bination therapy. Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

### Liver Histology before IFN therapy

Liver biopsy specimens were obtained percutaneously under observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Desmet et al (19). Baseline liver histology of chronic hepatitis prior to IFN therapy was classified according to the extent of fibrosis, into four stages: mild (F1), periportal expansion; moderate (F2), portoportal septa; severe (F3), portocentral linkage or bridging fibrosis; liver cirrhosis (F4). Patients with F1 or F2 were considered as non severe fibrosis. Patients with F3 or F4 were considered as severe fibrosis. In 47 patients, a liver biopsy was not available, because the patients declined to have a biopsy taken.

### Statistical analysis

Clinical and biochemical backgrounds before combination therapy among groups based on efficacy of treatment were analyzed using Krusukall Wallis test. We used multivariate analysis (multiple logistic regression analysis) to establish which factors contributed to the non-relapse after combination therapy. Results for each variable were transformed into categorical data consisting of two simple original numbers for multivariate analysis. $p<0.05$ was considered statistically significant. The variables that we use for multivariate analysis were age, sex, liver histology, body mass index (BMI), HCV-RNA load, continuance period of negative HCV RNA, attainment timing of negative HCV RNA, AST, ALT, hemoglobin, white blood cell, and platelet count. Significance of trends in values was determined with Cochran-Armitage trend test. A $p<0.05$ was considered statistically significant. The SPSS software package (SPSS 11.0 for windows; SPSS Inc., Chicago, IL, USA)was used for analyses.

## Results

### Clinical characteristics of the patients

A total of 366 patients were enrolled in the present study. Patients were classified into four groups according to the difference of response: RVR (n=37), EVR (n=161), LVR (n=131), and SLVR (n=37). The clinical and laboratory characteristics of the patients at baseline are shown in Table 1. There was a significant difference in the serum level of HCV RNA before treatment among the four groups classified based on the difference of response.

### Predictors of non-relapse in patients

Of the 366 patients originally included in this study, 241 had non-relapse and the non-relapse rates in each group were 89% (33/37)in RVR, 79% (127/161) in EVR, 54% (71/122) in LVR, and 27% (10/37) in SLVR. Next, predictors for non-relapse were assessed in the total 366 patients. Univariate analysis showed that the following ten factors significantly affected non-relapse as shown in Table 2A. Multivariate analysis indicated that non-relapse occurred when serum HCV RNA at week 12 was negative (p=0.003) and continuance of negative HCV RNA during treatment was $\leq$30 weeks (p=0.017) (Table 2B).

### Non-relapse based on the attainment time of negativity of serum HCV RNA and continuance period of negative HCV RNA

The non-relapse rate based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA during combination therapy are shown in Table 3. In RVR group, 26 of 27 patients with continuance of negative HCV RNA of $\leq$30 weeks during treatment had non-relapse. In EVR group, patients with period of negative HCV RNA of $\leq$40 weeks during treatment had SVR rate of 90% (71/79). In LVR and SLVR group, all nine patients with continuance of negative HCV RNA of$\leq$60 weeks during treatment had non-relapse.

## Discussion

We have described the incidence of non-relapse and treatment period to enhance the non-relapse rate in patients with undetectable HCV RNA during combination therapy of peginterferon and ribavirin for chronic hepatitis C. In the present study, 241 (66%) of 366 patients with undetectable HCV RNA during combination therapy of peginterferon and ribavirin for chronic hepatitis C showed non-relapse. One-third of patients with undetectable HCV RNA during combination therapy showed relapse. On the other hand, 128 with detectable HCV RNA during combination therapy showed detectable HCV RNA after the termination of combination therapy. The present study was limited to patients with genotype 1 and HCV-load of $\geq$100 KIU/ml. Other limitation is that the present study was not a randomized controlled study; the treatment period was varied.

However, several findings from the present study have direct implications for the combination therapy of chronic hepatitis C in the future. First, non-relapse in patients with undetectable HCV RNA during treatment was associated with attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA. Early undetectable HCV RNA and prolonged negativity of serum HCV RNA enhanced the non-relapse rate. Earlier studies have reported higher SVR rates in patients with undetectable HCV RNA at week 4 than in those with detectable HCV RNA (8, 20, 21). The present results coincided closely with these earlier results.

Second, in RVR group, patients with continuance of nega-

**Table 1. Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients**

| | Total | Response | | | | |
|---|---|---|---|---|---|---|
| | | RVR | EVR | LVR | SLVR | p |
| Patients, n | 366 | 37 | 161 | 131 | 37 | |
| Sex, male (%)† | 252 (69%) | 28 (76%) | 119 (74%) | 82 (63%) | 23 (62%) | .118 |
| Age (yrs)‡ | 51.6±10.4 | 51.1±7.0 | 51.5±10.4 | 52.0±10.2 | 52.3±11.7 | .399 |
| Weight (kg)‡ | 64.9±11.1 | 65.1±10.2 | 64.7±10.4 | 65.1±11.1 | 65.2±10.4 | .917 |
| BMI‡ | 23.5±2.9 | 23.3±2.8 | 23.3±2.8 | 24.1±2.9 | 24.0±3.2 | .248 |
| HCV RNA(KIU/ml)§ | 1400 (100->5000) | 430 (100-1200) | 1100 (110->5000) | 1750 (130->5000) | 1900 (300->5000) | <.001 |
| AST (IU/L)‡ | 66±43 | 59±62 | 64±43 | 66±41 | 70±52 | .419 |
| ALT (IU/L)‡ | 94±66 | 84±52 | 98±76 | 91±59 | 92±65 | .321 |
| WBC($10^3$/$mm^3$)‡ | 4.9±2.9 | 5.0±2.5 | 5.1±3.7 | 4.8±2.2 | 4.6±1.4 | .432 |
| Hb (g/dl)‡ | 14.7±1.5 | 14.7±1.6 | 14.8±1.7 | 14.7±1.3 | 14.8±1.3 | .887 |
| Platelet($10^4$/$mm^3$)‡ | 17.7±8.9 | 17.8±4.6 | 18.6±9.2 | 17.1±7.9 | 17.9±8.5 | .616 |
| Fibrosis staging* F1/F2/F3/F4 | 151/92/56/8 | 14/7/2/1 | 76/41/21/3 | 45/32/27/4 | 16/12/6/0 | .142 |
| Peginterferon/weight (μg/kg)‡ | 1.4±0.3 | 1.4±0.3 | 1.4±0.3 | 1.3±0.3 | 1.4±0.3 | .916 |
| Ribavirin/weight (mg/kg)‡ | 11.8±1.4 | 11.8±1.6 | 11.7±1.4 | 11.8±1.4 | 11.8±1.5 | .895 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early virological response; Hb, hemoglobin; HCV, hepatitis C virus; LVR, late virological response; RVR, rapid virological response; SLVR, super late virological response; WBC, white blood cell;
Normal reference ranges 6-50 IU/L for ALT, 11-38 IU/L for AST,

*Data are number of patients

†Data expressed as number of patients (percentage)

‡Data expressed as mean±standard deviation

§Data expressed as median (range)

tive HCV RNA of ≥30 week during treatment had non-relapse of >90%. Next in EVR group, patients with continuance of negative HCV RNA of ≥40 weeks during treatment had non-relapse of >85%.

Third, in LVR group, patients with continuance of negative HCV RNA of ≥60 weeks during treatment had a high non-relapse rate. The fact that all 9 patients in LVR or SLVR group showed non-relapse after continuance of negative HCV RNA of ≥60 week during treatment is important and impressive. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period to achieve non-relapse. As described above, for 85% of non-relapse, the desirable duration of negativity of serum HCV-RNA may be 30 weeks in RVR, 40 weeks in EVR and 60 weeks in LVR.

