

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

Masahiro Kobayashi,^{1*} Kenji Ikeda,¹ Tetsuya Hosaka,¹ Hitomi Sezaki,¹ Takashi Someya,¹ Norio Akuta,¹ Fumitaka Suzuki,¹ Yoshiyuki Suzuki,¹ Satoshi Saitoh,¹ Yasuji Arase,¹ Yuzo Miyakawa,² and Hiromitsu Kumada¹

¹Department of Hepatology, Toranomon Hospital, Tokyo, Japan

²Miyakawa Memorial Research Foundation, Tokyo, Japan

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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*Correspondence to: Masahiro Kobayashi, MD, Masahiro Kobayashi, Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. E-mail: mshkobayashi@toranomon.gr.jp

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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 R₁₅), 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 ($100 \times 10^3/\text{mm}^3$) | 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 |

^aRetention of indocyanine green at 15 min in percent.

^bNot 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 R₁₅) 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 (<i>n</i> = 270) | HBV (<i>n</i> = 60) | Differences (<i>P</i> -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 |

^aPercentage of deaths by decompensation is shown.

^bNot significant.

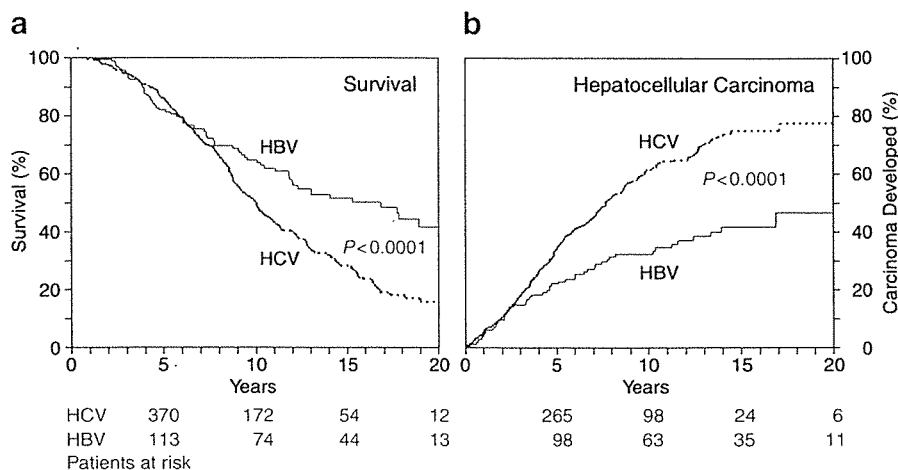


Fig. 1. Kaplan-Meier life tables for survival (a) and development of hepatocellular carcinoma (b) in patients with compensated cirrhosis in Child-Pugh Class A who were infected with HCV or HBV.

bilirubin levels, AFP, platelet counts, and ICG R₁₅ 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 R₁₅ >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 R₁₅ >30% (2.14), HBeAg (2.11), and platelet counts <100 × 10³/mm³ (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 | Dead | Differences (<i>P</i> -value) |
|---------------------|---|-------------|-------------|-----------------------------------|
| | | (n = 220) | (n = 270) | |
| 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 × 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; Benvegna 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 × 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 × 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 HBV, 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 respectively 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

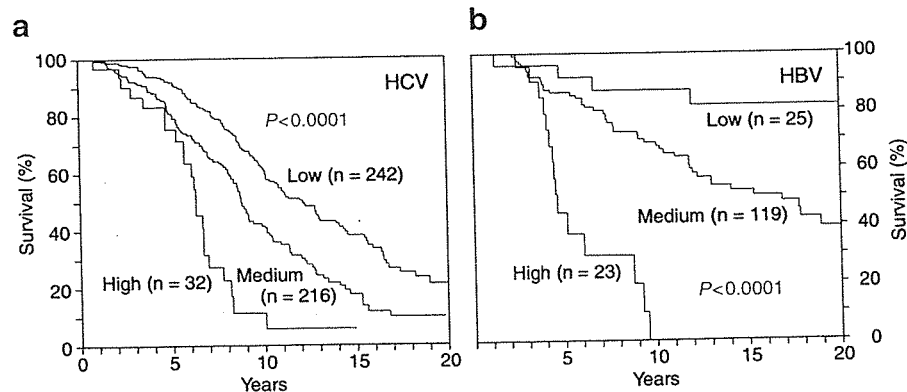


Fig. 2. Kaplan–Meier life tables for survival of patients with compensated cirrhosis who were infected with HCV (a) or HBV (b) and had low, medium, or high risk. High, medium, and low risk were defined by the sum of risk factors at 0–1, 2–3, and 4–5, respectively, in patients with HCV and that at 0–1, 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

