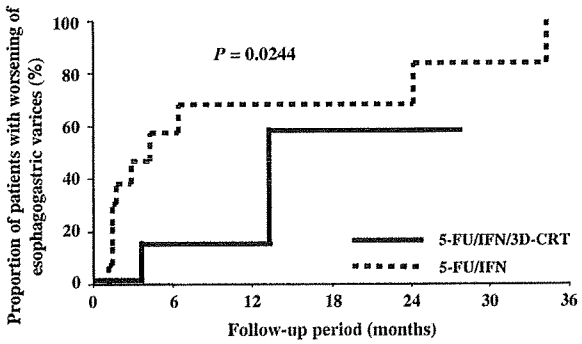

Fig. 2 Proportion of patients with worsening of esophagogastric varices. Comparison between patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

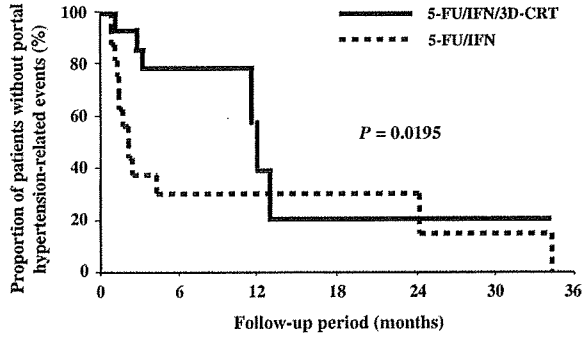

Fig. 3 Proportion of patients without portal hypertension-related events (variceal rupture, worsening of esophagogastric and emerging of uncontrollable ascites). Comparison between patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

difference in the cumulative PREs-free rate between the RT group and the non-RT group was significant (P = 0.0195, Fig. 3).

The median PS worsening-free periods were 6.6 (range 1.2–35.2) and 2.8 months (range 1.0–59.0) for the RT group and non-RT group, respectively. The median PS worsening-free period was longer in the RT group than in the non-RT group.

Survival

Data of the 32 patients showed that the median survival time (MST) was 7.9 months (95% CI, 4.6–11.2 months), and the cumulative survival rates at 6, 12 and 24 months were 61.3, 30.1 and 21.5%, respectively. The MST of the RT group [7.5 months (95% CI, 0.0–15.0 months)] was not significantly different from that of the non-RT group [7.9 months (95% CI, 6.1–9.7 months)] (P = 0.871, Fig. 4).

Univariate analysis identified positivity of HCV antibody (P = 0.0009), PS = 0 (P = 0.0003), absence of extrahepatic metastases (P = 0.0002) and objective response of both intrahepatic HCC and PVTT (P = 0.0020) as significant factors of overall survival. Multivariate analysis identified PS = 0 (P = 0.020), absence of extrahepatic metastases (P = 0.001) and objective response of intrahepatic HCC and PVTT (P = 0.005) as significant and independent factors of overall survival (Table 6).

Adverse reactions and complications

Table 7 lists the toxicity data for all patients during and after the treatment. Fever, fatigue, nausea and anorexia were the most common adverse events, but these were mostly NCI-CTC grade 1 or 2. NCI-CTC grade 3 or 4 adverse reactions relative to the RT group and the non-RT

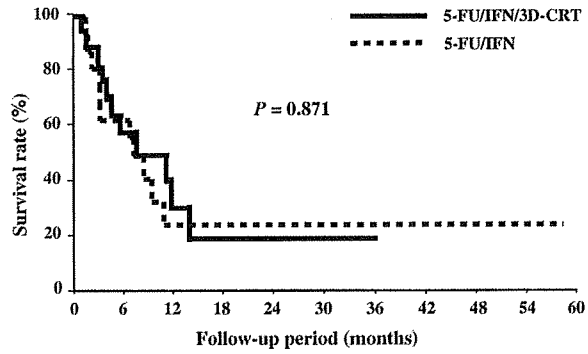

Fig. 4 Overall survival rate of patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

group were as follows: leukopenia was observed in nine (56.3%) and three (18.8%) patients, thrombocytopenia in six (37.5%) and five (31.3%) patients and anorexia in one (6.3%) and one (6.3%) patient, respectively. The proportion of patients with NCI-CTC grade 3 or 4 leukopenia tended to be higher in the RT group than in the non-RT group (P = 0.066). Two patients of the RT group developed NCI-CTC grade 4 leukopenia and required administration of granulocyte colony-stimulating factor. None required platelet transfusion. None developed upper gastrointestinal ulcers associated with 3D-CRT. Furthermore, none developed RILD, and hepatic failure related to 5-FU/IFN with 3D-CRT was not observed during the follow-up period.

