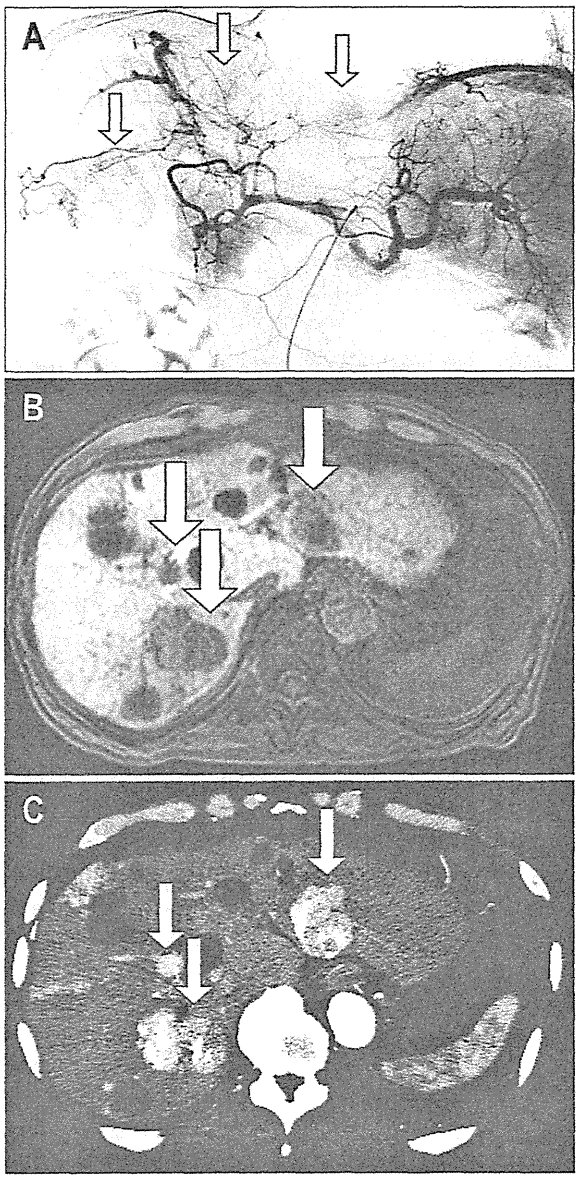

Fig. 3. Case 2. An 84-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemotherapy with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrows). (B) Magnetic resonance imaging (hepatobiliary phase) showed multiple HCCs (arrows). (C) Computed tomography performed 2 months after transcatheter arterial chemotherapy with miriplatin. The lesions showed accumulations of lipiodol (arrows). The treatment efficacy was assessed as a stable disease.

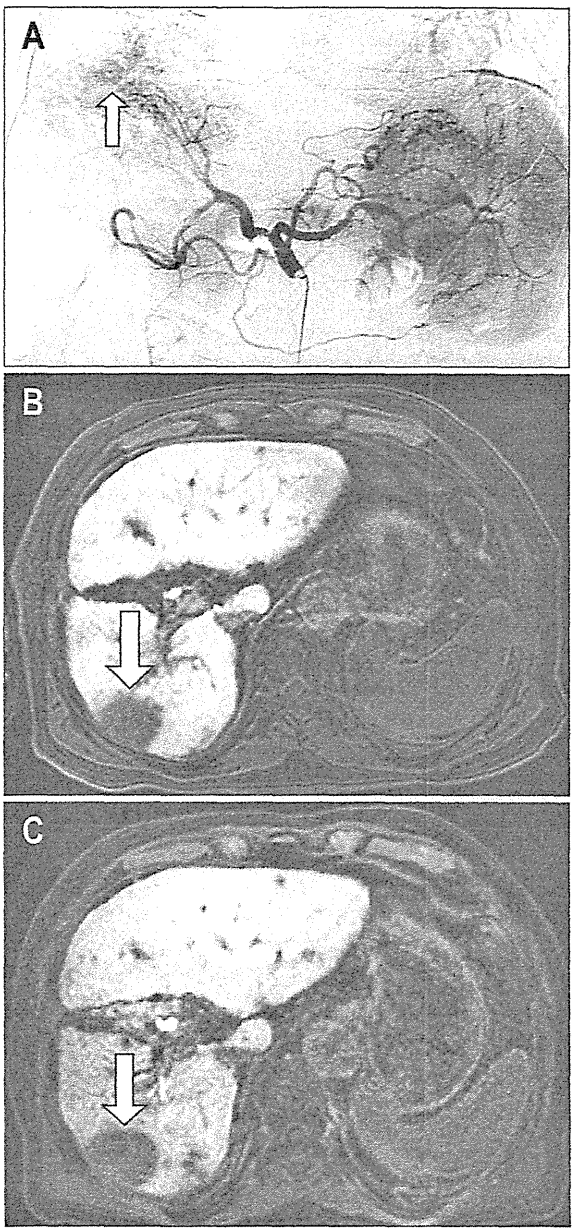

Fig. 4. Case 3. An 83-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) enhanced magnetic resonance imaging (MRI; hepatobiliary phase) showed multiple HCCs (arrow). (C) Gd-EOB-DTPA enhanced MRI performed 3 months after TACE. The lesions showed accumulations of lipiodol (arrow). The treatment efficacy was assessed as a stable disease.

motherapy of advanced HCC. It is one of the platinum agents, although hydration after administration is not necessary of its weak renal toxicity.

Various types of resistance to therapy can occur during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs.[23]

Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior antitumor effect in HCC with lower toxicity compared to cisplatin. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miripla-

tin.

According to pharmacokinetic studies, the plasma concentration of total platinum in patients treated with miriplatin is much lower than that after administration in patients administered intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer than the corresponding values for intra-arterial cisplatin.[17] Theoretically, therefore, it can be administered even in patients of advanced HCC patients with chronic renal failure if visceral angiography can be performed.

Clinical trials have shown that miriplatin is effective for the treatment of HCC, but the safety and efficacy of miriplatin has not been evaluated in HCC patients with chronic renal failure.[16,17] Herein we presented three HCC cases with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. In all three cases, no serious adverse events were observed, and serum creatinine level did not increase, even in the patient who had experienced renal failure due to cisplatin administration (Fig. 2). Repeated injection of miriplatin appears to be also safe in HCC patients with chronic renal failure.

The present results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. A prospective study is required to assess the most effective, least nephrotoxic anticancer agent among the various platinum derivatives. Miriplatin appears to be a promising agent for HCC patients with chronic renal failure.

CONFLICTS OF INTEREST

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan; Hiromitsu Kumada, MD, Kenji Ikeda, MD, Yasuji Arase, MD, Yoshiyuki Suzuki, MD, Fumitaka Suzuki, MD, and Norio Akuta, MD.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Kamada K, Nakanishi T, Kitamoto M, et al. Long-term prognosis of patients undergoing transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: comparison of cisplatin lipiodol suspension and doxorubicin hydrochloride emulsion. J Vasc Interv Radiol 2001;12:847-854.
3. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. Radiology 2002;224:47-54.
4. Ikeda M, Maeda S, Shibata J, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology 2004;66:24-31.
5. Takayasu K, Arii S, Ikai I, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131:461-469.
6. Marelli L, Stigliano R, Triantos C, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6-25.
7. Ikeda M, Maeda S, Ashihara H, Nagahama H, Tanaka M, Sasaki Y. Transcatheter arterial infusion chemotherapy with cisplatin-lipiodol suspension in patients with hepatocellular carcinoma. J Gastroenterol 2010;45:60-67.
8. Laragh JH, Cannon PJ, Bentzel CJ, Sicinski AM, Meltzer JI. Angiotensin Ii, Norepinephrine, and renal transport of electrolytes and water in normal man and in cirrhosis with ascites. J Clin Invest 1963;42:1179-1192.
9. Cardenas A, Ginès P, Uriz J, et al. Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. Hepatology 2001;34(4 Pt 1):671-676.
10. Moreau R, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. Hepatology 2003;37:233-243.
11. Huo TI, Wu JC, Lee PC, Chang FY, Lee SD. Incidence and risk factors for acute renal failure in patients with hepatocellular carcinoma undergoing transarterial chemoembolization: a prospective study. Liver Int 2004;24:210-215.
12. Huo TI, Wu JC, Huang YH, et al. Acute renal failure after transarterial chemoembolization for hepatocellular carcinoma: a retrospective study of the incidence, risk factors, clinical course and long-term outcome. Aliment Pharmacol Ther 2004;19:999-1007.
13. Hsu CY, Huang YH, Su CW, et al. Renal failure in patients with hepatocellular carcinoma and ascites undergoing transarterial chemoembolization. Liver Int 2010;30:77-84.
14. Maeda M, Uchida NA, Sasaki T. Liposoluble platinum(II) complexes with antitumor activity. Jpn J Cancer Res 1986;77:523-525.
15. Kishimoto S, Ohtani A, Fukuda H, Fukushima S, Takeuchi Y. Relation between intracellular accumulation and cytotoxic activity of cis-[{(1R, 2R)-1, 2-cyclohexanediamine-N, N')bis(myristato)] platinum(II) suspended in Lipiodol. Biol Pharm Bull 2003;26:683-686.
16. Fujiyama S, Shibata J, Maeda S, et al. Phase I clinical study of a novel lipophilic platinum complex (SM-11355) in patients with hepatocellular carcinoma refractory to cisplatin/lipiodol. Br J Cancer 2003;89:1614-1619.
17. Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y. Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. Invest New Drugs 2004;22:169-176.
18. Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T. Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. Cancer Sci 2009;100:189-194.
19. Hanada M, Baba A, Tsutsumishita Y, et al. Intra-hepatic arterial

administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. Cancer Chemother Pharmacol 2009;64:473-483.

20. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Kidney Disease Outcome Quality Initiative. Am J Kidney Dis 2002;39(Suppl 2):S7-S245.
21. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982-992.
22. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52-60.
23. Kawamura Y, Ikeda K, Hirakawa M, et al. Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin. Hepatol Res 2009;39:346-354.

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Original Article

Magnetic resonance laparoscopy: A new non-invasive technique for the assessment of chronic viral liver disease

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Aim: Laparoscopy-guided liver biopsy is the most accurate method for assessing liver fibrosis but have several limitations. We designed a non-invasive method, called magnetic resonance laparoscopy (MRL), based on gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging, to assess liver fibrosis in patients with chronic hepatitis B and C virus.

Methods: We prospectively analyzed 49 patients with normal liver and 353 patients with chronic viral hepatitis, laparoscopic liver biopsy was performed on 109 patients and 244 patients were diagnosed as having liver cirrhosis clinically. The MRL findings of the liver surface were classified into three categories: (i) smooth (essentially smooth surface of the entire liver or with limited areas of depression); (ii) partially irregular (several interconnected depressions on the surface mainly in the left lobe of the liver); and (iii) diffusely irregular (nodules present on the liver surface). Patients with diffusely irregular liver surface was diagnosed as liver cirrhosis.

Results: The liver surface changed with the progression of liver fibrosis from smooth, partially irregular to diffusely irregular, irrespective of viral type. The sensitivity, specificity, positive and negative predictive values for the diagnosis of cirrhosis according to the surface findings on MRL were 96%, 100%, 95% and 95%, respectively. The cirrhotic liver showed: (i) disappearance of impression of the right ribs; (ii) enlargement of the lateral segment; and (iii) atrophy of the right lobe according to Child–Pugh classification.

Conclusion: Our data indicated that MRL is a potentially useful non-invasive examination for evaluation of liver fibrosis associated with viral hepatitis.

Key words: chronic hepatitis, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid, laparoscopy, multiple resonance imaging, multiple resonance laparoscopy

INTRODUCTION

HEPATITIS C VIRUS (HCV) and hepatitis B virus (HBV) are common causes of chronic liver disease worldwide and often lead to liver cirrhosis and hepatocellular carcinoma (HCC).[1–7] The prognosis and clinical management of chronic liver diseases are highly dependent on the extent of liver fibrosis, as complications mainly occur in patients in the advanced stages. Therefore, accurate diagnosis of the extent of fibrosis is needed.

A definite diagnosis of chronic liver disease is established by histological examination of a biopsy specimen and by visualization of the liver surface at laparoscopy.[8–10] Laparoscopy-guided liver biopsy is considered the most accurate method for the diagnosis of liver disease, especially liver cirrhosis.[11,12] However, the use of laparoscopy as a diagnostic tool in liver has diminished over the past decade,[13,14] probably due to problems associated with safety and complexity compared with ultrasound-guided biopsy. Thus, ultrasound-guided liver biopsy is currently the gold standard in assessing liver histology. However, liver biopsy is also associated with at least a few problems even when performed by experienced hepatologists. In fact, obtaining a liver biopsy is an invasive procedure and associated sometimes with life-threatening complications.[15,16] Furthermore, sampling errors and intra- and interobserver variability may lead to over- or underestimation of fibrosis.[17–19]

Owing to the invasiveness of the biopsy procedure and its associated possible complications, the

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development of alternative non-invasive methods to characterize the condition of the liver is highly desirable. Non-invasive approaches to assess histology in patients with chronic liver disease include clinical symptoms and signs, routine laboratory tests, serum markers of fibrosis and inflammation, quantitative assays of liver function, and radiologic imaging studies.[20–31] Although laparoscopy is more invasive than percutaneous liver biopsy, some studies reported that laparoscopy was more accurate than liver biopsy in the diagnosis of cirrhosis and prediction of HCC in patients with chronic viral hepatitis.[12,32,33] However, due to its invasive nature, other non-invasive substitute techniques of laparoscopy are required.

Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) is a liver-specific contrast medium used for multiple resonance imaging (MRI). A bolus injection of Gd-EOB-DTPA allows the evaluation of tumor vascularity in a manner similar to evaluation with Gd-DTPA. Furthermore, this contrast medium accumulates in normally functioning hepatocytes in the hepatobiliary phase, which begins 20 min after injection, and enhances the liver parenchyma. While normally functioning hepatocytes are enhanced in hepatobiliary phase, tumors appear as hypointense lesions because they lack normally functioning hepatocytes.[34,35] Based on these features, we devised multiple resonance laparoscopy (MRL), which includes reconstructed 3-D images obtained from hepatobiliary phase images of Gd-EOB-DTPA-enhanced MRI, to visualize the liver surface. The aim of this study was to assess the utility of this technique as a substitute for laparoscopy and its usefulness for assessment of chronic liver disease.

METHODS

Patients

THIS STUDY INCLUDED 49 patients who each had a small solitary hemangioma with normal liver and underwent Gd-EOB-DTPA-enhanced MRI. They consisted of 29 women and 20 men with a median age of 55 years (range, 32–70). The inclusion criteria were: (i) body mass index within the normal range (median, 21; range, 17–24); (ii) aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within the normal range (median, 15 IU/L; range, 8–24 IU/L); (iii) negative for HCV RNA and hepatitis B surface antigen (HBsAg); (iv) maximum diameter of hemangioma less than 15 mm; (v) daily alcohol intake less than 10 mg/day; and (vi) absence of fatty liver as confirmed by ultrasonography. This study also included 101 hepatitis B patients and 252 hepatitis C patients who underwent Gd-EOB-DTPA-enhanced MRI between April 2008 and February 2011. These patients met the following criteria: (i) positive for HCV RNA or HBsAg; (ii) negative for antinuclear antibodies or antimitochondrial antibodies in the serum, as determined by radioimmunoassay or spot hybridization; and (iii) no history of treatment with corticosteroids, immunosuppressants or antiviral agents. Among these patients, laparoscopy-guided liver biopsy was performed in 52 hepatitis B patients and 57 hepatitis C patients; the other 49 hepatitis B patients and 195 hepatitis C patients were diagnosed with liver cirrhosis clinically. These 244 clinically diagnosed cirrhotic patients were classified into Child–Pugh class A–C according to clinical data. Table 1 summarizes the patients' characteristics. All patients diagnosed with stage F4 fibrosis by histology were classified as Child–Pugh class A. This study was approved by the Institutional Review Board of our hospital. Written informed consent was obtained from all patients.

Laparoscopy

Laparoscopy-guided liver biopsy was performed in 52 hepatitis B patients and 57 hepatitis C patients between April 2008 and February 2011. Details of the procedures of laparoscopy and laparoscopic biopsy were described previously.[32] Based on the irregularities of the liver surface, the laparoscopic findings were classified into three groups: (i) smooth (smooth liver surface or with limited areas of depression); (ii) irregular (the liver surface showed increased numbers of interconnected depressions, possibly resembling ripples or specks); and (iii) nodular (liver surface with nodular formations) as reported in our previous study.[32] The physicians in charge explained the purpose and method of the laparoscopy-guided liver biopsy to each patient, who gave their informed consent for participation. The criterion of laparoscopic diagnosis of liver cirrhosis was the demonstration of multiple nodular irregularities on the liver surface.

Histopathological evaluation

Liver biopsy specimens were obtained using a modified Vim Silverman needle of 2 mm internal diameter (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson-trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen used for examination contained more than six portal areas. Histopathological interpretation of

Table 1 Characteristics of included patients ($n = 353$)

Characteristic	Value		
	Hepatitis B virus ($n = 101$)	Hepatitis C virus ($n = 252$)	All ($n = 353$)
Median age (years)	52 (range 23–71)	62 (37–86)	58 (23–86)
Sex (male/female)	70/31	148/104	218/135
Histological diagnosis (METAVIR)			
F1	13	5	18
F2	10	14	24
F3	13	12	25
F4	16	26	42
Clinical diagnosis			
Child–Pugh A	42	101	143
Child–Pugh B	5	72	77
Child–Pugh C	2	22	24

the specimens was made by experienced liver pathologists who were blinded to the clinical information. Liver fibrosis was evaluated semiquantitatively according to the METAVIR scoring system.[18] Fibrosis was staged on a 0–4 scale (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; F4, cirrhosis).

Viral markers of HCV and HBV

The diagnosis of HCV infection was based on the detection of serum anti-HCV antibodies and positive RNA. Anti-HCV antibodies were tested by the second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). HCV RNA was measured by the Amplicor method (Cobas Amplicor HCV Monitor test ver. 2.0; Roche Diagnostic Systems, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories).