Masahiro Kobayashi, M.D.
 Kenji Ikeda, M.D.
 Tetsuya Hosaka, M.D.
 Hitomi Sezaki, M.D.
 Takashi Someya, M.D.
 Norio Akuta, M.D.
 Fumitaka Suzuki, M.D.
 Yoshiyuki Suzuki, M.D.
 Satoshi Saitoh, M.D.
 Yasuji Arase, M.D.
 Hiromitsu Kumada, M.D.

Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan.

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Address for reprints: Masahiro Kobayashi, M.D., Department of Gastroenterology, Toranomon Hospital, 2-2-two Toranomon, Minato-ku, Tokyo 105-8470, Japan; Fax: (011) 81 (44) 860-1623. E-mail: mshkobayashi@toranomon.gr.jp

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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.

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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

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

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, $\times 10^4/\text{mm}^3$ | 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.

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.

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 re-

order to confirm that tumor samples were correctly obtained. Sections of 3–6- μ m 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.

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

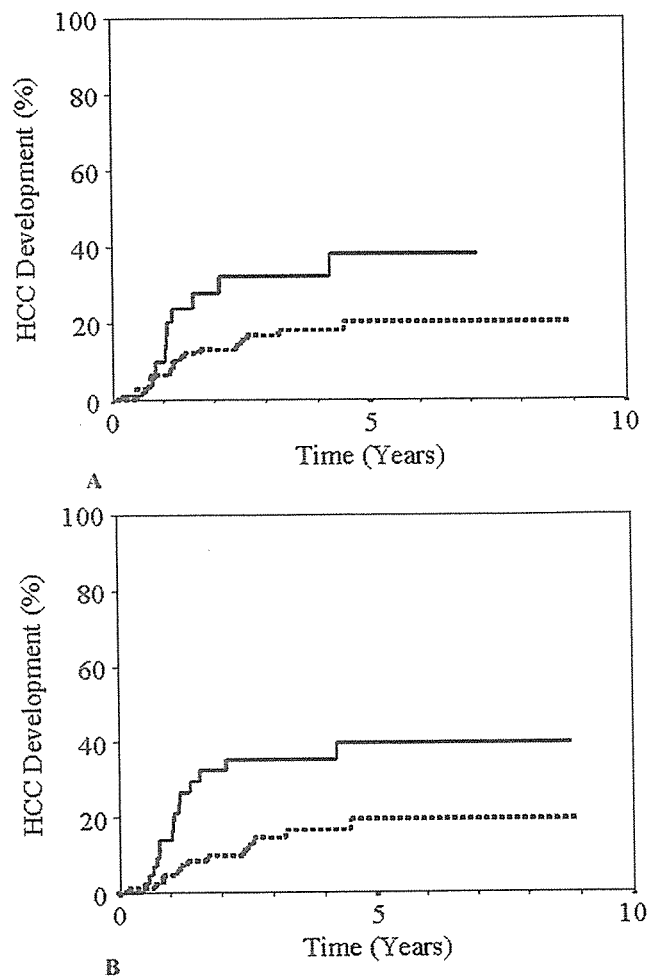


FIGURE 1. Cumulative HCC development rate of hepatic nodular lesions based on (A) detection by computed tomography (CT). Solid line: HCC developed from CT-detected nodules, dashed line: HCC from CT-undetected nodules (log-rank test: $P = 0.039$), and (B) CT-AP findings. Solid line: HCC from low-attenuating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

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, ×10 ⁴ /mm ^{3a} | 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).

