

Table 2. Predictive Factors for SVR in Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<50 / ≥50	1/0.09	0.01-0.97	.047
AST (IU/L)	≥76 / <76	1/0.46	0.18-1.17	.102
Age (years)	<60 / ≥60	1/0.22	0.04-1.42	.112
Platelet(10 ⁴ /mm ³)	<10 / ≥10	1/3.00	0.57-15.76	.306
WBC(10 ³ /mm ³)	<4 / ≥4	1/2.33	0.46-11.81	.367
Sex	Male / Female	1/0.71	0.14-3.58	.682
ALT (IU/L)	<100 / ≥100	1/0.75	0.13-4.29	.746
Total dose of IFN (IU/L)	<200 / ≥200	1/1.29	0.23-7.05	.772
Period of IFN therapy (week)	6 / 8	1/1.19	0.20-6.99	.851

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, HCV: hepatitis C virus, IFN: interferon, WBC: white blood cell count.

*p value calculated by logistic regression analysis.

types known to exist in Japan, as reported previously (19).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, HCV RNA level, liver histology, biochemical factors (AST (aspartate aminotransferase), ALT) before IFN therapy and methods of IFN administration. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Results

Patients' characteristics

Table 1 shows the characteristics of the 25 patients who

had performed IFN therapy. Clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. All the patients were categorized as Child-Pugh-Turcotte score class A. Of the 25 patients originally included in this study, in five patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at the time of 1-3 weeks after the initiation of IFN. Thus, the total dose of IFN was 228.0±79.2 million units (MU). The median (range) leukocyte and platelet count in patients with dose reduction were 3.400 (3.100-4.800)/mm³ and 95.000 (8.8-11.4)/mm³, respectively, while those in patients without dose reduction were 4.600 (3.000-6.900)/mm³ and 120.000 (80.000-120.000)/mm³. Both leukocyte and platelet count in patients without dose reduction were higher than those in patients with dose reduction (leukocyte; p=0.013, platelet; p=0.011).

Efficacy of treatment

Out of twenty-five patients enrolled on present study, 14 patients (56.0%) had SVR by the intention-to-treat analysis.

Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR	Non-SVR	p value
Age (years old) †	7/7	2/9	0.183
(<60/≥60)			
Sex (male/female) †	8/6	5/6	0.647
Period of IFN therapy †	9/5	8/3	0.986
(6 week/8 week)			
Total dose of IFN (MU) † (<200/≥200)	5/9	5/6	0.698
HCV-load (KIU/mL) *	16 (8-69)	66 (23-98)	0.021
AST (IU/L) *	63 (39-203)	85 (53-141)	0.730
ALT (IU/L) *	75 (27-434)	88 (34-230)	0.557
Hemoglobin (g/dL) *	13.7 (10.1-16.3)	11.7 (9.7-16.1)	0.139
Platelet(10 ⁴ /mm ³) *	12.2 (8.7-17.0)	10.0 (8.0-16.0)	0.096
WBC(10 ³ /mm ³) *	4.0 (3.1-6.9)	3.8 (3.0-5.3)	0.841

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, SVR: sustained virologic response, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients,

‡ p value calculated by the Mann-Whitney U test.

The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL (Table 2). Based on the difference of serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. The leukocyte count was 4,320±1,370/mm³ and the platelet count was 119,000±23,000/mm³ before the initiation of IFN therapy, whereas the values were 2,670±830/mm³ and 71,000±17,000/mm³,

respectively, two weeks after the initiation of the therapy. None of the patients withdrew from this treatment due to IFN-related side effects.

Discussion

The present study was limited by non-randomized controlled trial. Another limitation of the study was that the number of the patients was small. However, several findings from the present study have direct implications for the short-term IFN treatment of LC patients with genotype 2a and low virus load.

First, more than 50% of patients cleared HCV RNA. This result indicates that the 6- or 8-week regimen of IFN therapy was preferable to eradicate HCV RNA in LC patients with genotype 2a and low virus load. Second, the patients with HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL. On the treatment

period, the efficacy of the 6-week regimen of IFN therapy was almost the same as that of the 8-week regimen. Moreover, the efficacy of the total dose of IFN of <200 MU was not different from that by the total dose of ≥ 200 MU. These results indicate that in about two-thirds of LC patients with a genotype 2a and serum HCV RNA level of <50 KIU/mL and low virus load, HCV was eradicated by the 6-week regimen or total dose of IFN of <200 MU.

