was 2.6% at 1 year, 30.2% at 3 years, and 36.6% at 5 years. Similarly, HCC developed from RNs at a rate of 3.3% at 1 year, and 9.7% at 3 years, and 12.4% at 5 years. HCC developed more often from HGDN nodules than from LGDN and RN (log-rank test, $P < 0.0001$).

Predictive Factors for Development of HCC from Hepatic Nodular Lesions

To elucidate predictive factors for development of HCC from hepatic lesions, both patient and tumor characteristics were analyzed by the log-rank test. Age > 60 years, ICG R15 > 30%, tumor diameter >14 mm, detection of the nodule on conventional helical dynamic CT, decrease of portal blood flow in the hepatic

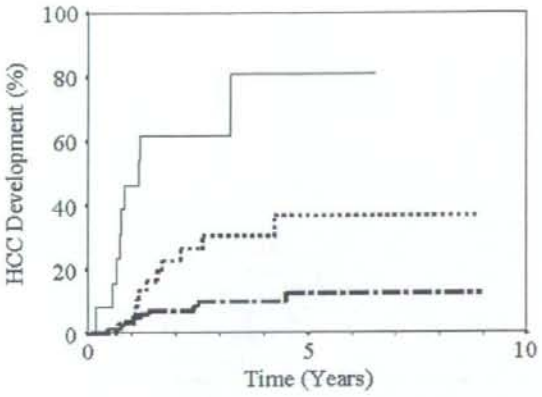

FIGURE 3. Cumulative HCC development rate from high-grade dysplastic nodules (HGDN, continuous line), low-grade DN (LGDN, dotted line), and regenerative nodules (RN, dashed line). HGDN developed into HCC more often compared with LGDN and RN ($P < 0.0001$).

TABLE 4
Factors Associated with HCC Progression from Hepatic Nodular Lesions by Multivariate Analysis (Cox Proportional Hazard Model)

Factors	Category	Hazard ratio (95% CI)	*P* value
Histology			
LRN	1	1	< 0.001
LGDN	2	2.96 (1.20–7.31)	
HGDN	3	16.8 (6.19–45.6)	
CT-AP			
Low attenuation, −	1	1	0.004
Low attenuation, +	2	3.04 (1.42–6.50)	

HCC: hepatocellular carcinoma; CI: confidence interval; LRN: large regenerative nodule; LDGN: low-grade dysplastic nodule; HGDN: high-grade dysplastic nodule; CT-AP: computed tomographic-arterial portography.

nodule on CT-AP, and liver histology on tumor biopsy were significant factors by univariate analysis. Furthermore, the etiology of chronic liver disease, serum albumin, serum bilirubin, prothrombin time, serum AFP level, serum DCP level, platelet count, US pattern, and hyperattenuation on CT-HA were not significant.

Subsequently, multivariate analysis by the Cox proportional hazard model was performed to adjust for the confounding effect on each variable. Histologic diagnosis was the most significant factor for development of HCC ($P < 0.0001$). Compared with RN, the rate of development of HCC in HGDN was as much as 16.8-fold higher (95% confidence interval [CI], 6.19–45.6), and in LGDN was 2.96-fold (95% CI, 1.20–7.31) higher. Decreased portal blood flow in the nodule on CT-AP was also significant (hazard ratio [HR], 3.04; 95% CI, 1.42–6.50; $P = 0.004$) (Table 4).

In addition, HCC developed at other sites of the liver during observation in 49 patients. However, there was no correlation between malignant transformation of the observed nodule and the subsequent development of HCC in other sites. In addition, no HCC developed from smaller nodules that were identified on first examination, during the follow-up period.

Case Reports of Hepatic Nodule Transforming to HCC during Four-Year Follow-Up

Figure 4 shows a case of HCC that progressed from a dysplastic nodule. This patient was a 61-year-old male with hepatitis C virus (HCV)-related cirrhosis. A 6-mm diameter hyperechoic nodule was found on US during the course of cirrhosis (Fig. 4A). Although detailed imaging analysis, including dynamic CT, hepatic angiography, CT-HA, and CT-AP, was carried out, the nodule was not detected with these modalities. FNAB was performed under US guidance, and the histologic diagnosis obtained from the specimen was LGDN (Fig. 5A). Therefore, the nodule was carefully followed up every 3 months on US. Three years later, the nodule was a little enlarged to 9 mm in diameter, and low-echoic foci appeared inside the nodule (Fig. 4B). Another year later, diameter of the tumor rapidly increased to 17 mm, and the nodule showed a "mosaic pattern," which is the typical sign of classical HCC on US (Fig. 4C). On this account, HCC development of the nodule was strongly suspected. Hepatic angiography was then carried out and showed typical hypervascular staining (Fig. 4D). The nodule was surgically resected afterward, and the specimen showed histologic features of moderately differentiated HCC (Fig. 5B).