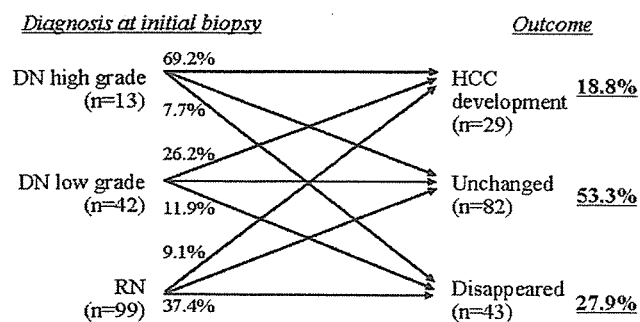


FIGURE 2. Initial histologic diagnoses and outcomes of 154 patients with small hepatic nodular lesions.

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 develop-

ment 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

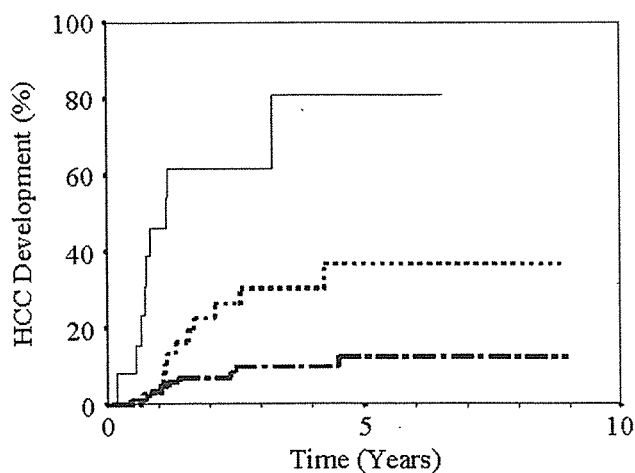


FIGURE 3. Cumulative HCC development rate from high-grade dysplastic nodules (HGDN, continuous line), low-grade DN (LGDN, dotted line), and regenerative nodules (RN, dashed line). HGDN developed into HCC more often compared with LGDN and RN ($P < 0.0001$).

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; LGDN: 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