Regarding the side effects of IFN, no patient withdrew the treatment due to IFN-related side effect. Okanoue et al (20) reported that side effects occurred when the daily IFN dose was increased. In the present study, five patients had to reduce the IFN dose due to IFN side effects. On the IFN therapy for LC patients, the physician in charge should check the clinical findings compared to the patients with chronic hepatitis C.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. However, prolonged

combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points of cost and side effect. Fortunately, in patients with low HCV-RNA levels, HCV RNA tends to be eradicated with a small dose of IFN (21-24). The present study indicates that in patients with a low HCV-RNA, HCV RNA can be eradicated with a small dose of IFN.

Conclusion

The present study indicates that the 6 or 8-week of IFN therapy is a possible selection of therapy for liver cirrhotic type C patients with genotype 2a and low virus load.

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References

- McHutchison JG, Poynard T, Pianko S, et al. The impact of interferon plus ribavirin on response to therapy in black patients with chronic hepatitis C. *Gastroenterology* **119**: 1317-1323, 2000.
- McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alpha-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* **339**: 1485-1492, 1998.
- Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha 2b plus ribavirin for 48 weeks or 24 weeks versus interferon alpha 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* **52**: 1426-1432, 1998.
- Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha 2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. *Lancet* **351**: 83-87, 1998.
- Schalm SW, Hansen BE, Chemello L, et al. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European Centers. *J Hepatol* **26**: 961-966, 1997.
- Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alpha-2a in patients with chronic hepatitis C. *N Engl J Med* **343**: 1666-1672, 2000.
- Lindsay KL, Treppe C, Heintges T, et al. Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alpha-2b to interferon alpha-2b as initial treatment for chronic hepatitis C. *Hepatology* **34**: 395-403, 2001.
- Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* **358**: 958-965, 2001.
- Tsubota A, Chayama K, Ikeda K, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology* **19**: 1088-1094, 1994.
- Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alpha therapy for chronic hepatitis C: A randomized, double blind placebo-controlled trial. *N Engl J Med* **321**: 1506-1510, 1989.
- Causse X, Godinot H, Chevallier M, et al. Comparison of 1 or 3 MU of interferon alpha-2b and placebo in patients with chronic non-A non-B hepatitis. *Gastroenterology* **101**: 497-502, 1991.
- Chayama K, Saitoh S, Arase Y, et al. Effect of interferon administration on serum hepatitis C virus RNA in patients with chronic hepatitis C. *Hepatology* **13**: 1040-1043, 1991.
- Reichard O, Glaumann H, Fryden A, et al. Two-year biochemical, virological and histological follow-up in patients with chronic hepatitis C responding in a sustained fashion to interferon alpha-2b treatment. *Hepatology* **21**: 918-922, 1995.
- Shiratori Y, Kato N, Yokosuka O, et al. Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. *Gastroenterology* **113**: 558-566, 1997.
- Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the non-structural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* **334**: 77-81, 1996.
- Ikeda K, Saitoh S, Kobayashi M, et al. Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical discriminant function using common laboratory data. *Hepatology* **18**: 252-266, 2000.
- Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* **36**: 862-865, 1998.
- Doglio A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebvre J. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. *J Clin Microbiol* **37**: 1567-1569, 1999.
- Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes; an investigation of type-specific differences in geographic origin and disease. *Hepatology* **19**: 13-18, 1994.
- Okanoue T, Sakamoto S, Itoh Y, et al. Side effects of high-dose interferon therapy for chronic hepatitis C. *J Hepatol* **25**: 283-291, 1996.
- Dalgard O, Mangia A. Short-term therapy for patients with hepatitis C virus genotype 2 or 3 infection. *Drugs* **66**: 1807-1815, 2006.
- Tabaru A, Narita R, Hiura M, Abe S, Otsuki M. Efficacy of short-term interferon therapy for patients infected with hepatitis C virus genotype 2a. *Am J Gastroenterol* **100**: 862-867, 2005.
- Sato Y, Tokue H, Kawamura N, et al. Short-term interferon ther-

apy for chronic hepatitis C patients with low viral load. Hepatogastroenterology 51: 968-972, 2004.
24. Fujiyama S, Chikazawa H, Honda Y, Tomita K. Effective inter-

feron therapy for chronic hepatitis C patients with low viral loads. Hepatogastroenterology 50: 817-820, 2003.