MRL

Multiple resonance imaging was obtained from all patients with a 1.5-T MRI system (Avanto, Siemens-Asahi Meditec, Tokyo, Japan) using a phased-array coil for signal detection. All patients underwent transverse 3-D of the liver with fat suppression (volumetric interpolated breath-hold examination [VIBE]) sequence in a single breath hold (18–20 s) at hepatobiliary phase of 20 min after injection of the contrast medium. Breathing was withheld at exhalation. The contrast medium used was Gd-EOB-DTPA (Primovist; Bayer Schering Pharma, Berlin, Germany) at a dose of 0.025 mmol/kg bodyweight (0.1 mL/kg). The MRI parameters were: TR, 4.3 msec; TE, 2.1 msec; flip angle, 15°; parallel imaging factor 2 (GRAPPA); slice thickness, 1.5 mm; matrix, 192 × 320; and field of view, 360 mm. 3-D reconstructions of the liver were rendered with enhanced MRI data using ZIOSTAION (Zio software, Tokyo, Japan). Various structures around the liver on the 3-D image were marked manually and then cut and removed digitally. Finally, the liver was extracted on the workstation. The following findings were noted on MRL: (i) irregular liver surface; (ii) impression of the right ribs; (iii) enlargement of the lateral segment; and (iv) atrophy of the right lobe. The MRL findings of the liver surface were classified into three categories: (i) smooth (essentially smooth surface of the entire liver or with limited areas of depression); (ii) partially irregular (several interconnected depressions on the surface mainly in the left lobe of the liver, with rippled or speckled appearance); and (iii) diffusely irregular (nodules present on the liver surface). The patient with diffusely irregular liver surface was diagnosed as liver cirrhosis.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, Chicago, IL, USA).

RESULTS

MRL of the normal liver

FIGURE 1 SHOWS the MRL images of the normal liver. The liver surface irregularities in patients with normal liver were classified as smooth ($n = 48$) and partially irregular ($n = 1$), and none showed diffuse

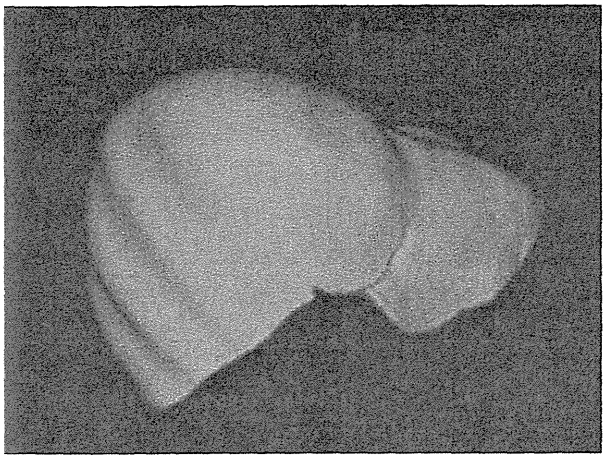

Figure 1 The image of normal liver by magnetic resonance laparoscopy: (i) irregularity of liver surface, smooth; (ii) impression of the right ribs, positive; (iii) enlargement of the lateral segment, negative; and (iv) atrophy of the right lobe, negative.

irregular pattern. Impression of the right ribs on the liver was observed in all patients. None of the patients showed enlargement of the lateral segment or atrophy of the right lobe.

Comparison of biopsy, laparoscopy and MRL

Figure 2(a–c) shows laparoscopic images and MRL image of a representative patient with chronic hepatitis C and fibrosis of F1 stage. The surface findings were well reproduced on the MRL images compared with laparoscopy. Although observation of the liver surface was restricted on laparoscopy, the entire liver surface was visualized on MRL. The correlation between laparoscopic, MRL and histological findings are shown in Table 2. The liver surface patterns on laparoscopy were distributed as follows: smooth, $n = 13$; irregular, $n = 20$; and nodular, $n = 19$. The liver surface patterns on MRL were distributed as follows in patients with HBV: smooth, $n = 14$; partially irregular, $n = 18$; and nodular, $n = 20$. The liver surface patterns on laparoscopy were distributed as follows: smooth, $n = 5$; irregular, $n = 25$; and nodular, $n = 27$. The liver surface patterns on MRL were distributed as follows in patients

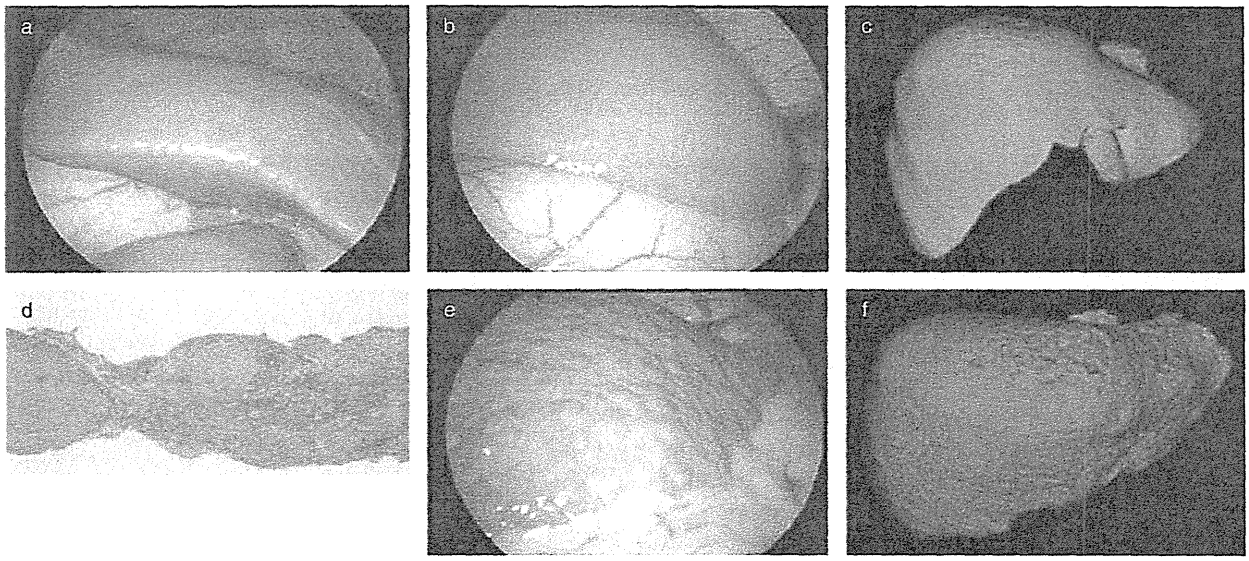

Figure 2 Comparison of laparoscopy, magnetic resonance laparoscopy (MRL) and biopsy. (a–c) Chronic hepatitis C with METAVIR score of F1 examined by laparoscopy and MRL. (a,b) The liver surface was smooth on laparoscopy. (c) On MRL: (i) irregularity of the liver surface, smooth; (ii) impression of the right ribs, positive; (iii) enlargement of the lateral segment, positive; and (iv) atrophy of the right lobe, negative. (d–f) Liver cirrhosis with hepatitis B virus infection. (d) Needle biopsy findings included numerous septa without cirrhosis, indicative of score F3 (hematoxylin-eosin staining, original magnification ×100). (e) Examination by laparoscopy showed nodular liver surface, confirming the diagnosis of cirrhosis. (f) Examination by MRL showed the following features: (i) irregularity of the liver, diffuse irregular; (ii) impression of the right ribs, negative; (iii) enlargement of the lateral segment, positive; and (iv) atrophy of the right lobe, negative.

Table 2 Comparison of laparoscopic, multiple resonance laparoscopic and histological findings

Hepatitis B virus patients ($n = 52$)				
Surface findings of multiple resonance laparoscopy	Surface findings of laparoscopy			
	n	Smooth ($n = 13$)	Irregular ($n = 20$)	Nodular ($n = 19$)
Smooth	14	12	2	0
Partial irregular	18	1	17	0
Diffuse irregular (nodular)	20	0	1	19
Histological findings (METAVIR)	Surface findings of laparoscopy			
	n	Smooth ($n = 13$)	Irregular ($n = 20$)	Nodular ($n = 19$)
F1	13	11	2	0
F2	10	2	8	0
F3	13	0	10	3
F4	16	0	0	16
Hepatitis C virus patients ($n = 57$)				
Surface findings of multiple resonance laparoscopy	Surface findings of laparoscopy			
	n	Smooth ($n = 5$)	Irregular ($n = 25$)	Nodular ($n = 27$)
Smooth	8	5	3	0
Partial irregular	23	0	22	1
Diffuse irregular (Nodular)	26	0	0	26
Histological findings (METAVIR)	Surface findings of laparoscopy			
	n	Smooth ($n = 5$)	Irregular ($n = 25$)	Nodular ($n = 27$)
F1	5	4	1	0
F2	14	1	13	0
F3	12	0	11	1
F4	26	0	0	26

with HCV: smooth, $n = 8$; partially irregular, $n = 23$; and nodular, $n = 26$. There were close similarities in liver surface findings between laparoscopic and MRL. Based on the surface findings on laparoscopy, the sensitivity, specificity, positive predictive value and negative predictive value for the diagnosis of cirrhosis by MRL were 96%, 100%, 95% and 95%, respectively. In comparison, the respective values were 86%, 98%, 97% and 91%, respectively, for the diagnosis by histological examination. These results indicate that MRL is more accurate than needle biopsy in the diagnosis of cirrhosis.