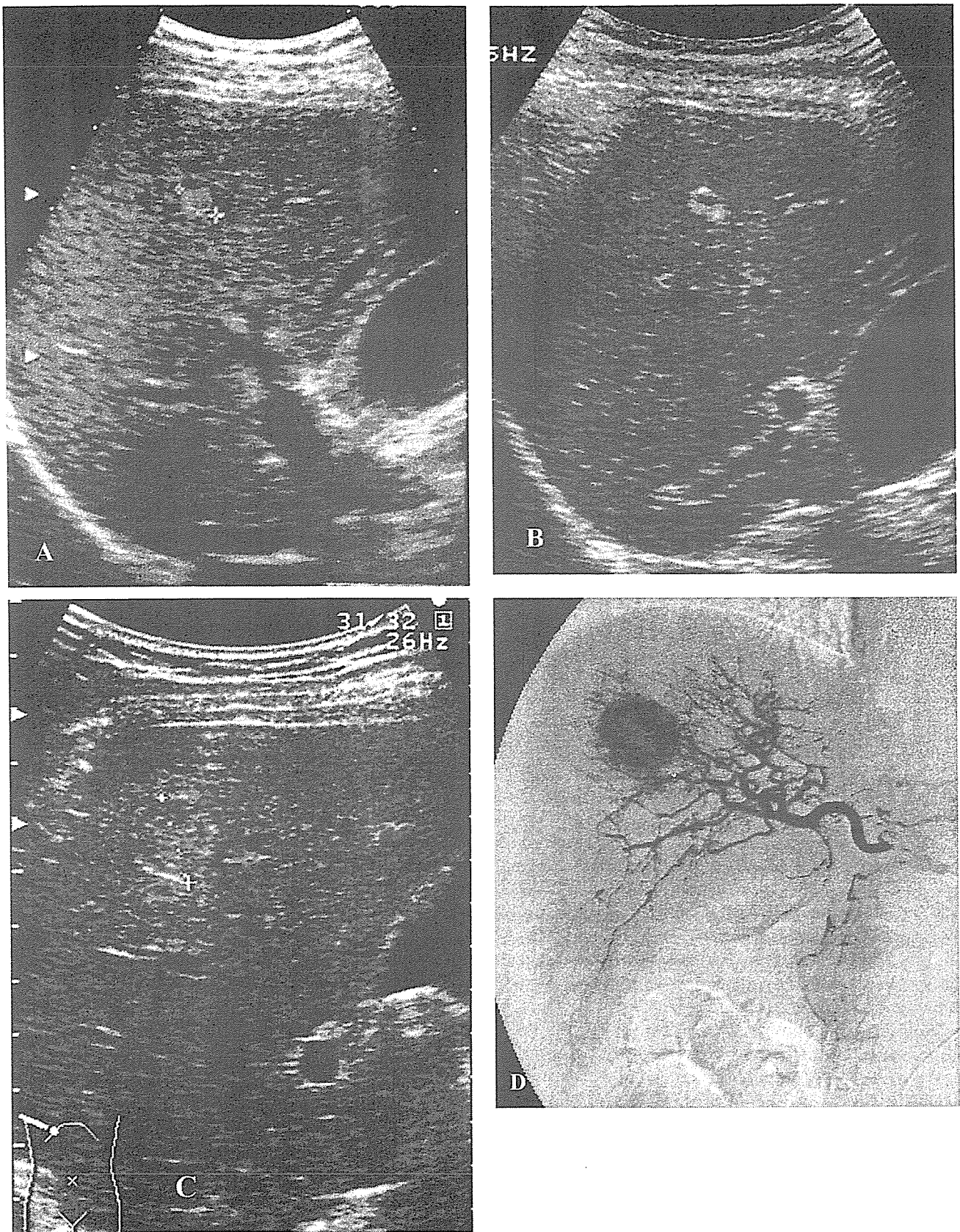


FIGURE 4. (A) A 6-mm diameter hyperechoic nodule appeared on ultrasonography (US) during the follow-up of HCV-related cirrhosis. (B) Four years later, the nodule increased in size to 9 mm in diameter and low-echoic foci appeared inside the nodule. (C) The nodule rapidly grew in size within 1 more year 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.

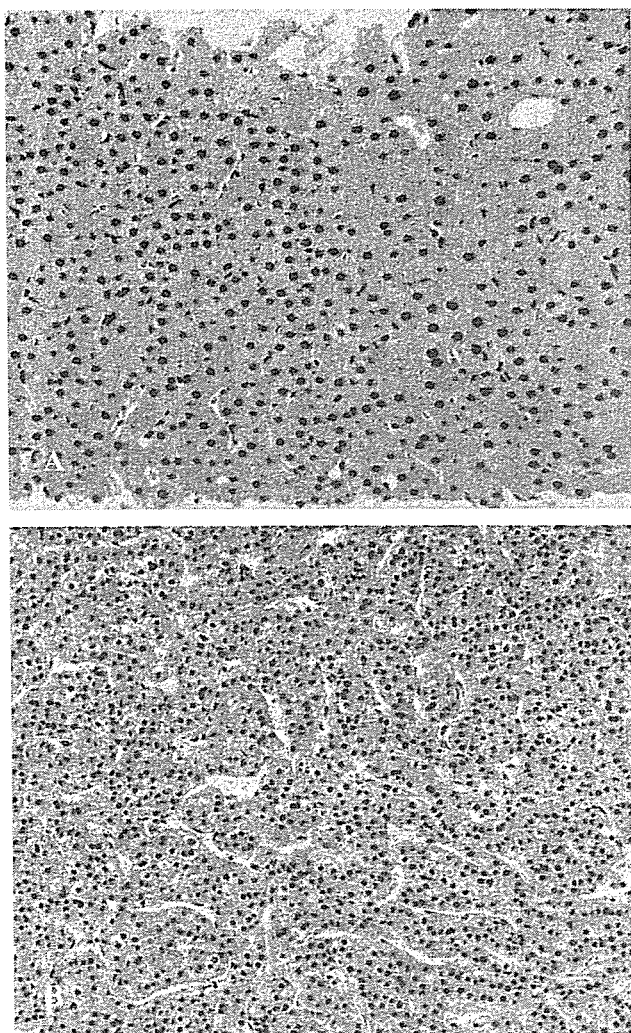


FIGURE 5. (A) Liver tissue sample obtained at first biopsy. The cell density and nuclear-cytoplasmic ratio was slightly increased (left) compared with the surrounding liver tissue (right). Small cell change was also observed and the histologic diagnosis was low-grade dysplastic nodule. (B) Liver tissue 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 $\times 100$.