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Suitable Treatment Period in Patients with Virological Response during Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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Abstract

Objective The aim of this study was to determine the suitable treatment period in patients who achieve virological response during combination therapy of peginterferon and ribavirin for chronic hepatitis C virus infection.

Methods Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of ≥ 100 KIU/ml before treatment, and negativity of serum HCV RNA during treatment. The 366 patients were enrolled in this retrospective cohort study. Patients were classified into four groups according to difference of response: rapid-virological response (RVR) at week 4 after the initiation of treatment ($n=37$), early-virological response (EVR) at week 5-12 ($n=161$), late-virological response (LVR) at week 13-24 ($n=131$), and superlate-virological response (SLVR) at week 25-48 ($n=37$). A non-relapse in patients with undetectable HCV RNA during therapy was defined as clearance of HCV RNA 6 month after the cessation of therapy.

Results Of the 366 patients, 241 had non-relapse and the non-relapse rate in each group was 89% (33/37) in RVR, 79% (127/161) in EVR, 54% (71/131) in LVR, and 27% (10/37) in SLVR. In RVR, 26 of 27 patients with continuance of negative HCV RNA of ≥ 30 weeks during treatment had non-relapse. In EVR, patients with period of negative HCV RNA of ≥ 40 weeks had non-relapse rate of 90% (71/79). In LVR and SLVR, all nine patients with continuance of negative HCV RNA of ≥ 60 weeks had non-relapse.

Conclusion A suitable treatment period of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA.

Key words: chronic hepatitis C, peginterferon, ribavirin, suitable treatment period

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Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-7). Hence, combination therapy of peginterferon and ribavirin for HCV has been recommended as a first choice for chronic hepatitis C. During combination therapy, the serum

level of HCV RNA becomes negative in about 70 to 80% of patients with genotype 1 and a high virus load. However, about 20 to 30% of patients with negativity of HCV-RNA during combination therapy relapse after termination of 48 week treatment. Thus, there is an ongoing need to refine treatment strategies because many patients do not respond or relapse after treatment.

Jensen et al reported that a rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of treatment with peginterferon α -2a plus ribavirin, was the

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single best predictor of SVR in genotype 1 patients treated for either 24 or 48 weeks in a randomized, multinational trial (8). This indicates that patients with RVR should be treated for a short course-regimen. In contrast, it may be necessary to treat patients without RVR using a long course-regimen.

The selection of duration of treatment and optimum doses of combination therapy is an area of active investigation (9-15). When the patients with chronic hepatitis C have an undetectable HCV RNA during combination therapy, it is not clear how long the physician should continue the treatment. Thus, in this present study, we performed a retrospective study to examine the relationship between attainment time of negativity of serum HCV RNA after the initiation of combination therapy and the continuance period of negative HCV RNA in patients with undetectable HCV-RNA during treatment.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of ≥ 100 KIU/ml before treatment; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay; 5) leukocytes $>2,000/\text{mm}^3$, platelet count $>80,000/\text{mm}^3$, and bilirubin <2.0 mg/ml; 6) negativity of serum HCV RNA within 48 weeks after initiation of combination therapy; 7) duration of treatment of ≥ 8 weeks; 8) the initiation time of treatment was from December 2004 to September 2006. We excluded from the study all the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The institutional ethics review board of our hospital approved our study. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for treatment. Informed consent was obtained after confirming the negativity of serum HCV RNA during combination therapy. A total of 366 HCV patients were enrolled in this retrospective cohort study at the study hospital.

Patients were classified into four groups according to the response of combination therapy: RVR, defined as undetectable HCV RNA at week 4 after the initiation of combination therapy; early virological response group (EVR), defined as undetectable HCV RNA at week of 5 to 12 of combination therapy; late virological group (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy; and super late virological group (SLVR), defined as undetectable HCV RNA at week 25 to 48 of combination therapy. A non-relapse in patients with undetectable HCV

RNA during combination therapy was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 month after the cessation of combination therapy (16).

Patients were classified into four groups and evaluated according to the difference of clinical and biochemical backgrounds. Next, predictors of no-relapse in patients with undetectable HCV RNA in serum during treatment were assessed by the multiple logistic regression analysis. Finally, the no-relapse rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy was examined.