It is desirable to expose patients with chronic hepatitis C to the shortest duration of treatment possible to reduce the likelihood of adverse events and minimize costs. Long-term treatment can be associated with serious side effects and is costly (22). The treatment of combination therapy is expensive; a 24-week treatment course costs approximately 20,000 US $. Regarding the side effects of treatment, previously unreported side effects were not observed in patients treated for more than one year. However, prolonged combination therapy may cause the serious side effects. Accordingly, careful selection of patients for long-term combination therapy is important.

## Conclusions

The results of this study underscore the importance of changing the duration of treatment based on the difference of attainment time of negativity of serum HCV RNA in combination therapy for chronic hepatitis C. To attain a non-relapse rate of >85% in patients with undetectable HCV RNA, continuance of negative HCV RNA during treatment are desirable was 30 weeks in RVR group, 40 weeks in

**Table 2. Univariate and Multivariate Analyses Identifying Predictors of Non-Relapse Patients with Genotype 1**

**Table 2A. Univariate Analyses Identifying Predictors of Non-relapse**

| Factor | Category | Odds ratio | 95% Confidence interval | p value |
|---|---|---|---|---|
| HCV RNA week 12* | -/+ | 1/0.29 | 0.18-0.47 | < .001 |
| HCV RNA week 24* | -/+ | 1/0.19 | 0.09-0.39 | < .001 |
| Continuance period of negative HCV RNA (week) | <30/ ≥30 | 1/4.03 | 2.34-6.93 | <.001 |
| Continuance period of negative HCV RNA (week) | <40/ ≥40 | 1/3.38 | 2.06-5.55 | <.001 |
| Age (years) | <50/≥50 | 1/0.44 | 0.27-0.72 | .001 |
| Continuance period of negative HCV RNA (week) | <50/≥50 | 1/2.67 | 1.26-5.65 | .010 |
| Continuance period of negative HCV RNA (week) | <60/≥60 | 1/6.66 | 1.56-28.46 | .011 |
| HCV RNA week 4† | -/+ | 1/0.24 | 0.07-0.82 | .022 |
| Liver fibrosis | F1,F2/ F3,F4 | 1/0.56 | 0.33-0.96 | .036 |
| HCV-RNA(KIU/mL) | <1000/≥1000 | 1/0.62 | 0.38-1.01 | .086 |
| ALT (IU/L) | ≤50 / >50 | 1/1.53 | 0.42-2.56 | .101 |
| Hb (g/dL) | <14/ ≥14 | 1/1.64 | 0.99-2.70 | .107 |
| Platelet ($x10^4/mm^3$) | <15 / ≥15 | 1/1.42 | 0.89-2.26 | .147 |
| Sex | Male / Female | 1/0.75 | 0.47-1.21 | .244 |
| WBC ($x10^3/mm^3$) | <4 / ≥4 | 1/1.29 | 0.78-2.12 | .325 |
| BMI | <25 / ≥25 | 1/0.79 | 0.47-1.31 | .351 |
| AST (IU/L) | ≤38 />38 | 1/1.08 | 0.64-1.83 | .952 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Hb, hemoglobin; HCV, hepatitis C virus; SVR, sustained virological response; WBC, white blood cell;

†HCV RNA at week 12, 24 after the initiation of treatment

**Table 2B. Multivariate Analyses Identifying Predictors of Non-Relapse**

| Factor | Category | Odds ratio | 95% Confidence interval | p value |
|---|---|---|---|---|
| HCV RNA week 12* | - / + | 1/0.42 | 0.24-0.74 | .003 |
| Continuance period of negative HCV RNA during treatment (week) | <30 / ≥30 | 1/2.27 | 1.16-4.47 | .017 |

HCV=hepatitis C virus,

*HCV RNA at week 12 after the initiation of treatment,

EVR, and 60 weeks in LVR.

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**Table 3. SVR Based on the Attainment Time of Negative HCV RNA and Continuance Period of Negative HCV RNA during Combination Therapy***

| Response of HCV RNA* | Continuance period of negative HCV RNA (week) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | ~19 | 20~29 | 30~39 | 40~49 | 50~59 | ≥60 | |
| RVR | 33(1/3) | 75(3/4) | 100(4/4) | 96(22/23) | 100(3/3) | ND | 89(33/37) |
| EVR | 30(3/10) | 50(3/6) | 76(50/66) | 88(51/58) | 100(10/10) | 91(10/11) | 79(127/161) |
| LVR | 0(0/6) | 37(10/27) | 53(27/51) | 60(9/15) | 72(18/25) | 100(7/7) | 54(71/131) |
| SLVR | 0(1/9) | 18(2/11) | 20(1/5) | 40(4/10) | ND | 100(2/2) | 27(10/37) |
| Total | 18(5/28) | 38(18/48) | 65(82/126) | 81(86/106) | 82(31/38) | 95(19/20) | 66(241/366) |

EVR, early virological response; HCV, hepatitis C virus; LVR, late virological response; RVR, rapid virological response; SLVR, super late virological response

*Response of HCV RNA means attainment time of negativity of serum HCV RNA after the initiation of combination therapy

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**Abbreviations:** ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, EVR: early virological response, Hb: hemoglobin, HCV: hepatitis C virus, LVR: late virological response, RVR: rapid virological response, SLVR: super-late virological response, WBC: white blood cell

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# Prolonged-Efficacy of Bisphosphonate in Postmenopausal Women With Osteoporosis and Chronic Liver Disease

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Osteoporosis is present often in postmenopausal women. The aim of this retrospective cohort study is to assess the cumulative appearance incidence and predictive factors for bone fracture in postmenopausal women with osteoporosis and chronic liver disease. The patients were 80 postmenopausal women with osteoporosis and chronic liver disease due to hepatitis virus B or C. These patients were given cyclic etidronate therapy within 3 months after diagnosis of osteoporosis (etidronate-group). Another 400 postmenopausal women with osteoporosis and chronic liver disease were selected as controls (control group). Patients in control group were matched 1:5 with etidronate-group for age. Patients in control group were not given any drugs after diagnosis of postmenopausal osteoporosis. The mean observation period was 8.1 years. Four patients in the etidronate-group and 46 in control group developed bone fracture. The 10th year cumulative appearance rates of bone fracture were 4.9% in etidronate-group and 13.8% in control group. Cox regression model showed that the appearance rate of bone fracture decreased with statistical significance in the following cases: (1) patients $< 65$ years ($P < 0.001$), (2) patients with serum albumin level of $\geq 3.5$ g/dl ($P = 0.003$), and (3) patients treated with etidronate ($P = 0.020$). The cumulative survival rate after bone fracture was 82.2% at the second year, and 57.6% at the fifth year. The present study suggests that a serum albumin level of $\geq 3.5$ g/dl and cyclic etidronate treatment reduce the appearance of bone fracture. ***J. Med. Virol. 80:1302–1307, 2008.*** © 2008 Wiley-Liss, Inc.

**KEY WORDS:** chronic hepatitis; osteoporosis; bisphosphonate; bone fracture

## BACKGROUND

Hepatitis C virus (HCV) or hepatitis B virus (HBV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C or B is an insidiously progressive form of liver disease that relentlessly but progresses silently to cirrhosis and/or hepatocellular carcinoma (HCC) over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992; Ikeda et al., 1993; Tsukuma et al., 1993]. Additionally, chronic infection due to hepatitis virus has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, autoimmune thyroiditis, and sialadenitis [Johnson et al., 1993; Gumber and Chopra, 1995; Pawlotsky et al., 1995].

Bone disease is one of the major complications of chronic liver disease. Bone fracture rate are increased in chronic liver disease, especially postmenopausal women [Rouillard and Lane, 2001]. Etidronate is an organic compound that inhibits osteoclast mediated bone resorption. Intermittent cyclical etidronate has been shown to be useful for the treatment of osteopenia [Storm et al., 1990; Watts et al., 1990; Miller et al., 1997; Emkey and Ettinger, 2006]. In addition, cyclical etidronate has been reported to be an effective means of increasing bone mineral density in patients with cirrhosis [Shiomi et al., 2002]. Bisphosphonates inhibition hold promise for the treatment of patients with hepatic osteodystrophy. However, there is little information on the yearly cumulative incidence and risk factors on the development rate of bone fracture in

Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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patients with chronic liver disease during prolonged follow-up.