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

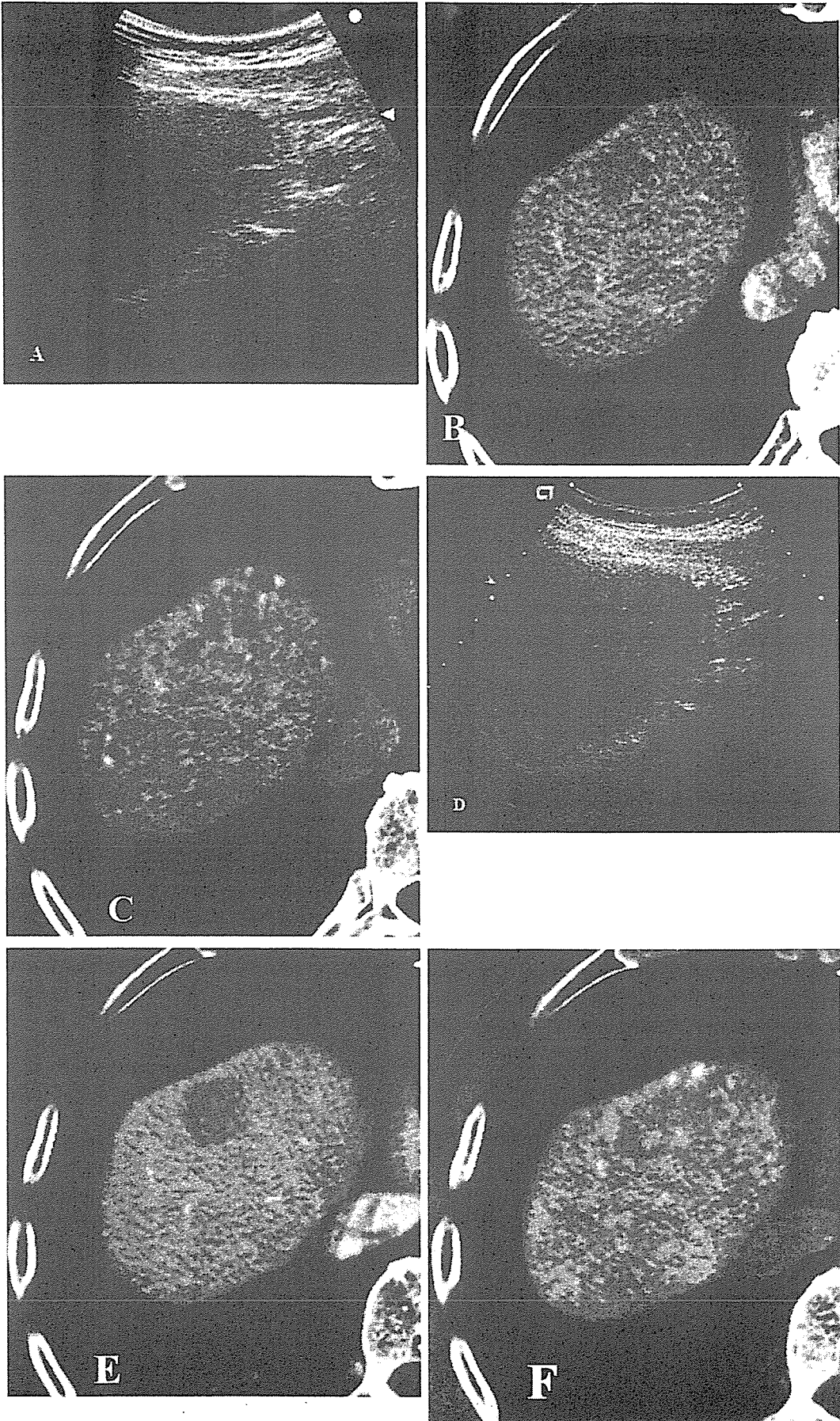


FIGURE 6

aging, and genetic mutation of oncogenes or tumor suppressor genes could occur at higher frequency with aging. We speculate that such alterations may generally increase risk of carcinogenesis with increasing age.