Changes in MRL findings with progression of liver fibrosis

Table 3 lists changes in MRL findings with disease progression. Liver surface irregularities increased with the progression of liver damage. The impression of the right ribs was observed in all livers including normal livers and those with chronic hepatitis. The incidence of impression of the right ribs decreased in patients with cirrhosis classified as Child–Pugh A. Furthermore, the impression of the right ribs disappeared in cases with advanced cirrhosis, namely, patients classified clinically as Child–Pugh B and C. The enlargement of the lateral segment was observed in chronic hepatitis with F2 grade fibrosis and more severe fibrosis. Atrophy of the right lobe was observed in patients with cirrhosis. The incidence of atrophy of the right lobe increased with the progression of Child–Pugh classification. All patients with F4 grade fibrosis were classified as Child–Pugh A, and the proportion of MRL findings was similar in the F4 group and clinically diagnosed group of Child–Pugh A. Figure 3 shows the MRL images for each histopathological and clinical stage, including changes in the liver surface.

Table 3 Proportion of liver surface findings of multiple resonance laparoscopy according to histological and clinical stage

	Irregularities of liver surface			Impression of the right ribs	Enlargement of the lateral segment	Atrophy of the right lobe
	Smooth	Partial irregular	Diffuse irregular (Nodular)			
Hepatitis B virus patients ($n = 101$)						
Histological diagnosis (METAVIR)						
F1 ($n = 13$)	92% (12/13)	8% (1/13)	0% (0/13)	100% (13/13)	46% (6/13)	0% (0/13)
F2 ($n = 10$)	20% (2/10)	80% (8/10)	0% (0/10)	100% (10/10)	90% (9/10)	0% (0/10)
F3 ($n = 13$)	0% (0/13)	69% (9/13)	31% (4/13)	100% (13/13)	100% (13/13)	0% (0/13)
F4 ($n = 16$)	0% (0/16)	0% (0/16)	100% (16/16)	88% (14/16)	100% (16/16)	19% (3/16)
Clinical diagnosis						
Child–Pugh A ($n = 42$)	0% (0/42)	0% (0/42)	100% (42/42)	74% (31/42)	100% (42/42)	19% (8/42)
Child–Pugh B ($n = 5$)	0% (0/5)	0% (0/5)	100% (5/5)	20% (1/5)	40% (2/5)	80% (4/5)
Child–Pugh C ($n = 2$)	0% (0/2)	0% (0/2)	100% (2/2)	0% (0/2)	0% (0/2)	100% (2/2)
HCV patients ($n = 252$)						
Histological diagnosis (METAVIR)						
F1 ($n = 5$)	100% (5/5)	0% (0/5)	0% (0/5)	100% (5/5)	0% (0/5)	0% (0/5)
F2 ($n = 14$)	21% (3/14)	79% (11/14)	0% (0/14)	100% (14/14)	86% (12/14)	0% (0/14)
F3 ($n = 12$)	0% (0/12)	92% (11/12)	8% (1/12)	100% (12/12)	100% (0/12)	0% (0/12)
F4 ($n = 26$)	0% (0/26)	4% (1/26)	96% (25/26)	85% (22/26)	100% (0/26)	12% (3/26)
Clinical diagnosis						
Child–Pugh A ($n = 101$)	0% (0/101)	9% (9/101)	91% (92/101)	83% (84/101)	100% (101/101)	13% (13/101)
Child–Pugh B ($n = 72$)	0% (0/72)	0% (0/72)	100% (72/72)	7% (5/72)	97% (70/72)	96% (69/72)
Child–Pugh C ($n = 22$)	0% (0/22)	0% (0/22)	100% (22/22)	0% (0/22)	45% (10/22)	100% (22/22)

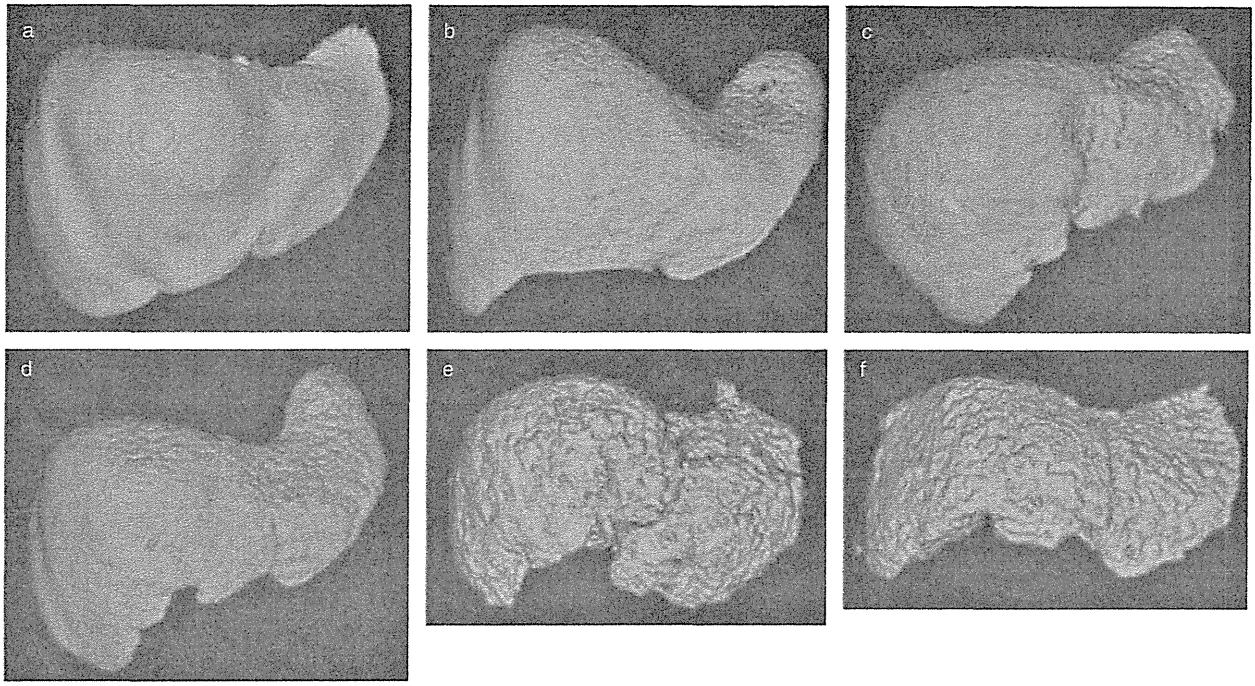

Figure 3 Magnetic resonance laparoscopy images of different stages of liver fibrosis. (a) METAVIR score F1, (b) METAVIR score F2, (c) METAVIR score F3, (d) cirrhosis, Child–Pugh A, (e) cirrhosis, Child–Pugh B, (f) cirrhosis, Child-Pugh C.

Comparison of MRL findings in patients with HBV and HCV

Table 3 shows proportion of liver surface findings of MRL in each stage of fibrosis which was diagnosed by histological examination. Diffuse irregular pattern was not observed in both HCV and HBV patients with F1 and F2 stage fibrosis. But in HBV patients with F3 stage fibrosis, higher proportion of diffuse irregular pattern was observed compare to HCV patients with F3 stage fibrosis. Almost all liver cirrhosis patients showed diffuse irregular pattern in MRL. In general, the MRL findings were almost similar in HBV- and HCV-related chronic liver disease.

DISCUSSION

ORLANDO *ET AL.* reported that evaluation of both laparoscopy and liver biopsy was useful. In his study, when the two techniques were considered separately, a final diagnosis of cirrhosis was possible in 78.4% by laparoscopy and in 78.8% by biopsy, whereas, doing both procedures improved the diagnostic yield to 97.7% by decreasing the percentage of false negatives for each technique.[36] Wetzke-Braun *et al.* also reported that diagnostic laparoscopy was more accurate than liver biopsy in recognizing cirrhosis in patients with chronic hepatitis C.[12] These results suggest that laparoscopic examination of the liver is superior for the diagnosis of liver cirrhosis compared with liver biopsy. In this study, comparison of MRL and liver biopsy in the diagnosis of liver cirrhosis showed sensitivity, specificity, positive predictive value and negative predictive value of 96% versus 86%, 100% versus 98%, 95% versus 97% and 95% versus 91%, respectively. Furthermore, the surface findings on MRL could be stratified according to the laparoscopic findings (Table 2). These results suggested MRL could evaluate the surface findings of liver in the same way as laparoscopy.

However, liver biopsy is currently considered as the gold standard for assessing liver fibrosis, this procedure has certain problems, though it is in general a safe procedure. It is an invasive technique with a morbidity rate of 3% and mortality rate of 0.003%.[15,16] In addition, liver biopsy examination carries intra- and interobserver variability in the staging of liver fibrosis, with sampling error mainly due to the small size of the harvested specimen, which represents at most 1/50 000 of the entire liver mass.[17–19] In this study, 19 HBV patients and 27

HCV patients were diagnosed as having cirrhosis by laparoscopy. Among these cirrhotic patients, three HBV patients and one HCV patient were diagnosed as having F3 fibrosis with liver biopsy. Liver cirrhosis was likely to be underestimated in HBV patients with liver biopsy. The higher proportion of diffuse irregular liver surface among HBV patients with F3 stage fibrosis was probably due to sampling error with needle biopsy. But there were no underestimated HBV patient diagnosed as having cirrhosis by laparoscopy (Table 2). With regard to cirrhosis, the surface findings of HBV and HCV are different. HBV-related cirrhosis is associated with the presence of large regenerative nodules. Thus, the discrepancy between MRL and needle biopsy was the type of viral infection. But this gap in diagnosis of liver cirrhosis can be filled by evaluating liver surface irregularities by MRL.