In Toranomon Hospital (Tokyo, Japan), a large number of patients with HCV or HBV-related hepatitis, were found often with hip fracture or vertebral fracture among elderly patients. With this background, the present retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of bone fracture among postmenopausal women with osteoporosis and chronic liver disease.

## MATERIALS AND METHODS

### Patients

The number of patients who were diagnosed with chronic HCV or HBV infection between April 1994 and March 2004 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 15,400. Out of these, 80 postmenopausal women with osteoporosis and chronic liver disease were treated with cyclical etidronate. These 80 consecutive patients treated with cyclical etidronate were regarded as the etidronate-group. Inclusion criteria except etidronate administration were as follows: (1) 55–75 years; (2) postmenopausal osteoporosis; (3) features of chronic hepatitis or cirrhosis diagnosed by ultrasonography and/or computed tomography; (4) positive for anti-HCV and HCV-RNA or hepatitis B surface antigens (HBsAg); (5) negative for antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; (7) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Diagnosis of osteoporosis was based on bone mineral density (AP spine by dual-energy X-ray absorptionetry) less than 2 SD of young adult mean and/or X-ray evidence of vertical trabecular loss. Patients with either of the following criteria were excluded from the study: (1) malignant tumor, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, and (3) a short follow-up period of 6 months or less. The patients in the etidronate-group received 200 mg of cyclical etidronate orally once a day for 2 weeks, followed by a 10-week period without cyclical etidronate.

In the same period, 1,960 women with osteoporosis and chronic liver disease were not treated with bisphosphonate, steroids or hormone replacement therapy. These patients were followed after exercise and taking calcium-rich foods or drugs for osteoporosis. The 1,215 of these 1,960 patients were considered with seven inclusion criteria and three exclusion criteria described in ethidronate group. Four hundred subjects in the control group were selected from these 1,215 patients by matching 1:5 with etidronate-group for age. The differences of the cumulative appearance rate of bone fracture in the etidronate-group and control group were compared. Next, predictive factors for bone fracture in both groups were assessed. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or the patients' family, who gave their informed consent for participation. This study had been approved by Institutional Review Board of Toranomon hospital.

### Viral Markers of HCV and HBV

Diagnosis of HCV infection was based on detection of serum HCV antibody and RNA. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., NJ). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored −80°C before the time of diagnosis of osteoporosis.

### Follow-Up

Patients were followed-up monthly to tri-monthly after the diagnosis of osteoporosis in the Toranomon hospital. Physical examination and biochemical tests were conducted at each examination together with a regular check-up using abdominal ultrasonography and/or computed tomography imaging in each patient. When a patient had any symptoms in relation to bone fracture, the physicians in charge further explored the possibility that patient having bone fracture. Forty-seven patients were lost to follow-up. Because the appearance of bone fracture and death was not identified in these 47 patients, they considered as censored data in statistical analysis [Harrington and Fleming, 1983].

### Statistical Analysis

Nonparametric procedures were employed for the analysis of background features of the patients, including the Mann–Whitney *U*-test. The cumulative appearance rate of bone fracture was calculated from the time of diagnosis of osteoporosis by using the Kaplan–Meier method. Differences in the development of bone fracture were tested using the log rank test. Independent factors associated with the incidence rate of bone fracture were analyzed by the Cox proportional hazard model. The following nine variables were analyzed for potential covariates for incidence of bone fracture at the time of diagnosis of osteoporosis at our hospital: age, state of liver disease (chronic hepatitis or liver cirrhosis), platelet count, albumin level, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), hepatitis virus, and etidronate therapy. A $P$ value of less than 0.05 in two-tailed test was considered significant. Data analysis was performed using the computer program SPSS version 11.0.

## RESULTS

### Patients' Characteristics

Table I shows the characteristics of the 480 women with postmenopausal osteoporosis and chronic liver disease. There were no significant differences in clinical profiles between the etidronate- and the control group.

TABLE I. Clinical Characteristics at the Time of Diagnosis of Osteoporosis[a]

| | Total | Etidronate-group | Control group | *P*-Value |
|---|---|---|---|---|
| N | 500 | 80 | 400 | |
| Age (years) | 61.0 ± 6.6 | 61.0 ± 6.9 | 61.0 ± 6.6 | 1.0 |
| Chronic hepatitis/liver cirrhosis | 370/130 | 59/21 | 291/109 | 0.319 |
| HBV/HCV | 82/398 | 10/70 | 72/328 | 0.860 |
| AST (IU/L) | 88.6 ± 71.6 | 90.8 ± 93.1 | 85.5 ± 120.3 | 0.234 |
| ALT (IU/L) | 102.0 ± 90.8 | 108.9 ± 105.5 | 100.6 ± 120.3 | 0.272 |
| Albumin (g/dl) | 4.1 ± 0.4 | 3.9 ± 0.5 | 4.1 ± 0.5 | 0.680 |
| γGTP (IU/L) | 51.4 ± 49.6 | 52.4 ± 51.1 | 50.2 ± 49.2 | 0.989 |
| Platelet count ($\times 10^4$ mm$^{-3}$) | 21.2 ± 18.5 | 19.5 ± 18.1 | 21.5 ± 18.6 | 0.556 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus.
[a]Data are number of patients or mean ± SD.

Three patients treated with etidronate had gastrointestinal episodes. However, they could continue treatment using etidronate. The observation period (mean ± SD) was 8.1 ± 3.5 years.

### Incidence of Bone Fracture

Fifty out of four hundred and eighty patients developed bone fracture. Thirty patients had vertebral fracture alone and nine patients had hip fracture alone. Three patients had both vertebral and hip fractures. Remaining eight patients had bone fractures except vertebral or hip fracture. The cumulative appearance rate of bone fracture was 3.7% at fifth year and 12.2% at 10th year in all the patients (Fig. 1). Four patients in etidronate-group and 46 in control group developed bone fracture. The 10th year cumulative appearance rates of bone fracture were 4.9% in etidronate-group and 13.8% in control group.

### Determinants of Incidence of Bone Fracture

Table II shows the factors associated with the incidence of a total of bone fracture in all the 480 women

