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

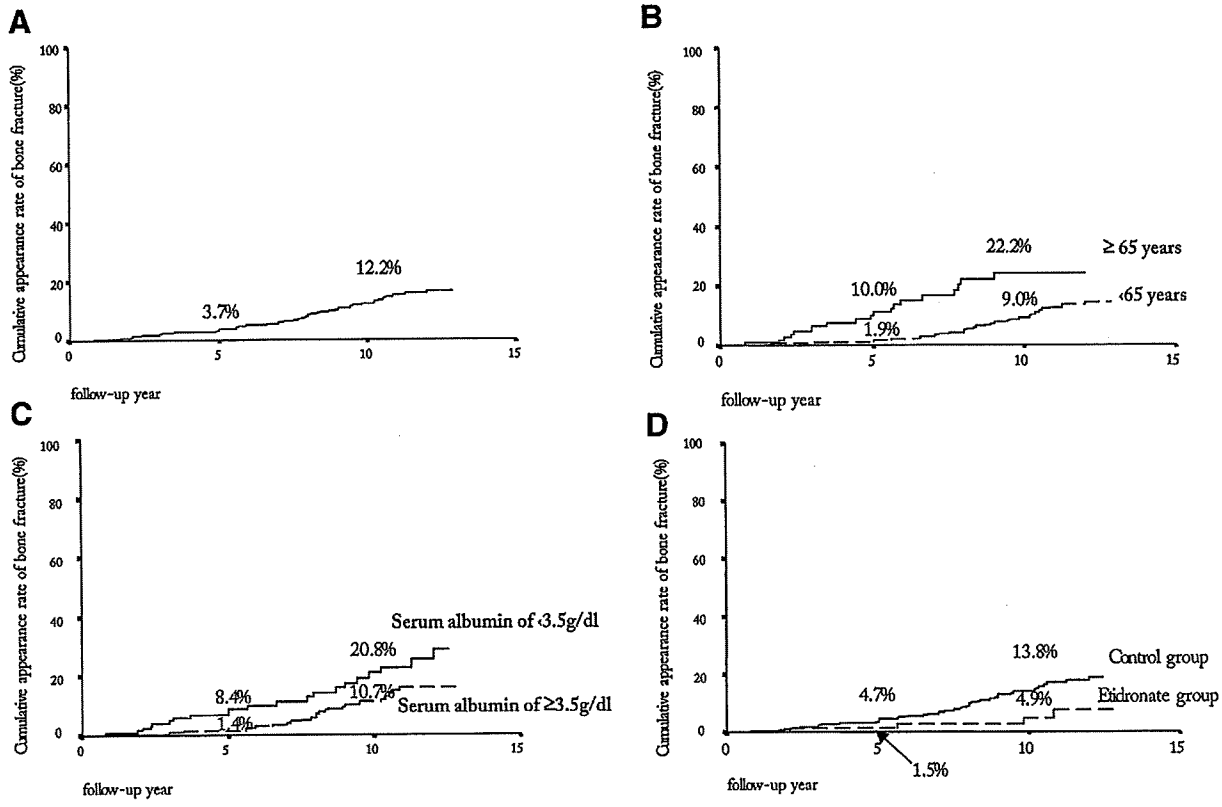

Fig. 1. Cumulative appearance rate of the bone fracture in women with osteoporosis and chronic liver disease. **Panel A**: Cumulative appearance rate of the bone fracture in a total of patients. **Panel B**: Cumulative appearance rate of the bone fracture based to difference of age (solid line, patients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