With respect to tumor diameter, it is intuitive that larger nodules could represent a more advanced stage in multistep carcinogenesis, and, thus, would progress to HCC more frequently than smaller ones. There is a problem of lead-time bias at this point. However, in our study, 133 of the 154 (86.4%) patients had undergone US screening before the first detection of hepatic nodules, and, thus, lead-time bias should have been minimized.

In multivariate analysis, histologic diagnosis and decrease of portal flow in CT-AP were independent factors for prediction of malignant transformation. In our study, we classified liver histology into three groups; HGDN, LGDN, and RN. According to this classification, as predicted, the progression rate of HCC from HGDN was significantly high, and the annual HCC development rate exceeded 30% in the first 2 years. The regression coefficient of HGDN in multivariate analysis was as much as 16.8 compared with RN. Therefore, we can conclude that HGDN was a true precancerous lesion of HCC.

Because of radiologic innovations, the relation between tumor progression and vascular supply of hepatic tumors is well documented. Hayashi et al.²⁹ followed up dysplastic nodules detected on CT-HA and CT-AP and described how portal blood flow in the nodule gradually decreased with acquisition of malignant tumor features. These results are consistent with our results in that in both univariate and multivariate analyses, reduced portal blood flow was a risk factor for HCC development. We realize that CT-AP and CT-HA are rather invasive methods and cannot easily be conducted repeatedly. Dysplastic nodules or RNs are usually isovascular or hypovascular.²⁹ In fact, in our patients, the arterial phase on dynamic CT was isoattenuating in 26 and low-attenuating in the remaining 9, and all 35 nodules were low-attenuating at portal venous phase and/or the equilibrium phase. Although

FIGURE 6. (A) A 16-mm diameter hyperechoic nodule was found on US during the course of cirrhosis. (B) A vague and slightly low-attenuating area was detected on both CT-AP. (C) The nodule showed slightly hypoattenuating area on CT-HA. (D) Two years later, the nodule increased in size to 20 mm in diameter on US. (E) Further detailed image diagnosis was performed and a relatively well bordered low-attenuating area was detected on CT-AP. (F) In addition, a slightly high-attenuating rim appeared in the low-attenuating area on CT-HA.