After 3D-CRT and one course of 5-FU/IFN, Child-Pugh classification did not change in the RT group: 13 patients, from A to B: 2 patients and from A to C: 1 patient. After one course of 5-FU/IFN, Child-Pugh classification did not change in the non-RT group: 13 patients, from A to B: 2 patients and from B to A: 1 patient. In the RT group, one

Table 6 Univariate and multivariate analyses of predictors of overall survival

Variable	Univariate analysis	Multivariate analysis		
	P value	Hazard ratio	95% CI	*P* value
Age (≤65 vs. >65)	0.2643			
Sex (M vs. F)	0.6535			
HCV antibody (positive vs. negative)	0.0009	–	–	0.136
Child Pugh stage (A vs. B, C)	0.4592			
PS (0 vs. 1)	0.0003	3.643	1.223–10.850	0.020
Intrahepatic tumor volume (≤50 vs. >50%)	0.4963			
Extrahepatic metastases (absence vs. presence)	0.0002	5.870	2.164–15.923	0.001
Vp (3 vs. 4)	0.3170			
AFP (≤1,000 vs. >1,000)	0.9743			
AFP-L3 (≤40 vs. >40)	0.7701			
DCP (≤10,000 vs. >10,000)	0.4399			
3D-CRT (combination with vs. without)	0.8710			
Objective response of PVTT only	0.4586			
Objective response of intrahepatic HCC + PVTT	0.0020	8.064	1.890–34.412	0.005
Variceal rupture	0.7345			

HCV hepatitis C virus, *PS* Eastern Cooperative Oncology Group performance status, *Vp3* tumor thrombus in the first branch of the portal vein, *Vp4* tumor thrombus in the trunk of the portal vein, *AFP* α-fetoprotein, *AFP-L3* lens culinaris agglutininreactive fraction of α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *3D-CRT* three-dimensional conformal radiotherapy, *PVTT* portal vein tumor thrombosis, *HCC* hepatocellular carcinoma

Table 7 Adverse reactions (NCI-CTC v3.0 grade 3/4) during and after the treatment

	5-FU/IFN combined with 3D-CRT		5-FU/IFN		*P* value
Grade:	3	4	3	4	
Leukopenia	7 (43.8%)	2 (12.5%)	3 (18.8%)	0	0.066
Anemia	0	0	0	0	NS
Thrombocytopenia	6 (37.5%)	0	5 (31.3%)	0	NS
Anorexia	1 (6.3%)	0	1 (6.3%)	0	NS

5-FU 5-fluorouracil, *IFN* interferon, *3D-CRT* three-dimensional conformal radiotherapy

patient with the Child-Pugh classification worsened from A to C. Hepatic failure in this patient was due to rapid progression of intrahepatic HCC and was not associated with 3D-CRT.

Causes of death

At the time of analysis, 10 patients were still alive, and 22 patients had died of disease. All patients died of cancer-related disease. Nineteen patients died of hepatic failure because of progression of intrahepatic HCC. Among the 19 patients, 4 died of HCC rupture. Three patients died of bleeding esophageal varices; all were in the non-RT group. In the RT group, none died of bleeding of esophageal varices. None of the 22 patients died of extrahepatic metastases.

Discussion

Advanced HCC is often accompanied by PVTT. However, there is no established standard therapeutic modality for HCC with PVTT, especially Vp3 and Vp4. The prognosis is often poor; the reported MST associated with symptomatic treatment of patients with HCC and PVTT is shorter than 90 days [9, 10], and the degree of portal vein tumor thrombosis is a fairly reliable factor in predicting survival [31]. The poor prognosis depends on (1) rapid progression of HCC by spread of tumor cells through the portal tract and (2) portal hypertension by PVTT causing various complications, such as variceal rupture, ascites and ischemic liver failure. Especially, Vp3/4 results in deterioration of PS of these patients, and consequently any treatment for HCC is considered contraindicated.