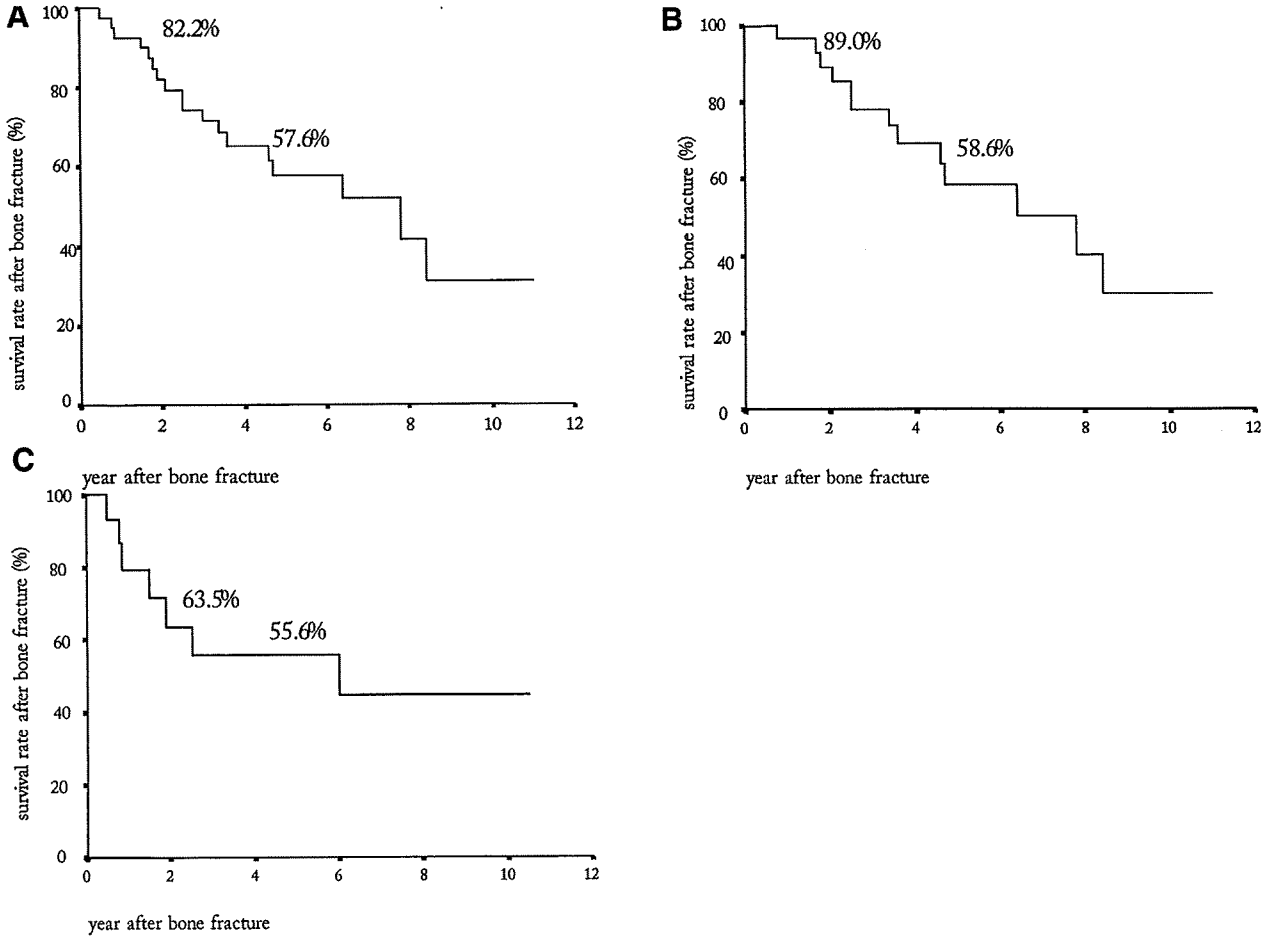

Fig. 2. Cumulative survival rate after the appearance of bone fracture in women with osteoporosis and chronic liver disease. **Panel A**: Cumulative survival rate after the appearance of bone fracture in a total of patients. **Panel B**: Cumulative survival rate after the appearance of hip bone fracture.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

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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.

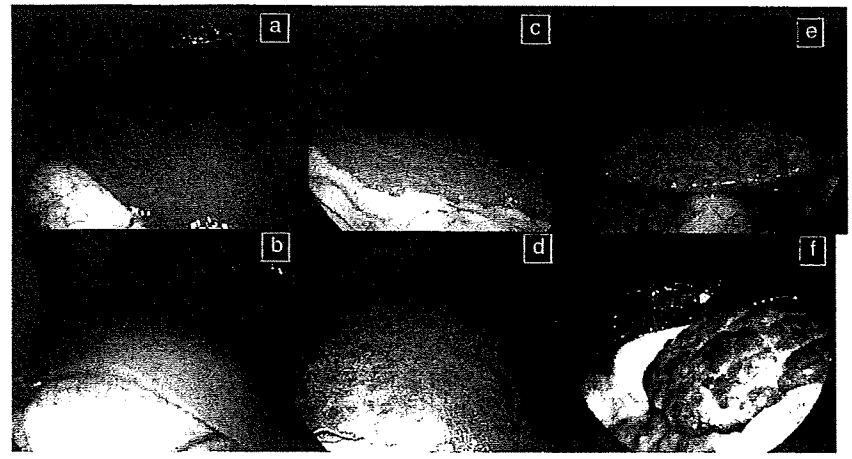

Figure 1 Based on the irregularities of the liver surface, laparoscopic findings were classified into three groups in progression order: smooth, an essentially smooth liver surface or with limited areas of depression (a,b); irregular, a liver surface showing increased numbers of interconnected depressions, 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 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 ($P < 0.0001$).