Combination therapy of peginterferon and ribavirin

For the treatment regimen, the peginterferon (Peg-intron, Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol, Schering-Plough Pharmaceutical Co.) were given at the dose described based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.4 $\mu\text{g}/\text{kg}$ (range, 1.3-1.8 $\mu\text{g}/\text{kg}$) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range, 9.5-15.4 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60 μg for ≤ 40 kg, 80 μg for >40 kg and ≤ 60 kg, 100 μg for >60 kg and ≤ 80 kg, 120 μg for >80 kg and ≤ 100 kg, and 150 μg for >100 kg). The ribavirin dose was adjusted according to body weight (600 mg for ≤ 60 kg, 800 mg for >60 kg and ≤ 80 kg, and 1,000 mg for >80 kg).

A total of 202 patients were treated with 48 week regimen and 113 patients were given combination therapy for > 48 week regimen. Treatment for the remaining 51 patients was discontinued because of treatment-related side effects within 48 weeks after the initiation of combination therapy. On the duration of treatment, 126 patients were given combination therapy based on regimen of clinical trial, 142 patients were treated for 48 weeks of within health insurance, and 98 patients were decided by physician's judgement.

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at -80°C until analysis. Using these blood samples, the HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (17). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (18). Serum alanine aminotransferase (ALT), aspartic aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay.

Follow-up protocol

The start of follow-up period was defined as the first day that showed negative HCV RNA after the initiation of com-

combination 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 Kruskal 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 ≤ 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 ≤ 30 weeks during treatment had non-relapse. In EVR group, patients with period of negative HCV RNA of ≤ 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 ≤ 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 ≥ 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				p
		RVR	EVR	LVR	SLVR	
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.

Acknowledgement

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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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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

Abbreviations: ALT: alanine aminotransferase, AST: aspartate

References

- Schalm SW, Weiland O, Hansen BE, et al. Interferon-ribavirin for chronic hepatitis C with and without cirrhosis: analysis of individual patient data of six controlled trials. Eurohep Study Group for Viral Hepatitis. *Gastroenterology* **117**: 408-413, 1999.
- Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* **358**: 958-965, 2001.
- Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* **347**: 975-982, 2002.
- McHutchison JG, Manns M, Patel K, et al. International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* **123**: 1061-1069, 2002.
- Hadziyannis SJ, Sette H Jr, Morgan TR, et al. PEGASYS International Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* **140**: 346-355, 2004.
- Shiffman ML, Di Bisceglie AM, Lindsay KL, et al. Antiviral Long-Term Treatment Against Cirrhosis Trial Group. Peginterferon alpha-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* **26**: 1015-1023, 2004.
- Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alpha-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* **132**: 103-112, 2007.
- Jensen DM, Morgan TR, Marcellin P, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* **43**: 954-960, 2006.
- Bronowicki JP, Ouzan D, Asselah T, et al. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2a plus ribavirin. *Gastroenterology* **131**: 1040-1048, 2006.
- Dalgard O, Bjørø K, Hellum KB, et al. Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* **40**: 1260-1265, 2004.
- Mangia A, Santoro R, Minerva N, et al. Peginterferon alpha-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* **352**: 2609-2617, 2005.
- Bruno S, Cammà C, Di Marco V, et al. Peginterferon alpha-2b plus ribavirin for naive patients with genotype 1 chronic hepatitis C: a randomized controlled trial. *J Hepatol* **41**: 474-481, 2004.
- Lindahl K, Stahle L, Bruchfeld A, et al. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* **41**: 275-279, 2005.
- von Wagner M, Huber M, Berg T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* **129**: 522-527, 2005.
- Krawitt EL, Gordon SR, Grace ND, et al. for the New York New England Study Team. A study of low dose peginterferon alpha-2b with ribavirin for the initial treatment of chronic hepatitis C. *Am J Gastroenterol* **101**: 1268-1273, 2006.
- Dogliu A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebvre J. Sec-

- ond generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. *J Clin Microbiol* **37**: 1567-1569, 1999.
17. Albadalejo J, Alonso R, Antinuzzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* **36**: 862-865, 1998.
18. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* **19**: 13-18, 1994.
19. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **19**: 1513-1520, 1994.
20. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* **38**: 645-652, 2003.
21. Ferenci P, Fried MW, Shiffman ML, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* **43**: 425-433, 2005.
22. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* **43**: 54-63, 2006.

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 ≥ 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 ≥ 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 erythematosus, rheumatic arthritis. Diagnosis of osteoporosis was based on bone mineral density (AP spine by dual-energy X-ray absorptiometry) less than 2 SD of young adult mean and/or X-ray evidence of vertebral 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 etidronate 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 (×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.