The indication for laparoscopy is limited due to its invasiveness. But MRL is derived from the construction of images of hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI, patients with contraindication for laparoscopy (e.g. bleeding tendency, old age) can be evaluated safely by the non-invasive MRL, as long as there are no contraindications for Gd-EOB-DTPA-enhanced MRI. Moreover, this examination can be repeated easily unlike laparoscopic examination. Further studies are needed to determine the usefulness of repeat MRL in the follow up of liver fibrosis.

Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid is a liver-specific contrast material for MRI and its safety and usefulness for detection of HCC has been reported.[35,37] The treatment strategy for HCC is different from other solid tumors because HCC develops in the cirrhotic liver. The Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy has gained wide acceptance because of its stratification capacity and its treatment indication.[38] The BCLC staging system is based on factors related to tumor stage, liver functional reserve and performance status because the prediction of prognosis is related to not only tumor stage but also underlying liver function in patients with HCC. In the present study, MRL showed that the liver surface findings changed with the progression of liver fibrosis. Especially, advanced liver cirrhosis was associated with increased incidence of right lobe atrophy (Table 3). In other words, advanced liver cirrhosis indicates reduction of functional liver reserve. Because patients of Child–Pugh B and C have impaired liver function, radical treatment for HCC must be avoided. As mentioned above, Gd-EOB-DTPA-enhanced MRI is used for evaluation of HCC stage. MRL is technically Gd-EOB-DTPA-enhanced MRI; this "one-stop-shop" method provides both HCC staging and liver functional reverse. Although transient elastography and multiple resonance elastography are useful non-invasive techniques for evaluation of liver fibrosis, they cannot be used to determine the stage of HCC. Thus, MRL may have an advantage in this respect.

Our study has certain limitations. First, because the 3-D shape of the liver is derived in our technique from MRI using Gd-EOB- DTPA contrast material, the technique does not allow evaluation of the color of the liver surface. Fujioka *et al.* reported that liver color was useful for the diagnosis of primary biliary cirrhosis.[39] Therefore, MRL is not entirely a complete substitute for laparoscopy in this regard. However, our method allows evaluation of the shape of the entire liver non-invasively similar to laparoscopy even in patients contraindicated for laparoscopy. Second, this study is based on the results of patients with chronic HBV or HCV infection. Patients with chronic liver diseases of other causes were not evaluated. However, HBV and HCV infections are two of the major causes of chronic liver disease and hepatocarcinogenesis, and were able to demonstrate the usefulness of MRL in high-risk groups. Further studies are needed to evaluate the usefulness of MRL in patients with chronic hepatitis of other etiologies. Third, the number of patients evaluated by laparoscopy-guided liver biopsy was small. So, further studies with a large number of patients are needed to strengthen usefulness of MRL.

Chronic viral liver disease is regarded as a high-risk for HCC. Gd-EOB-DTPA-enhanced MRI is a useful screening technique for HCC. The data of MRL are derived from Gd-EOB-DTPA-enhanced MRI. MRL and Gd-EOB-DTPA-enhanced MRI can be conducted simultaneously. In conclusion, MRL is a potentially useful non-invasive method for the evaluation of chronic viral hepatitis.

REFERENCES

1 Kiyosawa K, Fukura S. Review of hepatitis C in Japan. *J Gastroenterol Hepatol* 1991; **6**: 383–91.
2 Alter MJ, Margolis HS, Kwaczynski K *et al.* The natural history of community acquired hepatitis C in the United States. *N Engl J Med* 1992; **327**: 1889–905.
3 Liaw YF, Chu CH. Hepatitis B virus infection. *Lancet* 2009; **373** (9663): 582–92.
4 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; **28**: 930–8.

5 Hasan F, Jeffers LJ, De Medina M *et al.* Hepatitis C-associated hepatocellular carcinoma. *Hepatology* 1990; 12 (3 Pt 1): 589–91.
6 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; 328: 1797–801.
7 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospectice observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
8 Boyce HW. Diagnostic laparoscopy in liver and biliary disease. *Endoscopy* 1992; 24: 676–81.
9 Nord HJ. Complication of laparoscopy. *Endoscopy* 1992; 24: 693–700.
10 Poniachik J, Bernstein DE, Reddy KR *et al.* The role of laparoscopy in the diagnosis of cirrhosis. *Gastrointest Endosc* 1996; 43: 568–71.
11 Cardi M, Muttillo IA, Amadori L *et al.* Superiority of laparoscopy compared to ultrasonography in diagnosis of widespread liver diseases. *Dig Dis Sci* 1997; 42: 546–8.
12 Wietzke-Braun P, Braun F, Schott P, Ramadori G. Is laparoscopy an advantage in the diagnosis of cirrhosis in chronic hepatitis C virus infection? *World J Gastroenterol* 2003; 9: 745–50.
13 Gainai S, Gramantieri L, Venturoli N *et al.* What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. *J Hepatol* 1997; 27: 979–85.
14 Helmreich-Becker I, Meyer zum Buschenfelde KH, Lohse AW. Safety and feasibility of a new minimally invasive diagnostic laparoscopy technique. *Endoscopy* 1998; 30: 756–62.
15 Piccinino F, Sagenelli E, Pasquale G, Giusti G. complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986; 2: 165–73.
16 Montalto G, Soresi M, Carrocio A *et al.* Percutaneous liver biopsy: a safe outpatient procedure? *Digestion* 2001; 63: 55–60.
17 Regev A, Berho M, Jeffes LJ *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; 97: 2614–8.
18 Bedossa P, Poynaud T; The Metavir Cooperative Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; 20 (1 Pt 1): 15–20.
19 Westin J, Lagging LM, Wejstal R, Norkans G, Dhillon AP. Interobserver study of liver histology using the Ishak score in patients with chronic hepatitis C virus infection. *Liver* 1999; 19: 183–7.
20 Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis: relationship to cirrhosis. *Gastroenterology* 1988; 95: 734–9.
21 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518–26.
22 Sterling RK, Lissen E, Clumeck N *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43: 1317–25.
23 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. *Hepatol Res* 2000; 18: 252–66.
24 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705–13.
25 Ziol M, Handra-Luca A, Kettaneh A *et al.* Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; 41: 48–54.
26 Castéra L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343–50.
27 Tanimoto A, Yuasa Y, Shinmoto H *et al.* Superparamagnetic iron oxide-mediated hepatic signal intensity change in patients with and without cirrhosis: pulse sequence effects and Kupffer cell function. *Radiology* 2002; 222: 661–6.
28 Sandrasegaran K, Akisik FM, Lin C *et al.* Value of diffusion -weighted MRI for assessing liver fibrosis and cirrhosis. *AJR Am J Roentgenol* 2009; 193: 1556–60.
29 Boulanger Y, Amara M, Lepanto L *et al.* Diffusion-weighted MR imaging of the liver of hepatitis C patients. *NMR Biomed* 2003; 16: 132–6.
30 Munakata T, Griffiths RD, Martin PA, Jenkins SA, Shields R, Edwards RH. An *in vivo* ^{31}P MRS study of patients with liver cirrhosis: progress towards a non-invasive assessment of disease severity. *NMR Biomed* 1993; 6: 168–72.
31 Rouvière O, Yin M, Dresner MA *et al.* MR elastography of the liver: preliminary results. *Radiology* 2006; 240: 440–8.
32 Arase Y, Suzuki F, Suzuki Y *et al.* Potential of laparoscopy in chronic liver disease with hepatitis B and C viruses. *Hepatol Res* 2008; 38: 877–85.
33 Shiraki K, Shimizu A, Takase K *et al.* Prospective study of laparoscopic findings with regard to the development of the hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Gastrointest Endosc* 2001; 53: 449–55.
34 Saito K, Kotake F, Ito N *et al.* Gd-EOB-DTPA enhanced MR imaging for hepatocellular carcinoma: quantitative evaluation of tumor enhancement in hepatobiliary phase. *Magn Reson Med Sci* 2005; 4: 1–9.
35 Kogita S, Imai Y, Okada M *et al.* Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma:

correlation with histological grading and portal blood flow. *Eur Radiol* 2010; 20: 2405–13.
36 Orlando R, Lirussi F, Okolicsanyi L. Laparoscopy and liver biopsy: further evidence that the two procedures improve the diagnosis of liver cirrhosis. A retrospective study of 1,003 consecutive examinations. *J Clin Gastroenterol* 1990; 12: 47–52.
37 Bluemke DA, Sahani D, Amendola M *et al.* Efficacy and safety of MR imaging with liver-specific contrast agent: U.S. multicenter phase III study. *Radiology* 2005; 237: 89–98.
38 Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; 19: 329–38.
39 Fujioka S, Yamamoto K, Okamoto R *et al.* Laparoscopic features of primary biliary cirrhosis in AMA-positive and AMA-negative patients. *Endoscopy* 2002; 34: 318–21.