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

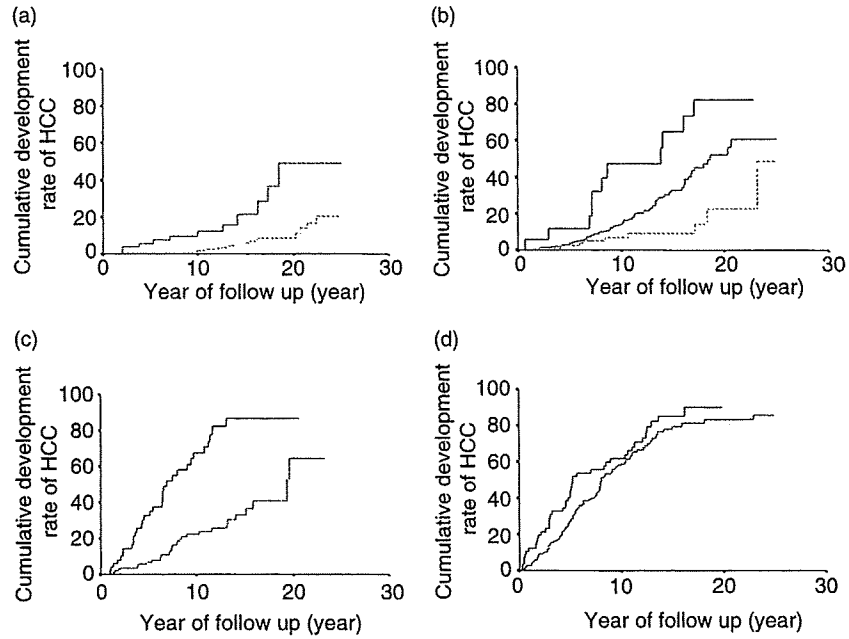

Figure 2 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings of hepatitis C virus patients. (a) Patients with stage 1 ($P < 0.0001$); (b) patients with stage 2 ($P < 0.0001$); (c) patients with stage 3 ($P < 0.0001$, $P = 0.055$); (d) patients with stage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

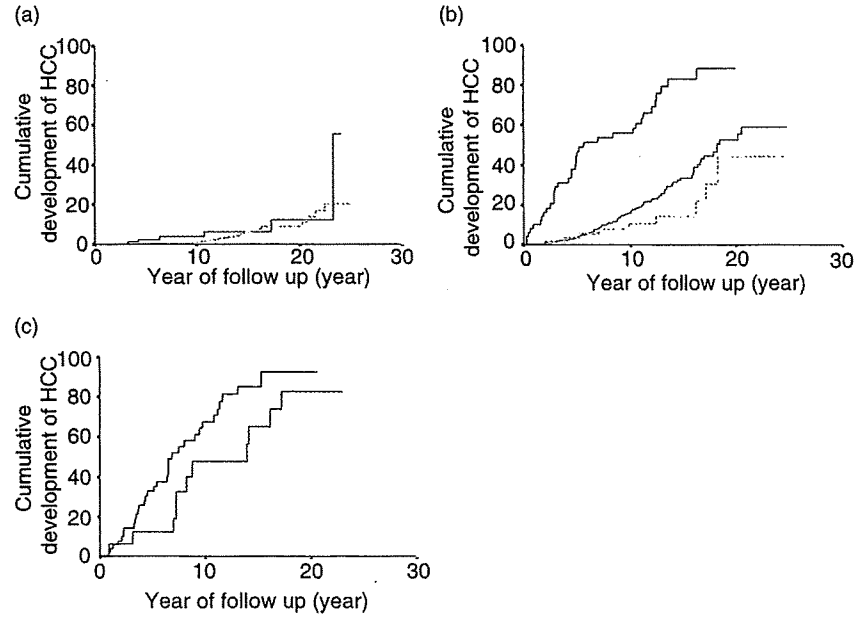

Figure 3 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis C virus patients. (a) Patients with smooth liver surface ($P < 0.391$); (b) patients with irregular liver surface ($P < 0.0001$); (c) patients with nodular liver surface ($P < 0.055$).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

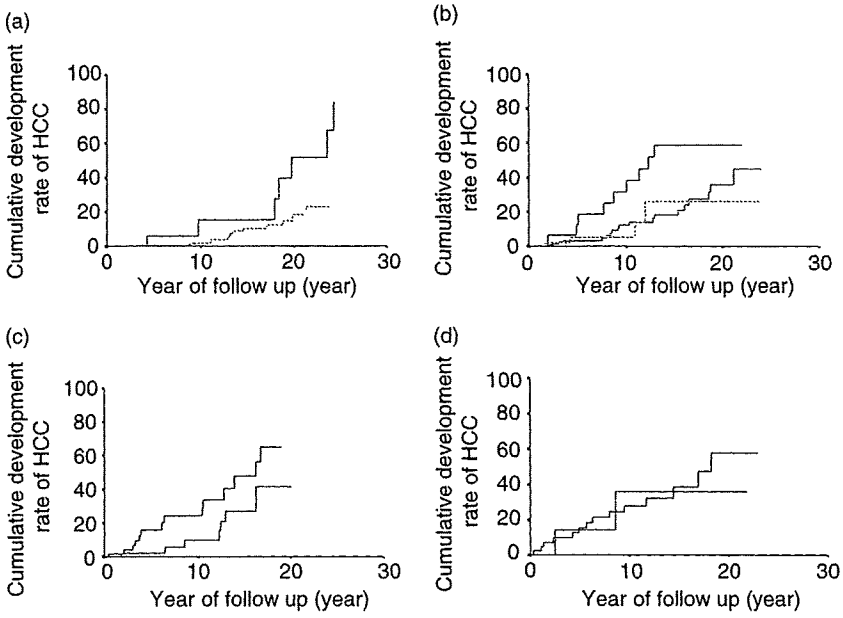

Figure 4 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings in hepatitis B virus patients. (a) Patients with stage 1 ($P < 0.013$); (b) patients with stage 2 ($P < 0.016$); (c) patients with stage 3 ($P < 0.116$); (d) patients with stage 4 ($P < 0.858$).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