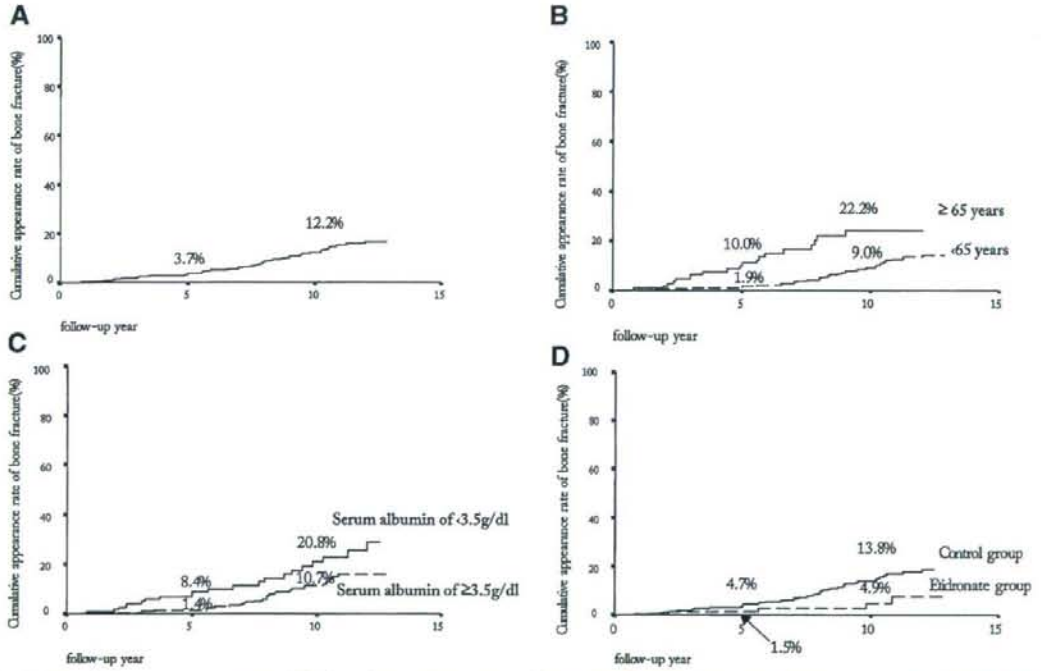


Fig. 1. Cumulative appearance rate of the bone fracture in women with osteoporosis and chronic liver disease. **Panel A**: Cumulative appearance rate of the bone fracture in a total of patients. **Panel B**: Cumulative appearance rate of the bone fracture based to difference of age (solid line, patients with ≥65 years; dotted line, patients with <65 years). **Panel C**: Cumulative appearance rate of the bone fracture based to difference of serum albumin level (solid line, patients with serum albumin level of <3.5 g/dl; dotted line, patients with serum albumin level of ≥3.5 g/dl). **Panel D**: Cumulative appearance rate of the bone fracture based to difference of treatment (solid line, controlled group; dotted line, etidronate-group).

TABLE II. Predictive Factors for a Total of Bone Fracture Development[a]

| Factor | Category | Odds ratio | 95% CI | *P*-Value |
|---|---|---|---|---|
| Univariate analysis | | | | |
| Age (years) | <65/≥65 | 1/2.95 | 1.65–5.24 | <0.001 |
| Albumin (g/dl) | <3.5/≥3.5 | 1/0.49 | 0.27–0.88 | 0.016 |
| Liver cirrhosis | −/+ | 1/1.86 | 1.04–3.32 | 0.036 |
| Etidronate | −/+ | 1/0.61 | 0.37–1.02 | 0.057 |
| AST (IU/L) | <76/≥76 | 1/0.49 | 0.22–1.08 | 0.076 |
| Platelet ($\times 10^4$ mm$^{-3}$) | <15/≥15 | 1/0.55 | 0.24–1.29 | 0.169 |
| ALT (IU/L) | <100/≥100 | 1/0.63 | 0.29–1.38 | 0.250 |
| Virus marker | HBV/HCV | 1/1.51 | 0.38–5.96 | 0.560 |
| γGTP (IU/L) | <110/≥110 | 1/0.78 | 0.19–3.28 | 0.734 |
| Multivariate analysis | | | | |
| Age (years) | <65/≥65 | 1/2.94 | 1.58–5.45 | 0.001 |
| Albumin (g/dl) | <3.5/≥3.5 | 1/0.48 | 0.26–0.87 | 0.016 |
| Etidronate | −/+ | 1/0.58 | 0.35–0.97 | 0.039 |

[a]ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus.

with postmenopausal osteoporosis and chronic liver disease. Univariate analysis identified the following four factors that influenced incidence of bone fracture: age ($P < 0.001$), serum albumin level ($P = 0.004$), liver staging ($P = 0.036$), and etidronate ($P = 0.057$). These four parameters were entered into multivariate Cox proportional hazard analysis. The cumulative appearance rate of bone fracture decreased with statistical significance in the following cases: (1) patients <65 years ($P < 0.001$), (2) patients who had serum albumin level of ≥3.5 g/dl ($P = 0.003$), and (3) patients who were given cyclic etidronate therapy ($P = 0.020$).

In the case of vertebral fracture, the cumulative appearance rate of bone fracture decreased with statistical significance in the following cases: (1) patients <65 years, (2) patients who had serum albumin level of ≥3.5 g/dl, and (3) patients who were treated with cyclic etidronate therapy (Table III). In the case of hip fracture, the cumulative appearance rate of bone fracture decreased with statistically significant in the patients without liver cirrhosis by the multivariate Cox proportional hazard analysis (Table IV).

### Mortality and Causes of Death After Bone Fracture

During the observation period after episode of bone fracture, 24 of the 50 patients died. Eight patients died of liver-related disease (HCC, decompensated liver cirrhosis, rupture of esophageal varices). On the other hand, 16 patients died of infection and aggravation of general condition. In these 24 died after bone fracture, liver-related death corresponded to 33.3% (8/24) of all deaths. The cumulative survival probability after bone fracture is shown in Figure 2. The cumulative survival probability after episode of bone fracture was 82.2% at the second year, and 57.6% at the fifth year in all.

## DISCUSSION

The incidence of bone fracture in postmenopausal women with osteoporosis and chronic liver disease are described. The present study was limited by a retrospective cohort trial in postmenopausal women with osteoporosis and chronic liver disease. Postmenopausal women of 55–75 years with osteoporosis were selected. The reason was as follows: (1) onset of bone

TABLE III. Predictive Factors for Vertebral Bone Fracture Development[a]

| Factor | Category | Odds ratio | 95% CI | *P*-Value |
|---|---|---|---|---|
| Univariate analysis | | | | |
| Age (years) | <65/≥65 | 1/3.01 | 1.47–6.18 | 0.003 |
| Albumin (g/dl) | <3.5/≥3.5 | 1/0.37 | 0.18–0.78 | 0.009 |
| Liver cirrhosis | −/+ | 1/1.86 | 1.04–3.32 | 0.036 |
| Etidronate | −/+ | 1/0.37 | 0.14–1.01 | 0.051 |
| Platelet ($\times 10^4$ mm$^{-3}$) | <15/≥15 | 1/0.43 | 0.15–1.21 | 0.110 |
| ALT (IU/L) | <100/≥100 | 1/0.55 | 0.19–1.53 | 0.247 |
| AST (IU/L) | <76/≥76 | 1/0.63 | 0.42–1.95 | 0.288 |
| γGTP (IU/L) | <110/≥110 | 1/0.81 | 0.19–4.86 | 0.670 |
| Virus marker | HBV/HCV | 1/1.10 | 0.14–5.64 | 0.930 |
| Multivariate analysis | | | | |
| Albumin (g/dl) | <3.5/≥3.5 | 1/0.38 | 0.18–0.81 | 0.012 |
| Age (years) | <65/≥65 | 1/2.48 | 1.16–5.31 | 0.020 |
| Etidronate | −/+ | 1/0.34 | 0.13–0.93 | 0.035 |

[a]ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus.

TABLE IV. Predictive factors for hip bone fracture development[a]

| Factor | Category | Odds ratio | 95% CI | *P*-Value |
|---|---|---|---|---|
| Univariate analysis | | | | |
| Liver cirrhosis | −/+ | 1/3.25 | 1.09–9.67 | 0.034 |
| Age (years) | <65/≥65 | 1/2.75 | 0.95–7.98 | 0.063 |
| Platelet ($\times 10^4$ mm$^{-3}$) | <15/≥15 | 1/0.60 | 0.31–3.36 | 0.310 |
| AST (IU/L) | <76/≥76 | 1/0.53 | 0.32–1.95 | 0.376 |
| Albumin (g/dl) | <3.5/≥3.5 | 1/0.60 | 0.19–1.89 | 0.382 |
| Etidronate | −/+ | 1/0.68 | 0.40–1.81 | 0.474 |
| Virus marker | HBV/HCV | 1/1.76 | 0.14–5.49 | 0.506 |
| γGTP (IU/L) | <110/≥110 | 1/0.65 | 0.35–2.75 | 0.600 |
| ALT (IU/L) | <100/≥100 | 1/0.66 | 0.13–3.43 | 0.623 |
| Multivariate analysis | | | | |
| Liver cirrhosis | −/+ | 1/3.25 | 1.09–9.67 | 0.034 |
| Age (years) | <65/≥65 | 1/2.82 | 0.94–8.46 | 0.064 |

[a]ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus.

fracture based on osteoporosis is rare in young female with <55 years and/or male, (2) the rate of patients with >75 years at the time diagnosing osteoporosis is small. Other limitations are the followings: (1) the control patients were not matched with patients treated with etidronate by bone density measurement, (2) serum levels of vitamin D were not measured, and (3) bone density measurement were not followed.