Figure 6 shows another case of HCC that progressed from HGDN. This patient was a 65-year-old male with HCV-related cirrhosis. A 16-mm diameter hyperechoic nodule was found on US during the course of cirrhosis (Fig. 6A). The nodule was not detected on helical dynamic CT. Hepatic angiography, including CT-AP and CT-HA, was carried out, and a vague slightly low-attenuating area detected on both CT-AP and CT-HA (Fig. 6B–C). FNAB was performed under US guidance, and the histologic diagnosis was HGDN (Fig. 7A). Two years later, the nodule was found slightly enlarged to 20 mm in diameter on US, and the nodule became detectable on dynamic CT. (Fig. 6D). Furthermore, detailed image diagnosis was performed. Although there was no hypervascular staining on hepatic angiography, a relatively well bordered low-attenuating area was detected on CT-AP (Fig. 6E). In addition, a slightly high-attenuating rim was noted in the low-attenuating area on CT-HA (Fig. 6F). The nodule was surgically resected afterward, and the

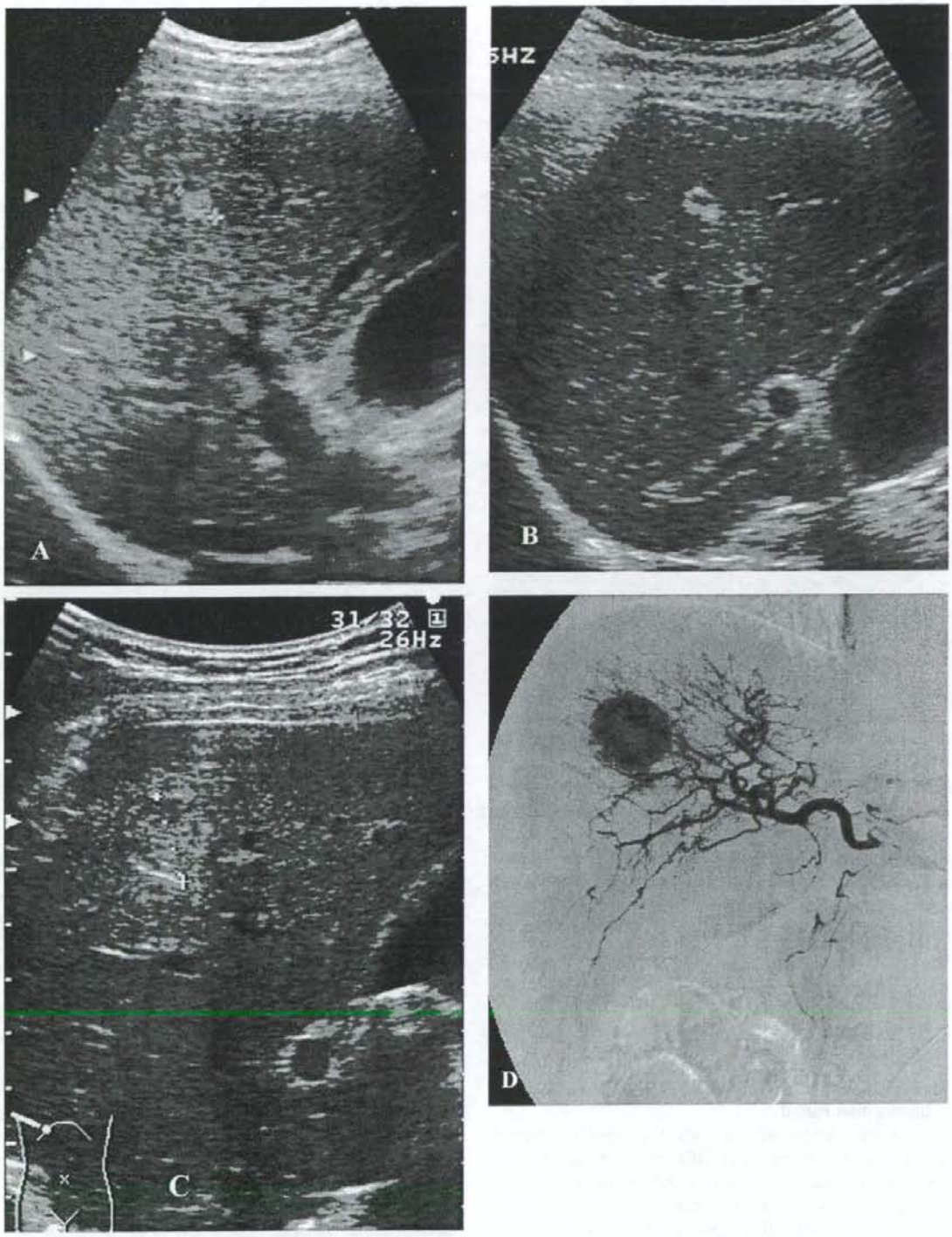

FIGURE 4. **(A)** A 6-mm diameter hyperechoic nodule appeared on ultrasonography (US) during the follow-up of HCV-related cirrhosis. **(B)** Four years later, the nodule increased in size to 9 mm in diameter and low-echoic foci appeared inside the nodule. **(C)** The nodule rapidly grew in size within 1 more year and showed a "mosaic pattern" on US. **(D)** Hepatic angiography was carried out before surgical resection. The angiogram showed typical hypervascular staining of HCC in the right lobe of the liver.