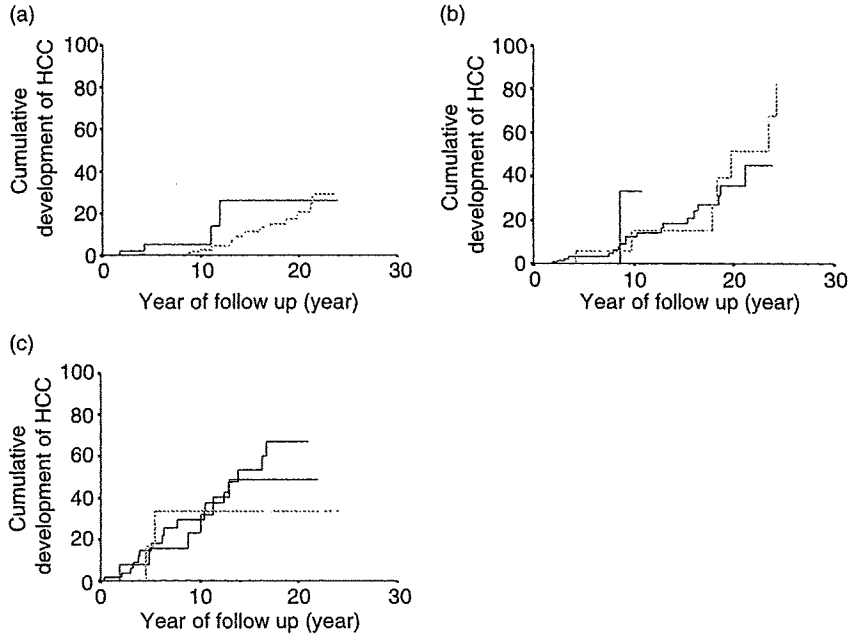

Figure 5 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis B virus patients. (a) Patients with smooth liver surface ($P < 0.252$); (b) patients with irregular liver surface ($P < 0.736$); (c) patients with nodular liver surface ($P < 0.785$). (—) 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 2: stage 2 3: stage 3 4: stage 4	2.56	2.34–2.79	<0.0001
Laparoscopic findings	1: smooth 2: irregular 3: nodular	4.31	3.74–4.97	<0.0001
Multivariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	1.63	1.39–1.92	<0.0001
Laparoscopic findings	1: smooth 2: irregular 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 2: stage 2 3: stage 3 4: stage 4	1.72	1.43–2.07	<0.0001
Laparoscopic findings	1: smooth 2: irregular 3: nodular	2.29	1.79–2.92	<0.0001
Multivariate analysis				
Histological findings	1: stage 1 2: stage 2 3: stage 3 4: stage 4	1.13	0.85–1.49	0.403
Laparoscopic	1: smooth 2: irregular 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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BASIC STUDIES

New ablation procedure for a radiofrequency liver tissue coagulation system using an expandable needle

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Keywords
expandable needle – liver cancer – radiofrequency ablation (RFA) – stepwise expansion

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Abstract

Objective: The stepwise hook extension technique for an expandable needle, which we reported previously, allowed roll-off in short time with low power. The aim of this study was to investigate experimentally the efficacy of a modified extension procedure. *Methods:* Three pigs underwent 10 radiofrequency ablation (RFA) procedures using the 10-hook electrode of LeVeen needle. The conventional technique was used in five RFA (group 1; the electrode was deployed in four steps to full extension), while the new technique was used in the other five RFA (group 2; the electrode was closed after the same three steps as group 1 and then fully extended). *Results:* The shape of the RFA-induced zone was cone-like or irregular in group 1 and oval-like in group 2. The diameter vertical to the shaft was larger in group 2 (37, range 33–42 mm) than in group 1 (23, range 20–29 mm). The median ablation time was longer in group 2 (10 min 13 s) than in group 1 (3 min 56 s). Although the required energy was higher in group 2 than in group 1, that per volume was comparable between the groups (median 0.9 vs. 1.4 kJ/mm^3). *Conclusions:* Our new procedure requires a longer session but produces larger necrosis of a uniform ellipsoid volume, making it potentially suitable for tumours more than 3 cm in diameter.

Percutaneous treatment including radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) is often used for small-size hepatocellular carcinoma (HCC) as it is less invasive than surgical therapy. RFA has become the first-choice local treatment because of the excellent outcome; the efficacy of RFA in HCC tumours measuring < 2 cm in diameter is similar to that of PEI but it requires fewer treatment sessions, and the efficacy in HCC tumours > 2 cm in diameter is better than with PEI (1). In addition, RFA is also more cost-effective than surgical resection of small HCC (2). Because the volume ablated during one RFA session is of a diameter < 3.0–4.0 cm in most cases, RFA therapy is now restricted to tumour < 3 cm. In this regard, previous studies reported that the necrotic area could be enlarged by a saline injection before RFA (3, 4), combination of RFA with PEI (5, 6), RFA with an ethanol–lipiodol injection (7), RFA with transcatheter arterial embolization (8) and RFA with transient arterial obliteration (9–11).