However, there are several findings with regard to bone fracture in postmenopausal women with

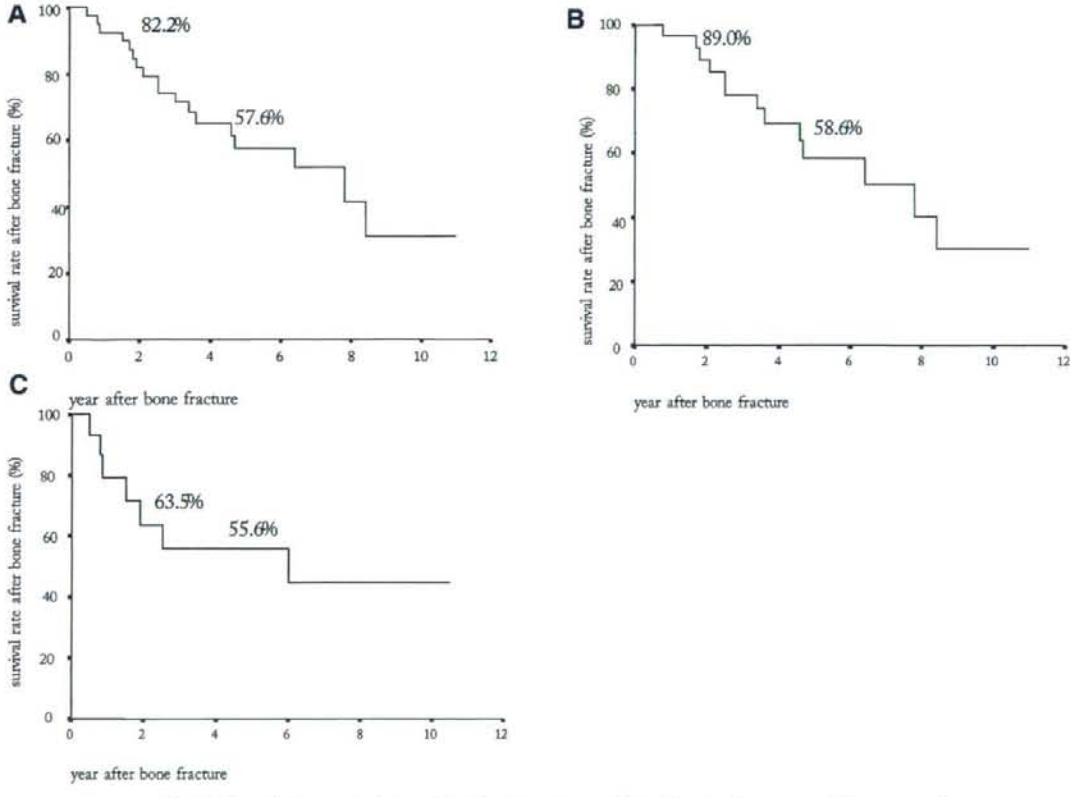


Fig. 2. Cumulative survival rate after the appearance of bone fracture in women with osteoporosis and chronic liver disease. **Panel A**: Cumulative survival rate after the appearance of bone fracture in a total of patients. **Panel B**: Cumulative survival rate after the appearance of hip bone fracture. **Panel C**: Cumulative survival rate after the appearance of vertebral bone fracture.

osteoporosis and chronic liver disease. First, the annual development rate of bone fracture among female patients with osteoporosis and chronic liver disease was one percent without treatment of osteoporosis.

Second, the appearance rate of bone fracture among postmenopausal women with osteoporosis and chronic liver disease was low with statistical significance in patients who had serum albumin of $\geq$3.5 g/dl and/or who were given intermittent cyclic therapy with etidronate. These results indicate that good nutrition and treatment using bisphosphonate for osteoporosis reduce the development of bone fracture in postmenopausal women with osteoporosis and chronic liver disease. In the case of vertebral fracture, good nutrition and etidronate therapy reduced the development of fractures. In the case of hip fracture, cirrhosis enhanced hip fracture. This result suggests that cyclical etidronate therapy could reduce significantly vertebral fracture compared to hip fracture.

Third, bone fracture reduced the survival rate. The survival rate after episodes of bone fracture was poor. About half of the postmenopausal women with osteoporosis died during the fifth year after the bone fracture. In patients who died, liver-related death corresponded to one-third. The remaining patients died of infection, aggravation of general conditions.

Recent studied have reported that osteodystrophy occurs not only in patients with alcoholic cirrhosis, but also in those with cirrhosis induced by hepatitis C or B virus. Due to improved treatment, patients with cirrhosis are living longer; an increasing proportion of such patients are found to have bone disease [Tsuneoka et al., 1996]. Intermittent cyclical therapy with etidronate has been reported to be increasing bone density and reducing the incidence of new vertebral fractures in postmenopausal women with osteoporosis [Fujita et al., 2007]. Although the potency of etidronate to inhibit bone resorption is relatively weak among some bisphosphonates, prolonged treatment with etidronate was reported to be effective, safe and well-tolerated. In the present study, none of the patients stopped the treatment due to adverse events.

In conclusion, the present retrospective study is the first to determine the annual incidence of bone fracture among postmenopausal women with osteoporosis and chronic liver disease in about 1% without treatment for osteoporosis. A serum albumin level of $\geq$3.5 g/dl and cyclic etidronate treatment reduce the development of bone fracture in postmenopausal women with osteoporosis and chronic liver disease.

## ACKNOWLEDGMENTS

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***Original Article***

# Potential of laparoscopy in chronic liver disease with hepatitis B and C viruses

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*Aim:* The definitive diagnosis of chronic liver disease is made either by a histological examination of a biopsy specimen or upon visualization of the liver surface at laparoscopy. The aim of this retrospective cohort study is to assess whether histological or laparoscopic findings are associated with hepatocellular carcinoma (HCC) development.

*Methods:* A retrospective review of paired laparoscopy and histology reports was performed on 4124 hepatitis virus-positive patients who underwent laparoscopy: 2804 patients had hepatitis C virus (HCV group) and 1320 patients had hepatitis B virus (HBV group). Based on the irregularities of the liver surface, the laparoscopic findings were classified into three groups in progression order: smooth, irregular, or nodular. The histological findings were classified according to the extent of fibrosis into four stages (stages 1–4) in progression order.

*Results:* The number of patients with HCC development was 565 in the HCV group and 115 in the HBV group. The Coxregression hazard model showed that HCC appearance in the HCV group was independently associated with laparoscopic findings (relative risk based on every progression of one rank [RR], RR = 4.31, $P < 0.0001$) and histological findings (RR = 2.56, $P < 0.0001$). In the HBV group, however, HCC appearance in was mainly associated with laparoscopic findings (RR = 2.12, $P < 0.0001$) compared to histological findings (RR = 1.13, $P = 0.403$).

*Conclusion:* Our data indicate that laparoscopic findings of the liver are dominant predictors for HCC development compared with histological findings in patients with HBV.

**Key words:** hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, laparoscopy, liver biopsy

## INTRODUCTION

HEPATITIS C VIRUS (HCV) or hepatitis B virus (HBV) is one of the common causes of chronic liver disease in the world. Chronic hepatitis C or B infection can be associated with progressive liver disease that may evolve insidiously to cirrhosis.[1–4] In addition, HCV or HBV is a major risk for hepatocellular carcinoma (HCC).[5–9] HCC is one of the major causes of death, especially in Asian countries. It is necessary for physicians to make an accurate diagnosis for the management for chronic hepatitis. A definitive diagnosis of chronic liver disease in patients with hepatitis B or C is important in the prognosis and management of patients. The definitive diagnosis of chronic liver disease is made either by a histological examination of a biopsy specimen or upon visualization of the liver surface at laparoscopy.