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Efficacy and Anticarcinogenic Activity of Ribavirin Combination Therapy for Hepatitis C Virus-Related Compensated Cirrhosis

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Hepatocellular carcinoma · Cirrhosis · Biochemical response

Abstract
Objective: Anticarcinogenic activity of ribavirin combination therapy for hepatitis C virus (HCV)-related compensated cirrhosis is still unclear. ***Methods:*** In study 1, in 157 consecutive patients with HCV-related compensated cirrhosis, treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b. In study 2, in 185 consecutive patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, hepatocarcinogenesis rates were evaluated according to the additional treatment, and they were classified into three groups: no treatment, interferon monotherapy, and ribavirin combination therapy. ***Results:*** In study 1, in HCV-1b, rates of sustained virological response and sustained biochemical response were 21 and 56%, respectively. In HCV-2a/2b, rates of sustained virological response and sustained biochemical response were 70 and 78%, respectively. In HCV-1b, sustained biochemical response rates were significantly higher than those of sustained virological response. In study 2, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy and no treatment, respectively. ***Conclusion:*** Ribavirin combination therapy for HCV-related compensated cirrhosis reduces the risk of hepatocarcinogenesis in comparison with interferon monotherapy, and higher rates of sustained biochemical response might be associated with lower hepatocarcinogenesis rates.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of hepatocellular carcinoma during the clinical course [3]. Because an effective and curative therapy for hepatocellular carcinoma remains

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Table 1. Profile and laboratory data at the start of ribavirin combination therapy in 157 patients with HCV-related compensated cirrhosis (study 1)

Demographic data	
Patients, n	157[1]
Sex (male/female), n	105/52
Age, years	58 (34–74)
Laboratory data	
Serum aspartate aminotransferase, IU/l	69 (7–235)
Serum alanine aminotransferase, IU/l	70 (14–585)
Leukocytes, /mm^3	4,100 (1,600–8,800)
Hemoglobin, g/dl	14.0 (9.4–17.6)
Platelet count, $\times 10^4$/mm^3	11.3 (6.1–32.2)
HCV genotype (1b/2a/2b), n	120/27/10
Levels of viremia, log IU/ml	6.1 (3.9–7.5)
Treatment	
Past history of interferon-based therapy, n	95 (60.5%)
PEG-IFNα-2b/IFNα-2b, n	110/47
Ribavirin dose, mg/kg	10.7 (2.7–15.1)
Duration of treatment, weeks	
Genotype 1b	48 (1–48)
Genotype 2a or 2b	24 (5–24)

Unless otherwise indicated, values represent median (range).

[1] 24 of the 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

limited at best, primary prevention of hepatocellular carcinoma in patients with chronic liver disease is of great importance at present.

Treatment of HCV-chronic hepatitis with interferon can induce viral clearance and marked biochemical and histological improvement [6, 7]. Furthermore, previous studies showed that interferon monotherapy reduced the risk of hepatocellular carcinoma [8–10]. However, an extended analysis of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) cohort recently showed that long-term peginterferon (PEG-IFN) monotherapy could not reduce the incidence of hepatocellular carcinoma among patients with advanced hepatitis C who did not achieve sustained virological response, and patients with cirrhosis who received PEG-IFN monotherapy had a lower risk of hepatocellular carcinoma than controls [11]. Thus, it is controversial whether interferon monotherapy for patients with liver cirrhosis might reduce hepatocarcinogensis. Furthermore, it is still unclear whether ribavirin combination therapy for patients with liver cirrhosis might reduce the risk of hepatocellular carcinoma, and there are also no reports on whether ribavirin combination therapy could reduce the risk in comparison with interferon monotherapy.

The present study investigated the efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis, especially in comparison with interferon monotherapy.

Materials and Methods

Study Population

Two retrospective cohort studies were performed to investigate treatment efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis.

In the study 1 cohort, 157 consecutive patients of HCV-related compensated cirrhosis were recruited into the study protocol of interferon (PEG-IFNα-2b or IFNα-2b) plus ribavirin combination therapy for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b, from 2001 to 2010 at Toranomon Hospital. In this retrospective study the rates of sustained virological response [HCV-RNA negativity at 24 weeks after the completion of therapy based on the COBAS TaqMan HCV test (Roche Diagnostics)] were evaluated as well as sustained biochemical response [normal level of serum alanine aminotransferase at 24 weeks after the completion of therapy (6–50 IU/l)]. Treatment efficacy was evaluated by intention-to-treat (ITT) analysis classified as treatment failure in patients who could not complete the treatment regimen and per protocol (PP) analysis. Table 1 summarizes the profiles and data of the 157 patients at the commencement of combination therapy with interferon plus ribavirin in study 1. They included 105 men and 52 women aged 34–74 years (median 58 years). 110 (70.1%) patients received PEG-IFNα-2b plus ribavirin, and the remaining 47 (29.9%) patients received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.3 μg/kg (range 0.5–1.9 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week). They also received oral ribavirin at a median dose of 10.7 mg/kg (range 2.7–15.1 mg/kg) daily. In 56 of the 157 (35.7%) patients, the dose of ribavirin was reduced during treatment due to a fall in hemoglobin concentration. The median total duration of treatment in 120 patients of HCV-1b was 48 weeks (range 1–48 weeks), and that in 37 patients of genotype 2a or 2b was 24 weeks (range 5–24 weeks).

In the study 2 cohort (fig. 1), 185 consecutive patients of HCV-related compensated cirrhosis, who showed no sustained virological response following at the first course of interferon monotherapy (≥24 weeks) from 1987 to 2010 at Toranomon Hospital, were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and were classified into three groups: no treatment (106 patients), interferon monotherapy (≥24 weeks; 55 patients), and ribavirin combination therapy (≥24 weeks; 24 patients). 106 patients without treatment did not receive the additional treatment because of concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression and car-

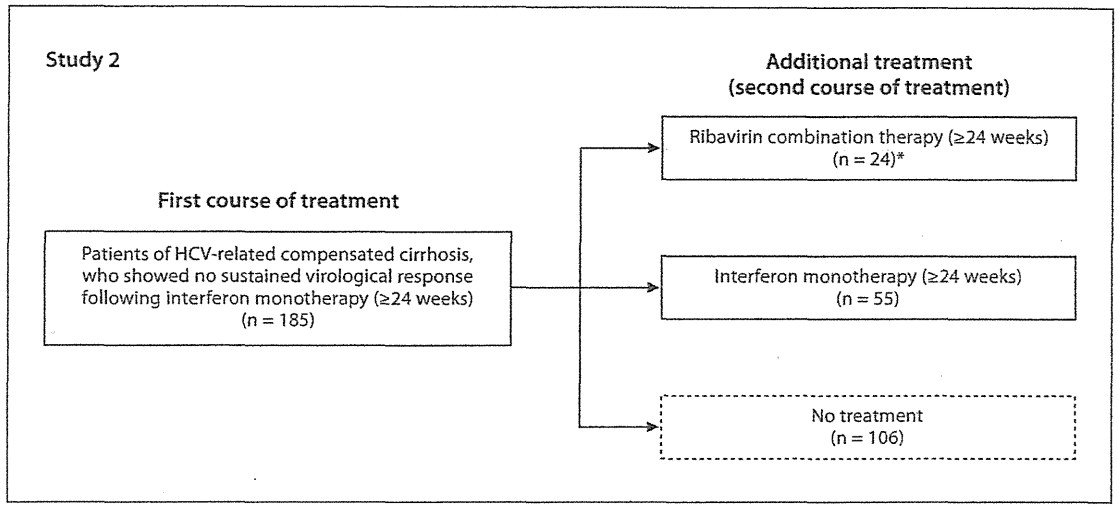

Fig. 1. For study 2, 185 patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks), were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and patients were classified into three groups: no treatment, interferon monotherapy (≥24 weeks), and ribavirin combination therapy (≥24 weeks). * 24 of 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2.

diopulmonary disease during and after the first course of interferon monotherapy or the lower levels of serum alanine aminotransferase. The median follow-up time, from the end of the first course of interferon monotherapy until the last visit, was 6.4 years (range 0.0–21.0 years). 24 of the 157 patients in study 1 were also included in study 2; they showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

At the additional treatment of interferon monotherapy, 43 patients (78.2%) received IFNα alone, and the remaining 12 patients (21.8%) received IFNβ alone. They received interferon monotherapy including initial aggressive induction therapy (every day for 8 weeks followed by 3 times per week), with a median treatment duration of 44 weeks (range 24–382 weeks) at a median dose of 3 million units (range 3–10 million units) intramuscularly each day.

At the additional treatment of ribavirin combination therapy, 11 patients (45.8%) received PEG-IFNα-2b plus ribavirin, and the remaining 13 patients (54.2%) received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range 0.8–1.7 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week), with a median treatment duration of 26 weeks (range 24–48 weeks). They also received oral ribavirin at a median dose of 11.0 mg/kg (range 3.0–12.5 mg/kg) daily.