Among the three commercially available RFA apparatuses, the radiofrequency tumour coagulation system (RTC system; Boston Scientific, Natick, MA, USA), radiofrequency interstitial tumour ablation system (RITA System, RITA Medical Systems Inc., Mountain View, CA, USA) and cool-tip RF system (Radionics Inc., Burlington, VT, USA), the first two types have adopted the expandable needle. We reported previously the efficacy of the stepwise hook extension technique for RFA therapy of HCC (12). The technique allows rapid roll-off at a lower power and reduces any possible increase in intratissue pressure that may cause scattering of intrahepatic metastasis (13–15). Additionally, we have designed a new technique involving full re-expansion after stepwise extension, that may ensure full expansion of the needle to enlarge the ablated zone. The aim of this study was to investigate experimentally the new expansion technique and to compare it with the conventional stepwise extension technique.

Materials and methods

We used the RTC system comprising the RF3000 generator and a slim expandable needle (30 mm,

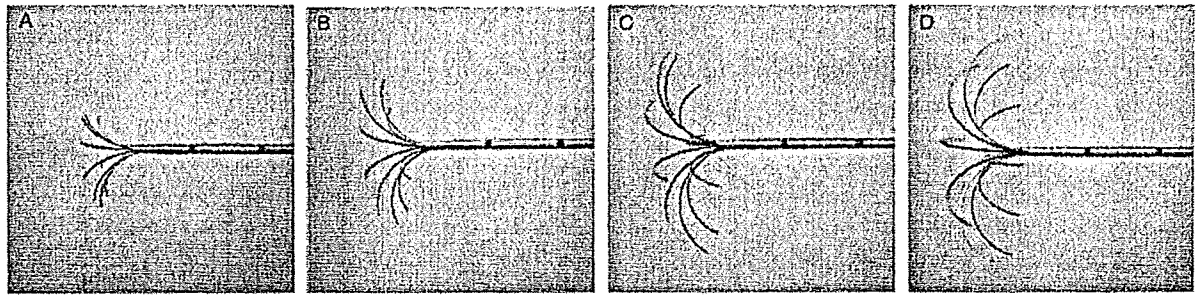

Fig. 1. The conventional four-stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) A full-length is expanded in the final step.

17-G LeVeen needle, SuperSlim®), which consists of 10 expandable monopolar array electrodes. RF was applied in the livers of three normal female domestic pigs (weight range: 58–62 kg) under general anesthaesia maintained until killing. The abdomen was opened so that the needle could be inserted directly into the upper region of the liver where the thickness was larger than about 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed four to six times in each liver respectively. Two electrode pads were placed on the skin. Instead of using the standard method recommended by the manufacturer, we adopted two types of stepwise hook extension techniques. In five RFA sessions, the conventional four stepwise expansion was performed (group 1); the electrode tines were expanded to a quarter, a half, three quarters of the length and full length in the first, second, third and final steps: The diameter of the array at each step was 15, 20, 25 and 30 mm, respectively, as shown in Figure 1. In the other five RFA sessions (group 2), the first, second and third steps were similar to those of group 1. After the third step, the tines were again closed within the shaft and then sharply and fully expanded (Fig. 2). The needle extension was observed under an X-ray at each step in the five examples.

Power was first applied at 30 W and then increased at 10 W increments every minute in each step. The necessary electric power and tissue impedance were recorded every 15 s. The procedure was applied continuously until an increase in impedance (caused by coagulation necrosis) with a corresponding decline in delivered power (a phenomenon called 'roll-off').

After completion of the experiments, the animal was killed and the ablated liver lobes were dissected out immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone. We measured the length of the ablated zone along the needle tract and the diameter of the area perpendicular to it. Using these values, we calculated the hypothetical volume of the ablated zone. The energy requirement for ablation was integration of the electric power (W) over the ablation time (s), which could be calculated approximately by summing a product of 15 (s) and the electric power measured every 15 s.

The duration of ablation, required energy and the size of the ablated zone were compared between the two groups using Mann–Whitney's *U*-test. All values are expressed as median. A *P*-value < 0.05 denoted the presence of a statistically significant difference.

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Results

Ablation time and required energy

Roll-off was achieved at each step of ablation in all 10 RFA procedures. The time to reach roll-off at each step and total ablation time are shown in Table 1. In group 1, the median time to reach roll-off at a quarter, a half, three quarters and full expansion was 84 (range 58–180), 49 (27–134), 45 (34–100) and 56 (52–148) seconds respectively. The total ablation time for group 1 was 3 min and 56 s (range 2 min and 56 s to 8 min and 36 s). For group 2, the median time to reach roll-off at each step was 107 (range 70–166), 23 (17–153), 61 (22–108) s, and 6 min and 19 s (4 min and 29 s to 6 min 40 s) respectively. The total ablation time was 10 min and 13 s (range 6 min and 34 s to 12 min and 29 s). These results indicate that the durations of the first step, second step and third step were similar for groups 1 and 2 ($P = 1.000$, 0.421 and 1.000), while that of the fourth step and total session were longer for group 2 than group 1 ($P = 0.008$, 0.032) as shown in Table 2. The energy required for one procedure was