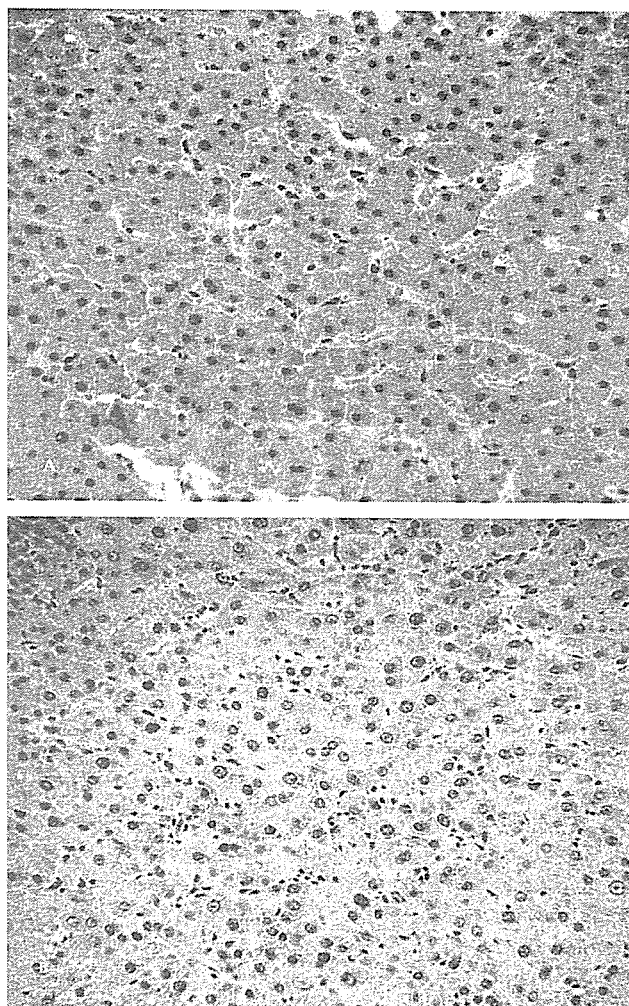


FIGURE 7. (A) The first histopathologic diagnosis based on examination of US-guided fine needle aspiration biopsy of the nodule was high-grade dysplastic nodule. (B) The nodule was subsequently resected surgically, and the specimen showed histopathologic features of well differentiated HCC. H&E staining in both panels; original magnification $\times 100$.

detection of the nodule on dynamic CT was significant for predicting HCC transformation, as determined by univariate analysis, this was not significant by multivariate analysis. This could mean that CT-AP is superior to dynamic CT in detecting reduced portal blood flow and, hence, a better predictor of progression to HCC.

With respect to evaluation of tumor arterial blood flow, although we reviewed the results of CT-HA in our patients, we could not determine the relation between arterial blood flow and tumor progression. Hepatic arterial flow decreased in dysplastic nodules and in early stage HCC, and then it increased as lesions progressed to classic HCC.²⁹ Such a two-phase arterial

flow change may have complicated our statistical analysis.

Previous studies showed the usefulness of MRI for characterization of hepatic nodular lesions.³²⁻³⁵ Earls et al.³² examined thin-section MRIs of explanted liver and reported that MRI depicted 41 of 42 (98%) hepatic nodular lesions, which included dysplastic nodules as well as HCC. Furthermore, Matsui et al.³³ demonstrated that hyperplastic adenomatous nodules were hyperintense on T1-weighted spin-echo imaging and hypointense on T2-weighted spin-echo imaging, and both features were useful for the differentiation of such borderline lesions from HCC in the cirrhotic liver. Furthermore, other groups also indicated the superiority of dynamic contrast-enhanced MRI and ferumoxides-enhanced MRI relative to CT for diagnosis of small HCC.^{34,35} We also examined dynamic MRI in 48 patients and ferumoxides MRI in 25 patients. Because the number of these patients was small, we did not include MRI results in our analysis. Choi et al.³⁴ indicated that ferumoxides-magnetic resonance imaging can be used instead of CT-AP and CT-HA, because both modalities have almost the same sensitivity and high specificity for diagnosis of HCC. Further studies are needed to confirm the usefulness of ferumoxides-MRI for diagnosis of malignant transformation of hepatic nodular lesions.

There are certain limitations in our study. First, tumor biopsy was usually carried out under US guidance, and, therefore, nodules that were not observed on US were not included in this study. In our clinical practice, we sometimes find hepatic nodules that show low attenuation on CT-HA and/or CT-AP, but they are barely observed on US. Tsuchiyama et al.³¹ examined repeated CT-HA and reported that 18.8% of small stained spots progressed to HCC during a mean follow-up of 29 months. Indeed, it is technically difficult to confirm such nodules histologically, and detailed investigation is required in the future.

Second, problems of sampling error and sampling variation are always inherent in this kind of examination. Indeed, nine patients in our study progressed to HCC despite histologic diagnosis of RN at first biopsy. In these cases, we cannot exclude sampling errors or sampling variation. However, to minimize such problems, we routinely recorded the scene of the US-guided biopsy on video recorder to confirm that the sample was actually from the nodule. In addition, it is possible that samples obtained by needle biopsy did not reflect the most malignant part of the nodule, particularly when the nodule was heterogeneous. On this account, we usually obtained samples from two or more parts of a nodule to prevent sampling variation.

If a nodule showed a heterogeneous US pattern, we obtained samples from each part of the nodule.

We recognized limitations of biopsy diagnosis for predicting HCC progression, as nine patients whose initial histologic diagnosis was RN later developed HCC. For this reason, it is necessary to include imaging diagnostic techniques such as CT-HA or CT-AP to predict liver cancer development.