^aData 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

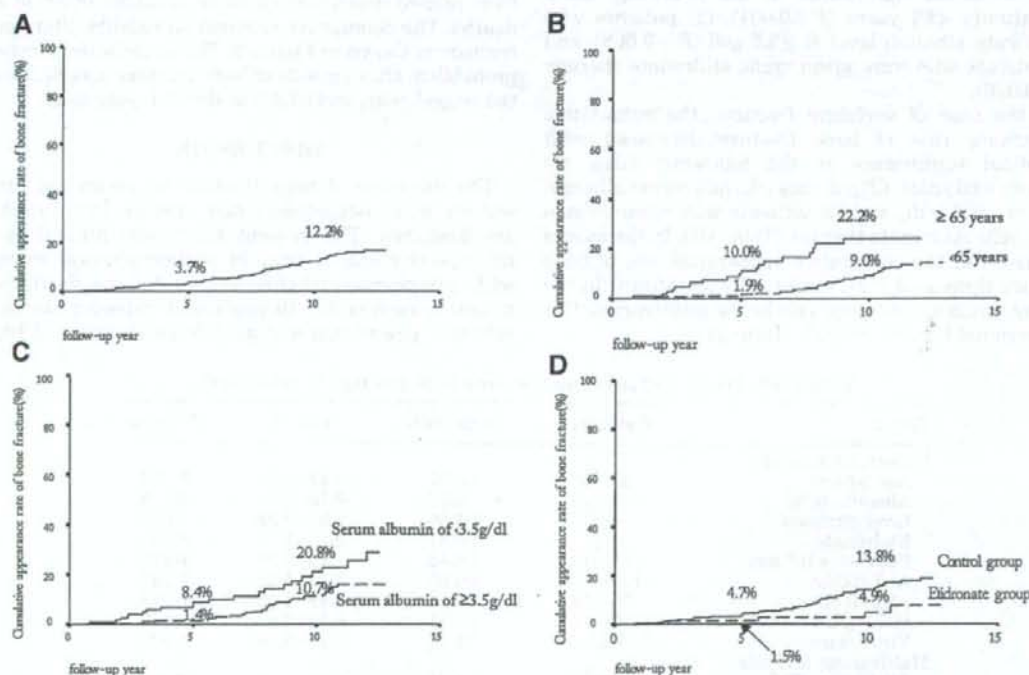


Fig. 1. Cumulative appearance rate of the bone fracture in women with osteoporosis and chronic liver disease. **Panel A:** Cumulative appearance rate of the bone fracture in a total of patients. **Panel B:** Cumulative appearance rate of the bone fracture based to difference of age (solid line, patients 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
γ GTP (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

^aALT, 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
γ GTP (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

^aALT, 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
γ GTP (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

^aALT, 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

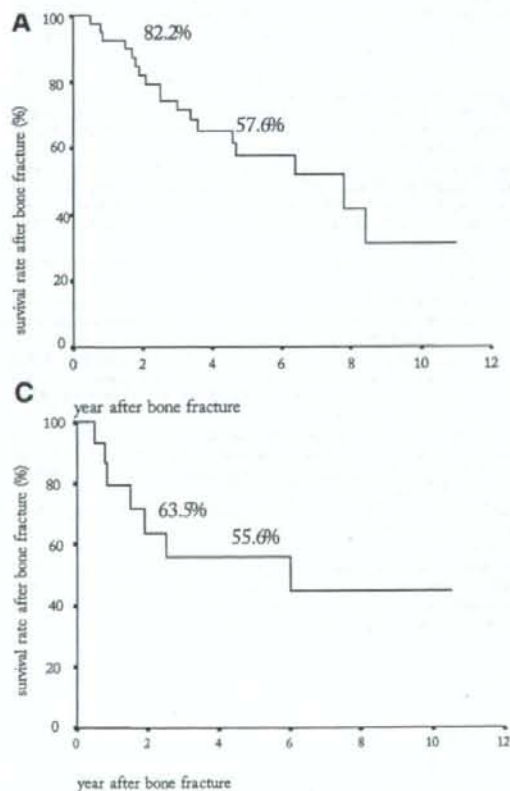


Fig. 2. Cumulative survival rate after the appearance of bone fracture in women with osteoporosis and chronic liver disease. **Panel A:** Cumulative survival rate after the appearance of bone fracture in a total of patients. **Panel B:** Cumulative survival rate after the appearance of hip bone fracture. **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 ≥ 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 studies 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 ≥ 3.5 g/dl and cyclic etidronate treatment reduce the development of bone fracture in postmenopausal women with osteoporosis and chronic liver disease.