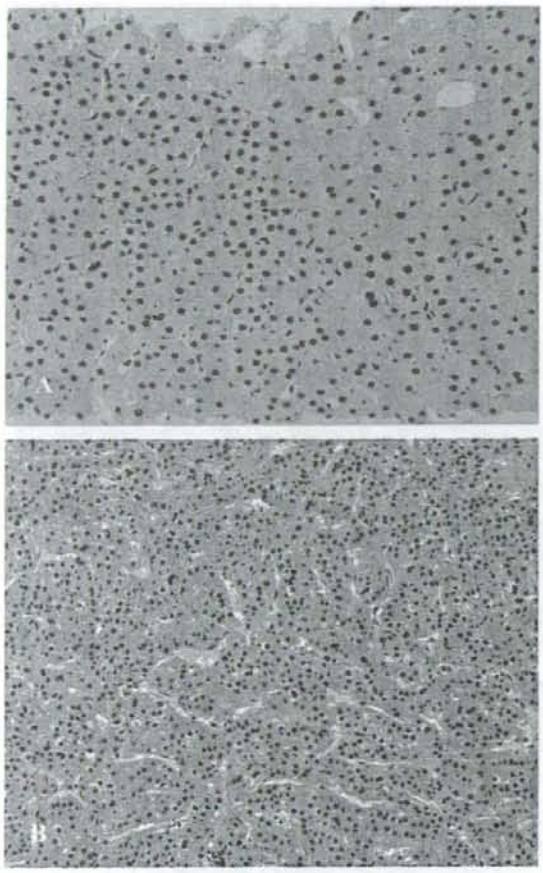

FIGURE 5. **(A)** Liver tissue sample obtained at first biopsy. The cell density and nuclear-cytoplasmic ratio was slightly increased (left) compared with the surrounding liver tissue (right). Small cell change was also observed and the histologic diagnosis was low-grade dysplastic nodule. **(B)** Liver tissue obtained by surgical resection. The tissue showed a trabecular pattern. The histologic diagnosis was well to moderately differentiated HCC. H&E staining in both panels; original magnification ×100.

specimen showed histologic features of well differentiated HCC (Fig. 7B).

Comparison of Tumor Diameter at First Biopsy and End of Observation Period

The above-mentioned analysis reviewed predictive factors for development of HCC by liver tissue diagnosis at the first tumor biopsy. When we observed a change in nodule diameter during follow-up of more than 5 mm, HCC developed in 10 of 17 patients, whereas HCC developed in only 12 of 137 nodules in which the diameter enlarged by less than 5 mm. The relation between tumor enlargement and HCC progression was statistically significant ($P < 0.001$).

DISCUSSION

The importance of dysplastic nodules as precancerous lesions of HCC is well established in Japan,[13,20–24] but less emphasized in Western countries.[25] Sakamoto et al. studied 320 resected liver tissues and concluded that multistep carcinogenesis is one pathway to HCC development.[13] Furthermore, several reports from Western countries in explanted whole liver from non-Japanese patients suggested that macroregenerative nodules may also represent precancerous lesions.[26,27]

However, consistent with previous reports, not all hepatic nodular lesions that we found on screening by US progressed to HCC.[23,24,28] Some nodules remained unchanged, and other nodules disappeared during long-term observation. Therefore, identification of true precancerous liver lesions, especially among patients with chronic liver disease, is important. The aims of the current study were to estimate the HCC progression rate of hepatic nodular lesions and to examine factors associated with malignant transformation.

Several reports have examined HCC development from borderline lesions;[23,24,28–31] however, no reports have included as many patients as the current study of 154 patients who were histologically diagnosed with dysplastic nodules and fully examined by imaging procedures before tumor biopsy. Notably, all patients also received imaging diagnosis every 3 months with a median follow-up of 2.8 years.

Twenty-nine of 154 (18.8%) hepatic nodules in our study transformed into HCC. The cumulative HCC development rates for such intrahepatic nodular lesions were 7.0% at 1 year, 19.9% at 3 years, and 27.4% at 5 years. Our findings are similar to those of Borzio et al.,[28] who reported an HCC rate of 31% in 90 large regenerative and dysplastic nodules and Seki et al.,[24] who reported HCC development in 12.1% of 33 dysplastic nodules measuring < 3 cm diameter at diagnosis.

With respect to predictive factors for development of HCC, age >60 years, ICG R15 > 30%, tumor diameter >14 mm, detection of a nodule on dynamic CT, decrease of portal blood flow in the hepatic nodule on CT-AP, and liver histology of tumor biopsy were significant factors by univariate analysis. The severity of background liver disease also has been reported as an important risk factor for development of HCC.[7–10] Therefore, high ICG R15 may affect potential HCC development. With respect to the effect of age, underlying liver disease would be expected to advance with