In the present studies, the patients were selected based on the following criteria. (1) Patients had compensated cirrhosis, but no decompensated cirrhosis or hepatocellular carcinoma. The diagnosis of compensated cirrhosis was based on clinical features (absence of signs for decompensation of ascites, encephalopathy, or gastrointestinal bleeding), laboratory tests, and peritoneoscopy or liver biopsy. (2) Patients were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA by qualitative or quantitative analysis. (3) Patients were free of coinfection with human immunodeficiency virus. (4) Lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (5) Patients were free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (6) Each patient signed a consent form of the study protocol that had been approved by the human ethics review committee.

Laboratory Investigations

Blood samples were frozen at –80° within 4 h of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [12]. HCV-RNA quantitative analysis was measured by branched DNA assay version 2.0 (Chiron Corp., Emeryville, Calif., USA), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems Inc., Pleasanton, Calif., USA), or COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). High viral load of viremia levels was defined as branched DNA assay ≥1.0 MEq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay <1.0 MEq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV-RNA qualitative analysis (Amplicor, Roche Diagnostics, Mannheim, Germany) was 100 copies/ml, and that of

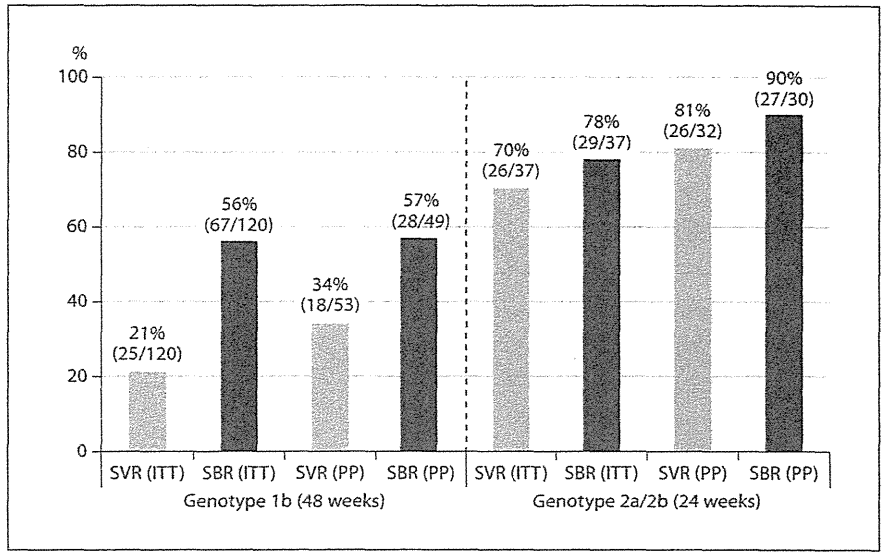

Fig. 2. In 157 patients with HCV-related compensated cirrhosis treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b or 24 weeks of genotype 2a/2b. In HCV genotype 1b, rates of sustained biochemical response (SBR) were significantly higher than those of sustained virological response (SVR; ITT analysis, $p < 0.001$, and PP analysis, $p = 0.028$).

COBAS TaqMan HCV test was 1.2 log IU/ml. The undetectable samples by HCV-RNA qualitative analysis or COBAS TaqMan HCV test were defined as negative HCV-RNA.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month before, during, and after treatment, and were also analyzed for levels of serum alanine aminotransferase and HCV-RNA at various time points.

Patients were examined for hepatocellular carcinoma by abdominal ultrasonography every 3–6 months. If hepatocellular carcinoma was suspected based on ultrasonographic results, additional procedures, such as computed tomography, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy if necessary, were used to confirm the diagnosis.

Statistical Analysis

χ^2 test, Fisher's exact probability test, and Mann-Whitney's U test were used to compare the background characteristics between groups. Multiple comparisons were examined by the Bonferroni test. The cumulative hepatocarcinogenesis rates were calculated using the Kaplan-Meier technique, and differences between the curves were tested using the log-rank test. Statistical analysis of the hepatocarcinogenesis rates according to groups was calculated using the period from the end of the first course of interferon monotherapy until the appearance of hepatocellular carcinoma or until the last visit or until the start of the third course of interferon-based treatment. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with hepatocarcinogenesis. The hazard ratio (HR) and 95% confidence interval were also calculated. Potential predictive factors associated with hepatocarcinogenesis included the following 13 variables: age, sex, serum aspartate aminotransferase, serum alanine aminotransferase, platelet count, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, HCV genotype, levels of viremia, total duration of additional treatment, and group of additional treatment. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All p values <0.05 and <0.1 by the two-tailed test were considered significant ($p < 0.05$) and marginally significant ($p < 0.1$), respectively. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were tested by multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Efficacy of Ribavirin Combination Therapy (Study 1)

Treatment efficacy of a 48-week regimen of interferon plus ribavirin combination therapy in 120 patients infected with HCV-1b was evaluated. In ITT analysis, rates of sustained virological response and sustained biochemical response were 21% (25 of 120 patients) and 56% (67 of 120 patients), respectively. In the PP analysis, rates of sustained virological response and sustained biochemical response were 34% (18 of 53 patients) and 57% (28 of 49 patients), respectively (fig. 2). In both analyses, rates of sustained biochemical response were significantly higher than those of sustained virological response (ITT analysis, $p < 0.001$, and PP analysis, $p = 0.028$).

Table 2. Profile and laboratory data of 185 patients with HCV-related compensated cirrhosis according to additional treatment groups (study 2)

	No treatment	Interferon monotherapy (≥24 weeks)	Ribavirin combination therapy[1] (≥24 weeks)
Demographic data			
Patients, n	106	55	24
Sex (male/female), n	64/42	37/18	20/4
Age, years	56 (30–75)[a]	56 (35–76)[b]	51 (34–68)
Laboratory data			
Serum aspartate aminotransferase, IU/l	75 (26–285)	83 (35–213)	62 (30–160)
Serum alanine aminotransferase, IU/l	92 (17–400)	104 (30–316)	93 (36–250)
Platelet count, $\times 10^4/mm^3$	10.7 (2.5–18.2)[c]	10.8 (5.7–19.8)[d]	13.0 (5.2–23.5)
Total cholesterol, mg/dl	165 (103–273)[h]	152 (101–220)	160 (111–211)
High-density lipoprotein cholesterol, mg/dl	46 (25–93)	43 (21–65)	47 (28–56)
Low-density lipoprotein cholesterol, mg/dl	93 (38–168)	87 (45–139)	100 (34–135)
Triglycerides, mg/dl	96 (36–437)	80 (51–215)	108 (52–206)
HCV genotype (1b/2a or 2b), n	70/36	39/16	17/7
Levels of viremia (high viral load/low viral load), n	84/16	37/15[e]	24/0
Additional treatment			
Duration of additional treatment, weeks	–	44 (24–382)[f]	26 (24–48)
Sustained virological response (ITT), n	–	11 (20%)	7 (29%)
Sustained biochemical response (ITT), n	–	25 (45%)[g]	16 (67%)

Unless otherwise indicated, values represent median (range).

Demographic data and laboratory data, at the start of the first course of interferon monotherapy, are shown.

[a] $p = 0.013$, [b] $p = 0.030$, [c] $p = 0.002$, [d] $p = 0.015$, [e] $p = 0.006$, [f] $p = 0.044$, [g] $p = 0.083$ compared with ribavirin combination therapy by Bonferroni test, Mann-Whitney U test, or χ^2 test. [h] $p = 0.039$ compared with interferon monotherapy by Bonferroni test.

[1] 24 of 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks), and were additionally treated with ribavirin combination therapy (≥24 weeks).

Treatment efficacy of a 24-week regimen of interferon plus ribavirin combination therapy in 37 patients infected with HCV-2a or 2b was evaluated. In the ITT analysis, rates of sustained virological response and sustained biochemical response were 70% (26 of 37 patients) and 78% (29 of 37 patients), respectively. In the PP analysis, rates of sustained virological response and sustained biochemical response were 81% (26 of 32 patients) and 90% (27 of 30 patients), respectively (fig. 2). In both analyses, rates of the sustained biochemical response were not significantly higher than those of the sustained virological response.

Profile, Laboratory Data, and Efficacy according to Additional Treatment Groups (Study 2)

Profile and laboratory data, at the start of the first course of interferon monotherapy of 185 patients with HCV-related compensated cirrhosis, are summarized in table 2. The age of patients with ribavirin combination therapy was significantly lower than that of patients with no treatment ($p = 0.013$; Bonferroni test) and interferon monotherapy ($p = 0.030$; Bonferroni test). The platelet count of patients of ribavirin combination therapy was significantly higher than that of patients without treatment ($p = 0.002$; Bonferroni test) and interferon monotherapy ($p = 0.015$; Bonferroni test). The total cholesterol level of patients with interferon monotherapy was significantly lower than that of patients without treatment ($p = 0.039$; Bonferroni test). Low viral load rates of patients with interferon monotherapy were significantly higher than those of patients with ribavirin combination therapy ($p = 0.006$; Bonferroni test). There were no other significant differences in clinical features at the start of the first course of interferon monotherapy among the three groups.