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REFERENCES

- Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. 1992. The natural history of community acquired hepatitis C in the United States. *N Engl J Med* 327:1899-1905.
- Emkey RD, Ettinger M. 2006. Improving compliance and persistence with bisphosphonate therapy for osteoporosis. *Am J Med* 119:S18-S24.
- Fujita T, Orimo H, Inoue T, Kaneda K, Sakurai M, Morita R, Yamamoto K, Sugioke Y, Inoue A, Takaoka K, Yamamoto I, Hoshino Y, Kawaguchi H. 2003. A multivariate analysis of risk factors for hepatocellular carcinoma: Reappraisal of a multicenter double-blind clinical trial comparing etidronate and alfacalcidol. *J Bone Miner Metab* 25:130-137.
- Gumber SC, Chopra S. 1995. Hepatitis C: A multifaceted disease—Review of extra hepatic manifestations. *Ann Intern Med* 123:615-620.
- Harrington DP, Fleming TR. 1983. A class of rank test procedures for censored survival data. *Biometrics* 62:553-566.
- Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. 1993. Clinical effect of bisphosphonate and vitamin D on osteoporosis: A multivariate analysis of risk factors for hepatocellular carcinoma: A prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 18:47-53.
- Kiyosawa K, Furuta S. 1991. Review of hepatitis C in Japan. *J Gastroenterol Hepatol* 6:383-391.
- Miller PD, Watts NB, Licata AA, Harris ST, Genant HK, Wasnich RD, Jackson PD, Hoseyni MS, Schoenfeld SL, Valent DJ, Chesnut GH III. 1997. Cyclical etidronate in the treatment of postmenopausal osteoporosis: Efficacy and safety after seven years of treatment. *Am J Med* 103:468-476.
- Pawlowsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, André C, Voisin MC, Intrator L, Zafrani ES, Duval J, Dhumeaux D. 1995. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 122:169-173.
- Rouillard S, Lane NE. 2001. Hepatic osteodystrophy. *Hepatology* 33:301-307.
- Shiomi S, Nishiguchi S, Kurooka H, Tamori A, Habu D, Takeda T, Ochi H. 2002. Cyclical etidronate for treatment of osteopenia in patients with cirrhosis of the liver. *Hepatol Res* 22:102-106.
- Storm T, Thamsborg C, Steiniche T, Genant HK, Soerensen OH. Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. 1990. *N Engl J Med* 322:1265-1271.
- Taukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. 1993. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 328:1797-1801.
- Tsuneoka K, Tameda Y, Takase K, Nakano T. 1996. Osteodystrophy in patients with chronic hepatitis and liver cirrhosis. *J Gastroenterol* 31:669-678.
- Watts NB, Harris ST, Genant HK, Wasnich RD, Miller PD, Jackson RD, Licata AA, Ross P, Woodson GC III, Yanover MJ. 1990. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. *N Engl J Med* 323:73-79.

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 Cox regression 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 anti-mitochondrial 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 laparoscopy-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 sedative or analgetics during the laparoscopic intervention. During laparoscopy, each patient was given continuously an isotonic electrolyte solution intravenously. Patients were monitored by pulseoxymetry 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.

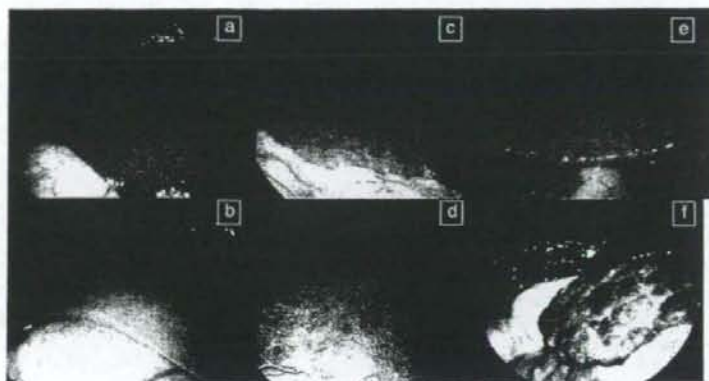
Histopathological evaluation

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.¹³

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 were considered as censored data in the statistical analysis.²⁰ Moreover, the patients treated with antiviral drugs were regarded as withdrawals at the time of starting the antiviral 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 χ^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 ($P < 0.0001$).