Keeling revolutionised investigations of liver disease using the original description of laparoscopy in 1923.[10] Laparoscopy-guided liver biopsy, however, is considered by many to be the most accurate method of diagnosing liver disease, especially liver cirrhosis.[11–16] However, the use of laparoscopy as a diagnostic tool in liver disease has decreased over the past decade.[17,18] The reason for decreasing laparoscopic examinations is that there are misconceptions about the overall safety and complication rate. The use of laparoscopy is generally more complex than that of ultrasonography (US)-guided biopsy.

With this in mind, the present cohort study aimed to compare the accuracy of liver descriptions made during laparoscopy with histological reports from biopsies in patients with chronic viral hepatitis. At the same time,

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we assessed whether laparoscopic or histological findings are associated with HCC appearance in patients with HCV or HBV.

## METHODS

### Patient population

THE NUMBER OF patients who were diagnosed by using both laparoscopy and histology between April 1985 and April 2000 in the Department of Hepatology, Toranomon Hospital (Tokyo, Japan) was 6640. Of these, 4124 patients met the following criteria: (i) positive for HCV-RNA or hepatitis B surface antigens (HBsAg); (ii) negative for antinuclear antibodies or antimitochondrial antibodies in the serum, as determined by radioimmunoassay or spot hybridization; (iii) no history of treatment with corticosteroids, immunosuppressive agents, or antiviral agents; (iv) no evidence of HCC nodules as shown by US and/or computed tomography (CT); and (v) macroscopic examination and classification by three laparoscopy experts (YA, KI, or HK) who have performed laparoscopy-guided biopsies of >1000 episodes. Patients with either of the following criteria were excluded from the study: (i) α-fetoprotein of 400 ng/mL or higher; (ii) positive for both HCV-RNA and HBsAg; and (iii) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites. The 2806 patients with HCV-RNA and without HBsAg were regarded as HCV group. The 1302 patients with HBsAg and without HCV-RNA were regarded as the HB group. The physicians in charge explained the purpose and method of the laparoscopic-guided liver biopsy to each patient and/or patients' family, who gave their informed consent for participation. This study was approved by the Institutional Review Board of our hospital. Written informed consent was obtained from all patients before the procedure commenced.

### Laparoscopy

Abdominal US, electrocardiogram, and a chest X-ray were performed before laparoscopy. The patients were deprived of food and water at the day of examination. Thirty minutes before exploration, 50 mg pethidin and 0.5 mg atropine were injected intramuscularly. If necessary, patients received sedativa or analgetics during the laparoscopic intervention. During laparoscopy, each patient was given continuously an isotonic electrolyte solution intravenously. Patients were monitored by pulsoxymetry and blood pressure manometer. After local anesthesia, the pneumoperitoneum was installed by puncturing at Kalk's point with the Verres needle followed by insufflation of 2–3 L nitrous oxide. After insertion of the laparoscope in a trocar with a safety shield at Kalk's point, macroscopic exploration of liver followed. Liver biopsies were taken generally from an area on the anterior surface of the right lobe of the liver using a Silbermann needle at least 3–4 cm from the liver edge, containing at least five portal areas. After laparoscopy-guided liver biopsies, hemostasis was achieved through gelatin sponge (Gelform, Nipponkayaku, Tokyo, Japan) placement to the biopsy site.

Based on the irregularities of the liver surface, the laparoscopic findings were classified into three groups in progression order: smooth (an essentially smooth liver surface or with limited areas of depression), irregular (a liver surface showing increased numbers of interconnected depressions, possibly resembling ripples or speck), and nodular (a liver surface with nodular formations) as shown Figure 1.

### Histopathological evaluation

Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of the specimens for the examination was more than six portal areas. Histopathological interpretation of specimens was made by experienced liver pathologists who had no clinical information. Baseline liver histology of chronic hepatitis was classified according to the extent of fibrosis into four stages in progression order: stage 1, periportal expansion; stage 2, portoportal septa; stage 3, portocentral linkage or bridging fibrosis; and stage 4, liver cirrhosis.[19]

### Viral markers of HCV and HBV

The diagnosis of HCV infection was based on the detection of the serum HCV antibody and positive RNA. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV monitor test version 2.0, Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The used serum samples were stored −80°C at the time of the laparoscopic examination.

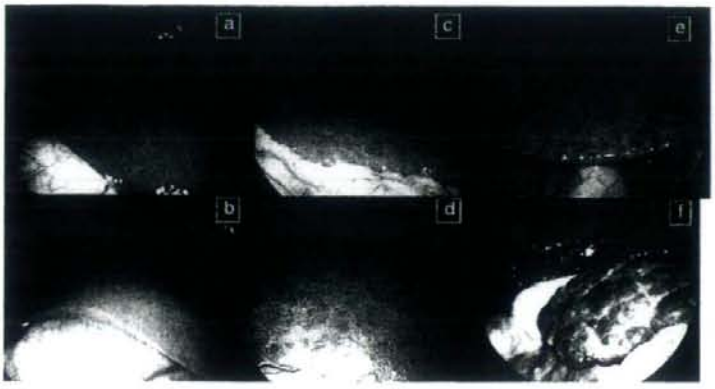


**Figure 1** Based on the irregularities of the liver surface, laparoscopic findings were classified into three groups in progression order: smooth, an essentially smooth liver surface or with limited areas of depression (a,b); irregular, a liver surface showing increased numbers of interconnected depressions, possibly resembling ripples or specks (c,d); and nodular, a liver surface showing nodular formations with or without specks (e,f).

### Follow up

Patients were followed up monthly to tri-monthly after the first medical examination at our hospital. The physical examinations and biochemical tests were conducted at each examination together with regular check-ups using abdominal CT or US imaging in each patient. Three hundred and nineteen patients were lost to follow up. Because the appearance of HCC and death was not identified in these 319 patients, they wee considered as censored data in the statistical analysis.[20] Moreover, the patients treated with antivirus drugs were regarded as withdrawals at the time of starting the antivirus drugs. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on CT and US. A microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical HCC image.

### Statistical analysis

Non-parametric procedures were employed for the analysis of background features of the patients, including the Mann–Whitney *U*-test and $\chi^2$-test. The cumulative appearance rate of HCC was calculated from the time of the laparoscopy examination to the appearance of HCC, using the Kaplan–Meier method. Differences in the development of HCC were tested using the log–rank test. We analyzed whether laparoscopic or histological findings were associated with the incidence rate of HCC by the Cox proportional hazard model. A *P*-value of less than 0.05 in the two-tailed test was considered significant. Data analyses were performed using the SPSS computer program version 11.0 (SPSS, Chicago, IL, USA).

## RESULTS

### Patients' characteristics

THE CHARACTERISTICS OF the 4124 patients with HCV or HBV are shown in Table 1. These patients comprised of 2804 with HCV infection (HCV group) and 1320 with HBV infection (HBV group). There were significant differences in many backgrounds between the two groups as shown in Table 1. The number of patients with HCC development was 565 in the HCV group and 115 in the HBV group.

### Relationship between laparoscopic findings and histological stage

The relationship between the laparoscopic findings and histological stage in the HCV group are shown in Table 2. Almost all the patients with a smooth liver surface had stage 1 or 2; 89.9% (879/981) of patients with an irregular liver surface had stage 2 or 3; and although patients with a nodular liver surface had mainly stage 4, 21.8% (71/326) of these patients had stage 1, 2, or 3.

The relationship between the laparoscopic findings and histological stage in the HBV group are shown in Table 3. Almost all the patients with a smooth liver surface had stage 1 or 2; 88.7% (361/407) of patients with an irregular liver surface had stage 2 or 3; and although 58.5% (120/206) of patients with a nodular liver surface had mainly stage 4, approximately 40% of these patients had stage 1, 2, or 3. The incidence of stage 4 in HBV patients with a nodular surface was smaller than that in HCV patients ($P < 0.0001$).