Our present results allow us to conclude that dysplastic nodules, in particular HGDN, are true precancerous lesions of HCC. Hemodynamic changes in these nodules predicted progression to HCC; however, angio-computed tomography is an invasive examination, and repeated studies are not feasible. Recently, it was reported that HCC and borderline lesions, like dysplastic or regenerative nodules, can be discriminated by Levovist contrast-enhanced US.^{36,37} Because commercial use of Levovist was not possible until 1999 in our country, we have not included this technique in the current study. We are very interested in whether contrast-enhanced harmonic US is useful in predicting HCC development from hepatic nodular lesions, as this examination is less invasive than CT-HA or CT-AP and does not require hospitalization.

On the basis of changes observed during follow-up, our results indicated that enlargement of tumor diameter was the most important factor suggesting malignant transformation. Changes in US pattern also indicated HCC development. Indeed, low-echoic foci appeared in the center of hyperechoic nodules preceding HCC diagnosis in four patients who developed HCC. This finding is consistent with multistep carcinogenesis in HCC.

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Prediction model of hepatocarcinogenesis for patients with hepatitis C virus-related cirrhosis. Validation with internal and external cohorts

Kenji Ikeda^{1,*}, Yasuji Arase¹, Satoshi Saitoh¹, Masahiro Kobayashi¹, Takashi Someya¹, Tetsuya Hosaka¹, Norio Akuta¹, Yoshiyuki Suzuki¹, Fumitaka Suzuki¹, Hitomi Sezaki¹, Hiromitsu Kumada¹, Akihisa Tanaka², Hideharu Harada²

¹Department of Gastroenterology, Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Toranomon 2-2-2, Minato-ku, Tokyo 105-8470, Japan

²Department of Gastroenterology, Tokyo National Hospital, National Hospital Organization, Tokyo, Japan

See Editorial, pages 1013–1016

Background/Aims: To estimate hepatocarcinogenesis rates in patients with hepatitis C virus (HCV)-related cirrhosis, an accurate prediction table was created.

Methods: A total of 183 patients between 1974 and 1990 were assessed for carcinogenesis rate and risk factors. Predicted carcinogenesis rates were validated using a cohort from the same hospital between 1991 and 2003 ($n = 302$) and an external cohort from Tokyo National Hospital between 1975 and 2002 ($n = 205$).

Results: The carcinogenesis rates in the primary cohort were 28.9% at the 5th year and 54.0% at the 10th year. A proportional hazard model identified alpha-fetoprotein (≥ 20 ng/ml, hazard ratio 2.30, 95% confidence interval 1.55–3.42), age (≥ 55 years, 2.02, 95% CI 1.32–3.08), gender (male, 1.58, 95% CI 1.05–2.38), and platelet count ($< 100,000$ counts/mm³, 1.54, 95% CI 1.04–2.28) as independently associated with carcinogenesis. When carcinogenesis rates were simulated in 16 conditions according to four binary variables, the 5th- and 10th-year rates varied from 9 to 64%, and 21–93%, respectively. Actual carcinogenesis rates in the internal and external validation cohorts were similar to those of the simulated curves.

Conclusions: Simulated carcinogenesis rates were applicable to patients with HCV-related cirrhosis. Since, hepatocarcinogenesis rates markedly varied among patients depending on background features, we should consider stratifying them for cancer screening and cancer prevention programs.

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Keywords: Cirrhosis; Hepatocellular carcinoma; Carcinogenesis; Hepatitis C virus; Simulation; Proportional hazard model; Validation; Prediction

1. Introduction

There is increasing evidence that chronic hepatitis C virus (HCV) infection is closely associated with the occurrence of hepatocellular carcinoma (HCC) [1–4]. The

incidence of patients with HCV-related HCC has increased recently in several parts of the world [5–9]. In Japan, blood transfusion and parenteral drug use became prevalent in 1960s, and patients with HCV-related cirrhosis gradually increased around 1980s. Since, an effective and truly curative therapy for a large and advanced HCC still remains limited at best, evaluation and assessment of carcinogenesis in chronic liver disease and detection at an early stage of HCC are of great importance. Reports of HCC development rates in HCV-cirrhosis differ [10–13], probably due to

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* Corresponding author. Tel.: +81 44 877 5111; fax: +81 44 860 1623.

E-mail address: ikedakenji@tora.email.ne.jp (K. Ikeda).