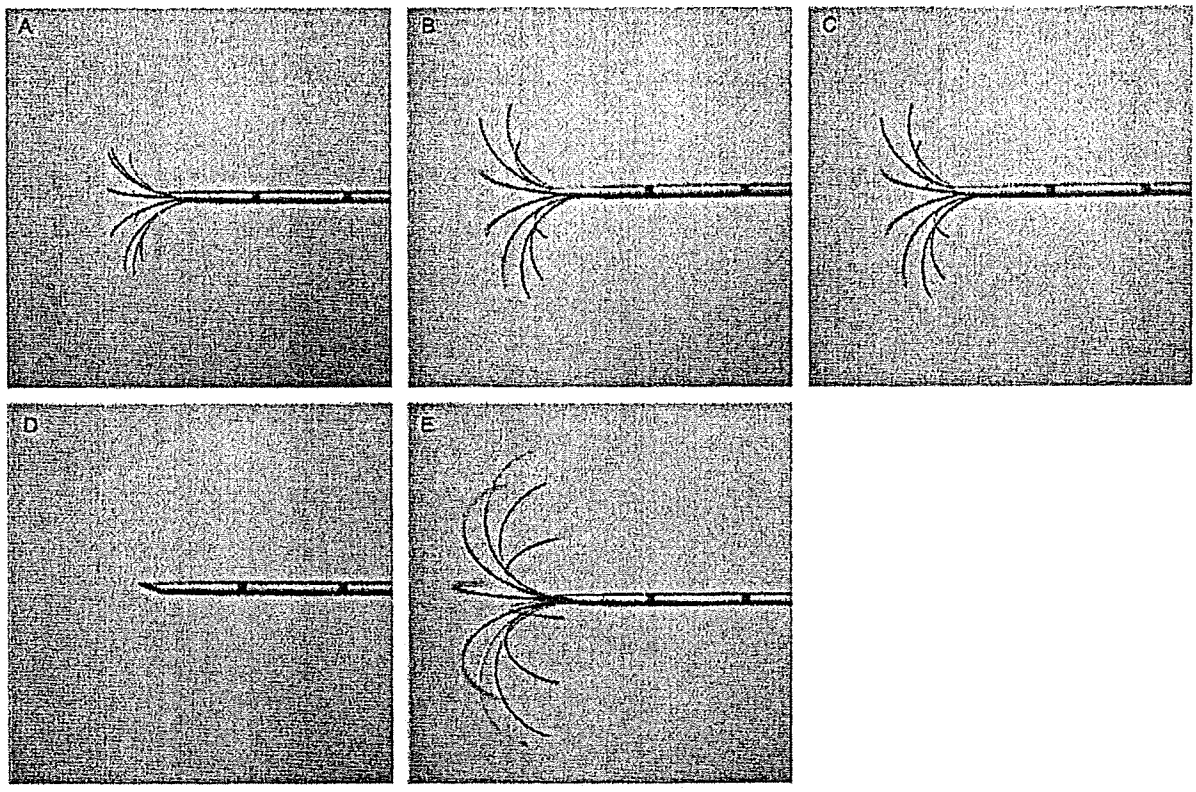

Fig. 2. The new stepwise procedure. (A) A quarter-length of the electrode tines is expanded in the first step. (B) A half-length is expanded in the second step. (C) A three-quarter length is expanded in the third step. (D) The electrode tines are closed in the shaft. (E) Tines are expanded to full length in the final step.

Table 1. Duration of ablation (in minutes seconds) and RF-induced area in groups 1 and 2

	Group 1					Group 2				
	1	2	3	4	5	1	2	3	4	5
Duration										
First step	2′ 55″	3′ 00″	1′ 18″	1′ 24″	0′ 58″	1′ 47″	2′ 15″	2′ 46″	1′ 42″	1′ 10″
Second step	2′ 14″	1′ 28″	0′ 49″	0′ 27″	0′ 32″	1′ 02″	2′ 33″	0′ 18″	0′ 17″	0′ 23″
Third step	0′ 58″	1′ 40″	0′ 35″	0′ 45″	0′ 34″	1′ 48″	1′ 01″	1′ 26″	0′ 22″	0′ 32″
Fourth step	0′ 44″	2′ 28″	1′ 14″	0′ 56″	0′ 52″	5′ 36″	6′ 40″	6′ 19″	6′ 29″	4′ 29″
Total	6′ 51″	8′ 36″	3′ 56″	3′ 32″	2′ 56″	10′ 13″	12′ 29″	10′ 49″	8′ 50″	6′ 34″
RF-induced area										
Transverse diameter, mm	20	28	25	23	22	33	42	38	37	35
Longitudinal length, mm	27	24	30	30	32	20	30	27	27	34
Shape	Irregular	Cone-like	Cone-like	Cone-like	Cone-like	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid

larger in group 2 than that in group 1 (group 1: 5.5 kJ range 4.0–14.8 kJ, group 2: 25.0 kJ range 13.4–30.6 kJ, $P = 0.016$) respectively.