**Table 1** Clinical characteristics at laparoscopy and liver biopsy†

| | HBV group | HCV group | *P*-value |
|---|---|---|---|
| *n* | 1320 | 2804 | |
| Age | 36.6 ± 10.5 | 51.1 ± 11.6 | <0.001 |
| Sex (male percentage) | 76.6% (1011) | 57% (1597) | <0.001 |
| AST (IU/L) | 113.5 ± 154.9 | 89.5 ± 102.2 | <0.001 |
| ALT (IU/L) | 194.7 ± 255.7 | 130.1 ± 145.7 | <0.001 |
| Total bilirubin (mg/dL) | 0.82 ± 0.45 | 0.84 ± 0.43 | 0.553 |
| γ-GTP (IU/L) | 64.8 ± 82.9 | 98.2 ± 83.1 | <0.001 |
| Platelet count ($\times 10^4/mm^3$) | 15.6 ± 12.6 | 20.3 ± 17.4 | <0.001 |

†Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidases; HBV, hepatitis B virus; HCV, hepatitis C virus.

## Cumulative appearance rates of HCC

The cumulative development rates of HCC based on the histological findings in the HCV group are shown in Figure 2. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HCV group are shown in Figure 3. In the patients with laparoscopic findings of an irregular liver surface, the HCC development rates differed due to differences in the histological findings. However, in the patients with laparoscopic findings of a smooth or nodular liver surface, the HCC development rates were not significantly different due to difference in the histological findings.

The cumulative development rates of HCC based on the histological findings in HBV group are shown in Figure 4. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to difference in laparoscopic findings. However, in patients with stage 3 or 4, HCC development rates were not statistically different in spite of the differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HBV patients are shown in Figure 5. In the patients with the same laparoscopic findings, the HCC development rates did not differ due to differences in the histological findings.

HCC appearance rates based on laparoscopic and histological findings were evaluated by the Cox propor-

**Table 2** Relationship between laparoscopic findings and histological stage in patients with chronic type C hepatitis

| Laparoscopic finding | Histological stage | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Total |
| Smooth | 1390 (92.7%) | 106 (7.1%) | 4 (0.3%) | 0 | 1500 |
| Irregular | 65 (6.6%) | 631 (64.5%) | 248 (25.4%) | 37 (3.8%) | 981 |
| Nodular | 3 (0.9%) | 16 (4.9%) | 52 (16.0%) | 255 (78.2%) | 326 |
| Total | 1458 | 753 | 304 | 292 | 2807 |

**Table 3** Relationship between laparoscopic findings and histological stage in patients with chronic type B hepatitis

| Laparoscopic findings | Histological stage | | | | |
|---|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Total |
| Smooth | 645 (91.2%) | 61 (8.6%) | 1 (0.1%) | 0 | 707 |
| Irregular | 40 (9.8%) | 275 (67.6%) | 86 (21.1%) | 6 (1.5%) | 407 |
| Nodular | 4 (1.9%) | 20 (9.7%) | 62 (30.1%) | 120 (58.5%) | 206 |
| Total | 693 | 352 | 159 | 126 | 1320 |

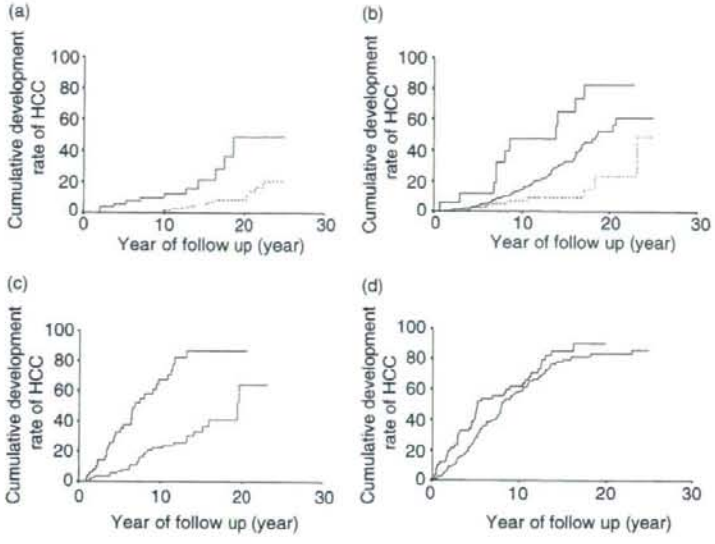


**Figure 2** Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings of hepatitis C virus patients. (a) Patients with stage 1 ($P < 0.0001$); (b) patients with stage 2 ($P < 0.0001$); (c) patients with stage 3 ($P < 0.0001$, $P = 0.055$); (d) patients with stage 4 ($P = 0.081$). (—) Nodular, (—) Irregular, (- - -) Smooth.

tional hazard model as shown in Tables 4 and 5. The multivariate Cox regression hazard model using two factors of laparoscopic and histological findings showed that HCC development in the HCV group were independently associated with laparoscopic findings (relative risk based on every progression of one rank [RR], RR = 4.31, $P < 0.0001$) and histological findings (RR = 2.56, $P < 0.0001$). In the HBV group, however, HCC development in was mainly associated with laparoscopic findings (RR = 2.12, $P < 0.0001$) compared to histological finding (RR = 1.13, $P = 0.403$).

The multivariate analysis showed that both laparoscopic and histological findings were associated with HCC appearance in the HCV group. However, in the HBV group, laparoscopic findings were important predictors compared to histological findings.

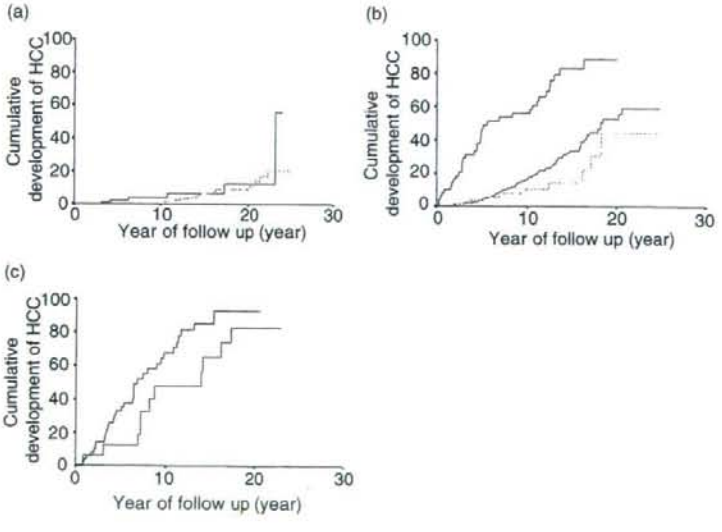


**Figure 3** Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis C virus patients. (a) Patients with smooth liver surface ($P < 0.391$); (b) patients with irregular liver surface ($P < 0.0001$); (c) patients with nodular liver surface ($P < 0.055$). (—) Stage 4, ( ) Stage 3, (—) Stage 2, (- - -) Stage 1.