Additional treatment duration of only 1 patient, who was diagnosed with hepatocellular carcinoma during additional treatment, was evaluated using the period from the start of the second course of interferon monotherapy

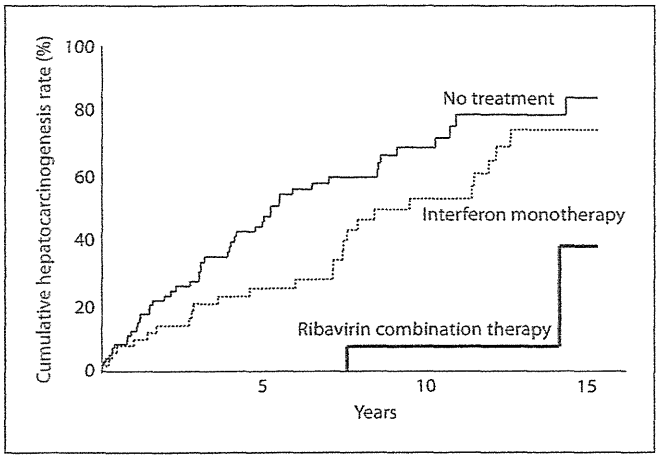

Fig. 3. Cumulative hepatocarcinogenesis rates in the three groups of additional treatment. The rates in no treatment were significantly higher than those in interferon monotherapy ($p = 0.047$; log-rank test) and ribavirin combination therapy ($p < 0.001$; log-rank test), and the rates in interferon monotherapy were significantly higher than those in ribavirin combination therapy ($p < 0.001$; log-rank test).

Table 3. Factors associated with hepatocarcinogenesis in 185 patients of HCV-related compensated cirrhosis identified by multivariate analysis (study 2): Cox proportional hazard model

Factors/category	Hazard ratio (95% confidence interval)	p
Additional treatment		
Ribavirin combination therapy	1	
Interferon monotherapy	4.47 (1.04–19.3)	0.045
No treatment	9.14 (2.19–38.2)	0.002
Age		
<55 years	1	
≥55 years	2.87 (1.76–4.67)	<0.001
Aspartate aminotransferase		
<58 IU/l	1	
≥58 IU/l	2.11 (1.20–3.74)	0.010

until the appearance of hepatocellular carcinoma. During additional treatment, the total duration of interferon monotherapy was significantly longer than that of ribavirin combination therapy ($p = 0.044$; Mann-Whitney U test). In ITT analysis, sustained virological response rates of ribavirin combination therapy (29%) were not different from those of interferon monotherapy (20%), but sustained biochemical response rates of ribavirin combination therapy (67%) tended to be higher than those of interferon monotherapy (45%; $p = 0.083$; χ^2 test) (table 2).

Predictive Factors Associated with Hepatocarcinogenesis by Multivariate Analysis

The data for the whole population sample were analyzed to determine those factors that could predict hepatocarcinogenesis. Hepatocarcinogenesis rates in older patients (≥55 years), in patients with higher levels of aspartate aminotransferase (≥58 IU/l), and lower levels of platelet count (<15.0 × 10^4/mm^3) were significantly higher than those in younger patients (<55 years), in patients with lower levels of aspartate aminotransferase (<58 IU/l), and higher levels of platelet count (≥15.0 × 10^4/mm^3), respectively ($p < 0.001$, $p = 0.006$, and $p = 0.017$; log-rank test). Furthermore, the rates in no treatment were significantly higher than those in interferon monotherapy ($p = 0.047$; log-rank test) and ribavirin combination therapy ($p < 0.001$; log-rank test), and the rates in interferon monotherapy were significantly higher than those in ribavirin combination therapy ($p < 0.001$; log-rank test) (fig. 3). Thus, univariate analysis identified four parameters that significantly correlated with hepatocarcinogenesis. These factors were entered into multivariate analysis, which then identified three parameters that significantly influenced hepatocarcinogenesis independently: additional treatment (no treatment; HR 9.14, $p = 0.002$), age (≥55 years; HR 2.87, $p < 0.001$), and levels of aspartate aminotransferase (≥58 IU/l; HR 2.11, $p = 0.010$) (table 3).

The data for 167 patients, except for 18 patients who showed a sustained virological response following additional treatment, were also analyzed to determine those factors that could predict hepatocarcinogenesis. Hepatocarcinogenesis rates in older age (≥55 years) and higher levels of aspartate aminotransferase (≥58 IU/l) were significantly higher than those in younger age (<55 years) and lower levels of aspartate aminotransferase (<58 IU/l), respectively ($p < 0.001$ and $p = 0.007$; log-rank test). Furthermore, the rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy ($p < 0.001$; log-rank test) and no treatment ($p < 0.001$; log-rank test) (fig. 4). Thus, univariate analysis identified three parameters that significantly correlated with hepatocarcinogenesis. These factors were entered into multivariate analysis, which then identified three parameters that significantly influenced hepatocarcinogenesis independently: additional treatment (no treatment; HR 7.87, $p = 0.005$), age (≥55 years; HR 2.52, $p < 0.001$), and levels of aspartate aminotransferase (≥58 IU/l; HR 2.13, $p = 0.010$) (table 4).

Discussion

One of our previous studies indicated that the cancer-suppressive activity of interferon monotherapy in patients with HCV-RNA eradication was similar to that in patients with alanine aminotransferase normalization without HCV-RNA elimination [9]. Other studies also indicated a higher incidence and more rapid development of hepatocellular carcinoma in HCV patients with high levels of alanine aminotransferase [13, 14]. Collectively, these results suggest that the carcinogenic process in patients with chronic HCV infection is enhanced by high levels and fluctuations of alanine aminotransferase, and indicate a close relationship between suppression of inflammatory necrosis of hepatocytes and a lower incidence of hepatocellular carcinoma in patients with HCV-associated chronic liver disease. Recent studies based on interferon plus ribavirin combination therapy also showed that the attainment of sustained virological response or lower levels of alanine aminotransferase after ribavirin combination therapy could reduce the rates of hepatocellular carcinoma [15, 16], but the small numbers of patients with compensated cirrhosis (5% or less of all patients) were recruited. The present study 1 based on the patients with compensated cirrhosis showed that rates of sustained virological response and sustained biochemical response in HCV-2a/2b were high rates of 70 and 78%, and that rates of sustained biochemical response (57%) were significantly higher than those of sustained virological response (34%) in HCV-1b. Furthermore, the present study 2 based on the patients with compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, also showed that sustained biochemical response rates of ribavirin combination therapy (67%) tended to be higher than those of interferon monotherapy (45%). Thus, in ribavirin combination therapy for compensated cirrhosis, higher rates of sustained biochemical response might be associated with lower rates of hepatocarcinogenesis. One limitation is that the present study was performed based on the small numbers of patients who showed no sustained virological response with interferon monotherapy. In further prospective studies a larger number of patients need to be investigated to confirm this finding.

Previous studies have shown that gender, age, fibrosis stage, alanine aminotransferase, and interferon regimen are important pretreatment predictors of hepatocarcinogenesis [9, 10, 17]. In the present study 2 based on the patients with compensated cirrhosis, higher age and aspartate aminotransferase were associated with higher hepatocarcinogenesis rates in the whole population sample and in the sample which excluded patients who showed sustained virological response following additional treatment. Furthermore, as treatment-related factors, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy. Thus, in patients with compensated cirrhosis representing a high-risk group of hepatocarcino-

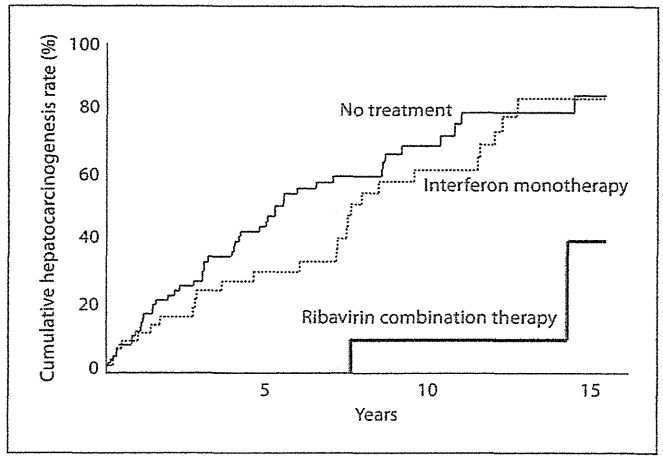

Fig. 4. Cumulative hepatocarcinogenesis rates in the three groups of additional treatment, except for patients who showed sustained virological response following additional treatment. The rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy ($p < 0.001$; log-rank test) and no treatment ($p < 0.001$; log-rank test).

Table 4. Factors associated with hepatocarcinogenesis in 167 patients of HCV-related compensated cirrhosis, except for 18 patients who showed sustained virological response following additional treatment identified by multivariate analysis (study 2): Cox proportional hazard model

Factors/category	Hazard ratio (95% confidence interval)	p
Additional treatment		
Ribavirin combination therapy	1	
Interferon monotherapy	4.68 (1.08–20.3)	0.039
No treatment	7.87 (1.89–32.9)	0.005
Age		
<55 years	1	
≥55 years	2.52 (1.54–4.11)	<0.001
Aspartate aminotransferase		
<58 IU/l	1	
≥58 IU/l	2.13 (1.20–3.79)	0.010