Size and shape of ablated tissue

Table 1 shows the shape and size of the RF-induced areas in groups 1 and 2. In group 1, the shape of the ablated zone was cone-like or was sometimes irregularly shaped. The length along the shaft was longer than the vertical diameter as shown in Figure 3A. In group 2, the ablated zone was near-oval in shape, with the short axis equivalent to the shaft (Fig. 3B). As shown in Table 2, the area perpendicular to the shaft and the ablation volume were larger in group 2 than in group 1: vertical diameter: 23 (range 20–28) mm vs. 37 (range 33–42) mm ($P = 0.008$). This indicates that our technique produced a larger area of necrosis following

Table 2. Comparison of ablation time (in minutes seconds) and RF-induced areas between groups 1 and 2

	Group 1	Group 2	*P*
Incidence of roll-off	5/5	5/5	1.000
Duration of the first step	1′ 24″ (0′ 58″–3′ 00″)	1′ 47″ (1′ 10″–2′ 46″)	1.000
Second step	49″ (0′ 27″–2′ 14″)	23″ (17″–2′ 33″)	0.421
Third step	45″ (0′ 34″–1′ 40″)	1′ 1″ (22″–1′ 48″)	1.000
Fourth step	56″ (0′ 44″–2′ 28″)	6′ 19″ (4′ 29″–6′ 40″)	0.008
Total ablation time	3′ 56″ (2′ 56″–8′ 36″)	10′ 13″ (6′ 34″–12′ 29″)	0.032
Required energy for ablation, kJ	5.5 (4.0–14.8)	25.0 (13.4–30.6)	0.016
Diameter of the cross-section vertical to the axis, mm	23 (20–28)	37 (33–42)	0.008
Axial length, mm	30 (24–32)	27 (20–34)	0.841
Shape of RF-induced area			
Ellipsoid	0	5	
Cone-like	4	0	
Irregular	1	0	

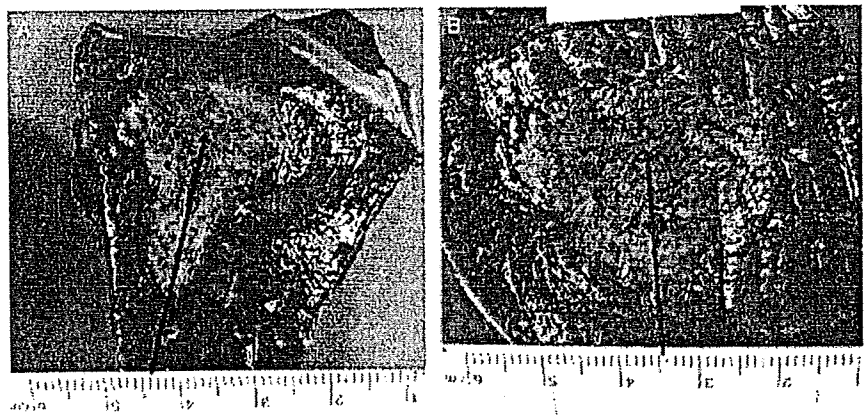

Fig. 3. Photographs of the coagulated area. Arrow shows the direction of the needle shaft. (A) The shape of the area produced by the conventional procedure is cone-like (RFA#3). (B) The shape of the area produced by the new procedure is ellipsoid in shape (RFA#3).

one session of RF. Although the axial length of the ablation zone showed no significant difference between the two groups, that in group 2 seemed a slightly shorter than that in group 1: axial length: 30 (range 24–32) mm vs. 27 (range 20–34) mm ($P = 0.841$). Based on the assumption that the shape of the necrotic area was a combination of a hemisphere and a cone in group 1 and an ellipsoid in group 2, the estimated volume of the ablated liver tissue was 5.7 (range 3.8–7.8) μm^3 for group 1 and 20 (range 11–28) μm^3 for group 2. Using this value and the total required energy for ablation, the calculated energy required for ablation per volume was 0.9 (range 0.7–2.5) J/mm^3 for group 1 and 1.4 (range 0.6–1.8) J/mm^3 for group 2 ($P = 1.000$).

Needle expansion

Figures 4 and 5 show X-ray images of the electrode tines in the pig liver at each step. Both in the second step and the third step, the progress of the tines' spread was smaller than at the first step. The needle expansion at the third step did not reach three quarters length in both groups 1 and 2. The extent of the expansion at the final step was nearly similar to that at the second and third steps in group 1, while it was nearly complete in group 2.

Discussion

Radiofrequency ablation therapy is one of the curative therapies for HCC measuring < 30 mm in diameter, while surgical resection is the only curative treatment for HCC more than 30 mm and < 50 mm in diameter. However, surgical resection cannot be performed in patients with cirrhotic liver and liver dysfunction. Thus, a technique that widens the RF-ablated area can improve, at least theoretically, the survival of cirrhotic patients with HCC over 30 mm in diameter.