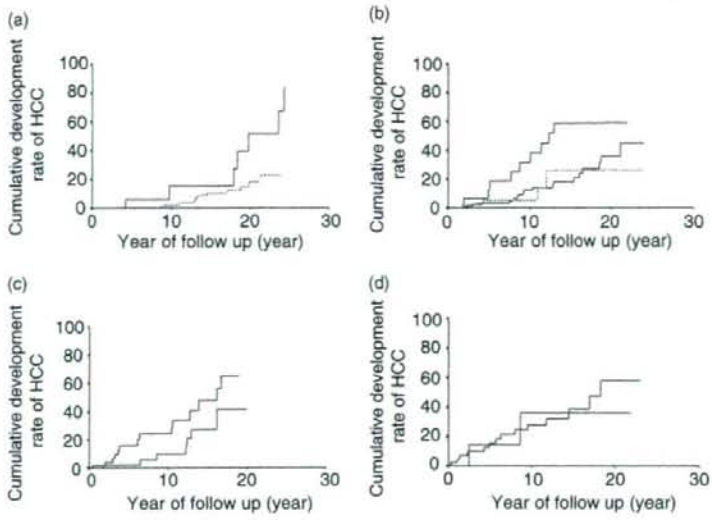


**Figure 4** Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings in hepatitis B virus patients. (a) Patients with stage 1 ($P < 0.013$); (b) patients with stage 2 ($P < 0.016$); (c) patients with stage 3 ($P < 0.116$); (d) patients with stage 4 ($P < 0.858$). (—) Nodular, (—) Irregular, (—) Smooth.

## Adverse event

Seven patients had the following major operative complications: pneumothorax ($n = 6$) and shock due to vasovagal reflux ($n = 1$) at the time of liver biopsy. There were no complications of bleeding at the site of the liver biopsy.

## DISCUSSION

WE HAVE DESCRIBED the difference of laparoscopic and histological findings in patients with HCV or HBV. The present study was limited by a retrospective cohort trial. Other limitations of the study were

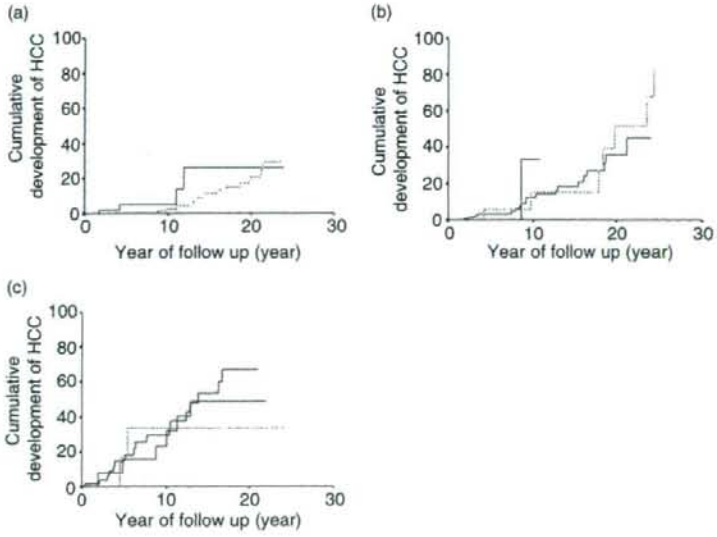


**Figure 5** Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis B virus patients. (a) Patients with smooth liver surface ($P < 0.252$); (b) patients with irregular liver surface ($P < 0.736$); (c) patients with nodular liver surface ($P < 0.785$). (—) Stage 4, ( ) Stage 3, (—) Stage 2, (—) Stage 1.

**Table 4** Predictive factors for hepatocellular development in hepatitis C virus group

| Factor | Category | Risk ratio | 95% CI | *P*-value |
|---|---|---|---|---|
| Univariate analysis | | | | |
| Histological findings | 1: stage 1<br>2: stage 2<br>3: stage 3<br>4: stage 4 | 2.56 | 2.34–2.79 | <0.0001 |
| Laparoscopic findings | 1: smooth<br>2: irregular<br>3: nodular | 4.31 | 3.74–4.97 | <0.0001 |
| Multivariate analysis | | | | |
| Histological findings | 1: stage 1<br>2: stage 2<br>3: stage 3<br>4: stage 4 | 1.63 | 1.39–1.92 | <0.0001 |
| Laparoscopic findings | 1: smooth<br>2: irregular<br>3: nodular | 2.31 | 1.78–2.99 | <0.0001 |

Risk ratio, relative risk based on every progression of one rank in histological and laparoscopic findings. CI, confidence intervals.

that clinical backgrounds of the HCV- and HBsAg-positive patients were significantly different in many factors and the physicians had different experiences on the diagnosis of chronic liver disease. The macroscopic classification by the use of the laparoscopic examination may be subjective, which introduces a further bias; this heterogeneity makes it slightly difficult to interpret the results of the study.

However, the features of the present study are the large study population and prolonged observation study. Moreover, based on irregularities of the liver surface, the laparoscopic findings were classified into only three groups to minimize the subjective bias. The present study shows several findings with regard to laparoscopic and histological examinations in HCV- or HBV-positive patients. First, some patients with a nodular liver surface

**Table 5** Predictive factors for hepatocellular development in hepatitis B virus group

| Factor | Category | Risk ratio | 95% CI | *P*-value |
|---|---|---|---|---|
| Univariate analysis | | | | |
| Histological findings | 1: stage 1<br>2: stage 2<br>3: stage 3<br>4: stage 4 | 1.72 | 1.43–2.07 | <0.0001 |
| Laparoscopic findings | 1: smooth<br>2: irregular<br>3: nodular | 2.29 | 1.79–2.92 | <0.0001 |
| Multivariate analysis | | | | |
| Histological findings | 1: stage 1<br>2: stage 2<br>3: stage 3<br>4: stage 4 | 1.13 | 0.85–1.49 | 0.403 |
| Laparoscopic | 1: smooth<br>2: irregular<br>3: nodular | 2.12 | 1.44–3.14 | <0.0001 |

Risk ratio, relative risk based on every progression of one rank in histological and laparoscopic findings. CI, confidence intervals.

were not diagnosed as having liver cirrhosis when only histological samples were used; in the HBV patients, approximately 45% of patients with a nodular liver surface were not diagnosed as having liver cirrhosis when only histological samples were used. These results suggest that HBV-positive patients with a nodular liver surface tend to have a sampling error compared to HCV-positive patients. A typical liver biopsy represents approximately 1/50 000 of the entire liver surface.[16] Based on this information, we presume that a significant sampling error will be found when diagnosis is based on a single, blind liver biopsy as reported previously.[12–15] Seven patients with a nodular surface were diagnosed as having stage 1, histologically. These seven patients had macro nodular liver surface laparoscopically and showed a part of septa of liver cirrhosis, histologically.

Second, cumulative HCC appearance rates based on the difference of liver surface were more accurate than those based on the difference of the histological findings; this was particularly the case for the HBV patients. In HBV-positive patients with the same histological findings of stage 1, 2, or 3, the HCC development rates differed due to differences in the laparoscopic findings. Using the Cox proportional hazard model, laparoscopic findings were important predictors for HCC development compared to histological findings in the HBV group. On the other hand, both laparoscopic and histological findings were important predictors for HCC development in the HCV group.

Third, there were no complications of bleeding at the biopsy site in our series of 4106 laparoscopies. On the other hand, five of approximately 4100 patients who received US-guided liver biopsy between 1985 and 2000 had bleeding at the biopsy site. The reason for good hemostasis in laparoscopy-guided biopsies is as follows; (i) operators could use gelatin sponge (Gelform) placement to the biopsy site; and (ii) operators could directly check the hemostasis. Laparoscopy offers the advantage of visualization of the liver surface, which leads to greater success in the diagnosis of chronic liver disease than biopsy alone. Although diagnostic laparoscopy is carried out to make an accurate diagnosis of chronic liver disease, the use of laparoscopy accompanies some severe complications. Thus laparoscopy should be examined under the experienced physicians given many training. Moreover, when patients have contraindications, the physician in charge should avoid doing laparoscopy-guided liver biopsy.

The present study suggests that laparoscopic findings are superior to histological findings for predicting HCC development in HBV patients. Based on our results, we recommend that laparoscopy should be considered in the evaluation of chronic liver disease for patients with HBV. When the patients with HBV or HCV are diagnosed without laparoscopic examination, the physician in charge should constantly consider the possibility of cirrhosis and HCC appearance.

In conclusion, our data indicate that laparoscopic findings of the liver are dominant predictors for HCC development compared to histological findings in patients with HBV.

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