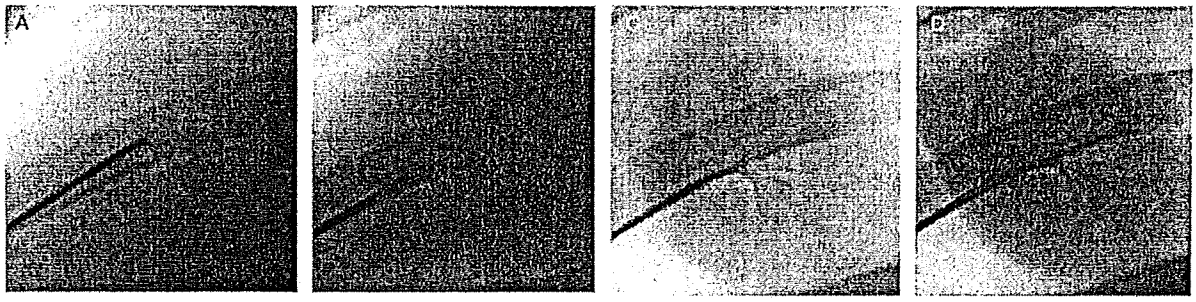

Fig. 4. Electrode tines in a pig liver during the conventional four-stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step. The extent of the expansion at the final step was nearly similar to that at the second and third steps.

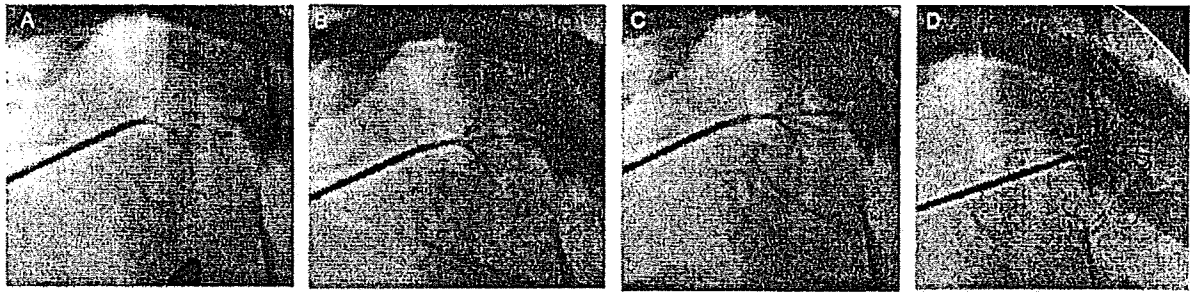

Fig. 5. Electrode tines in a pig liver during the new stepwise extension procedure. (A) First step. (B) Second step. (C) Third step. (D) Final step. At the second step, the third step and the final step, the progress of the tines' spread is smaller in comparison with that at the first step. The extent of the expansion at the final step was nearly complete.

The shape of the ablated zone depends on the needle type (6). For example, the path along the shaft is longer than the transverse diameter when using the cool-tip electrode (Radionics System; Radionics, Burlington, VT, USA), shorter when using the expandable needle of RTC system and compatible with each other when using the LeVeen needle (RTC system). The shorter path is less disadvantageous than the shorter perpendicular diameter, because the ablated zone along with the needle trace can be enlarged by repeating the procedure as the needle is extracted while that perpendicular to the tract cannot be enlarged during one insertion. Although it is often difficult to achieve roll-off during a single-step full expansion procedure using the LeVeen needle, our stepwise procedure has overcome this difficulty and produced an oval ablation zone similar to the single-step procedure.

The LeVeen needle, which had a diameter of 14 G in the first stage, has been made slender for the ease and safety of insertion into the liver. The needle now available in the market has a diameter of 17 G. The slim needle may be easier to deform during insertion and difficult to fully extend within the liver by the conventional stepwise method. The liver tissue resistance consists of resistance acting on the needle tip and that on the side. The strength of the former is proportional to the cross-section and that of the latter is to the surface area. Based on this, the slender shaft is subjected to a large stress and strain resulting in larger deformation, although its resistance is smaller. Thus, the hooks of the slim needle hardly extend as expected; it cannot be fully extended when expanded slowly as shown in Figure 4. This is because the shaft is pushed back as the electrode is inserted towards the liver. To overcome this inconvenience, we investigated a new technique: full re-expansion after stepwise extension, which allows a sharper and definite expansion of the slim needle to full length. Thus, this technique is suggested to be more advantageous in a slimmer needle; this procedure has not been examined in needles 14 or 15 G in diameter.

The additional reason for the larger ablation zone made by the new method is that the tanned tumour or parenchymal tissue would be removed from the surface of the multiple tines when they are once closed in the shaft. The tan was observed on the tip of the shaft

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when the needle was extracted from the liver. The tan adhering on the tine may prevent the uniform electric current, which results in a decrease in the electric efficiency. Thus, the removal of tan can result in an increase in the effectiveness of RF ablation procedure and that in the ablation zone.

A larger ablation zone at the final step of the new technique required a longer coagulation time and a higher input energy during the final step and during the total session; the ablation zone, ablation time and the required energy by our method were larger than those by the conventional stepwise method. The required energy per volume, on the other hand, was almost identical.

In conclusion, the new extension procedure for the expandable needle allows coagulation of larger and more oval area even when using the slim needle. This method may be useful to expand the application of RFA for hepatic tumours.

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