It was reported that surgical resection of HCC with Vp3/4 is limited to highly selected group of patients such as those with good hepatic reserve and relatively small primary tumors. The reason for the limitation is related to the potential intraoperative death or the high recurrence rate postoperatively [32, 33]. Although TACE was widely applied to unresectable HCC, the reported outcome was poor, especially for HCC with Vp3/4 [34, 35]. Previous studies also reported the efficacy of HAIC for PVTT. Several groups reported the combination therapy of intraarterial 5-FU and CDDP for HCC with Vp3/4 [36, 37]. Recent reports described the efficacy and survival benefits of combination therapy of intraarterial 5-FU and systemic IFN-α [15–18]. IFN-α acts as a modulator by increasing the level of thymidine phosphorylase, which is an enzyme responsible for biochemical activation of 5-FU [38, 39]. In addition, IFN-α suppresses cancer cells directly and/or indirectly via several pathways, such as inhibition of the cell cycle, boosting p53 activation and activation of immunocytes [40–45]. The response rate to 5-FU/IFN in HCC patients with PVTT seems superior to 5-FU/CDDP [15–18, 36, 37, 46, 47]. The combination therapy of 5-FU/IFN for Vp3/4 has been reported with MST, with the response rate ranging from 6.9 to 11.8 months and 44–52%, respectively [17, 18].

Other studies also reported the safety and efficacy of local radiotherapy for PVTT. 3D-CRT monotherapy for Vp3/4 has been reported with MST, with the response rate in Vp3/4 ranging from 9.6 to 10.7 months and 45–46%, respectively [19, 20].

Despite the development of chemotherapies and radiotherapies, the prognosis of HCC patients with Vp3/4 is still less than 1 year, and the response rate is less than about 50% [17–20].

Recent studies reported the synergistic effects of chemotherapy combined with radiotherapy in several malignancies, such as lung cancer and esophageal cancer [21–24]. The combination of chemotherapy and radiotherapy interacts in several ways. For example, the combination of RT, 5-FU and carboplatin raises the concentration of 5-FU in head and neck tumors [48]. Furthermore, the combination of RT, 5-FU and doxorubicin or CDDP improved the radiosensitivity of HepG2 cell lines [49]. With regard to HCC, radiotherapy was first used in combination with TACE [50, 51]. Recently, Han et al. [25] reported the use of 3D-CRT with HAIC consisting of low-dose cisplatin and 5-FU, with a 13-month MST and 45% response rate. However, to our knowledge, there are no reports about the combination of 5-FU/IFN and 3D-CRT. Accordingly, we applied this combination therapy for advanced HCC with Vp3/4.

In this study, the response rate of PVTT was significantly higher ($P = 0.012$) in the RT group than in the non-RT group (75 vs. 25%). The response rate to 5-FU/IFN/3D-CRT was higher than that reported in a previous study using 3D-CRT alone (45%), which also applied RECIST for evaluation of the response rate, similar to the present study [20]. In addition, in the same study, the reported response rate of PVTT was 22% for <44 Gy and 80% for ≥44 Gy [20]. In our study, a positive response of PVTT was observed in 4/5 (80%), 6/9 (67%) and 2/2 (100%) patients who received RT at a dose of 30, 39 and 45 Gy. Comparison with the above study indicates a higher response rate with lower RT dose. These results suggest that RT acts synergistically with 5-FU/IFN and improves the outcome.

In the present study, the high response rate (75%) of PVTT in the RT group was associated with excellent secondary benefits. Shrinkage of PVTT improved portal hypertension and avoided PREs. In other words, it significantly reduced the rate of variceal rupture and worsening of varices, compared with the non-RT group ($P = 0.040$ and 0.0244, respectively). It is noteworthy that none of the patients of the RT group and three patients of the non-RT group required preventive therapy for varices, such as Hassab's operation, EIS and EVL, during the follow-up period. In addition, uncontrollable ascites was less likely to develop in patients of the RT group than non-RT group.

The response rates of intrahepatic tumor in the RT group and the non-RT group were similar. We believe this is due to the limitation of the irradiation area to PVTT.

There is almost no information regarding the toxicity of combination therapy of 3D-CRT and 5-FU/IFN. Previous studies reported the safety of 3D-CRT alone for PVTT. The reported rate of 0–1.7% RILD was observed by 3D-CRT alone for PVTT with a dose range of 18–54 Gy [19, 20]. In our study, RILD was not observed, and changes in the hepatic reserve before and after treatments were similar in the two groups. NCI-CTC grade 3 or 4 leukopenia tended to be more frequently observed in the RT group than in the non-RT group ($P = 0.066$), but there was no statistical difference between the two groups. Two patients with grade 4 leukopenia required treatment with granulocyte colony-stimulating factor, but no discontinuation of the chemotherapy based on well-preserved hepatic reserve.

In this study, three patients in the non-RT group died of bleeding from esophageal varices. When variceal ruptures occurred, these three patients received the best supportive care because of deterioration of HCC. We estimated their prognosis at that stage to be only a few months, even if ruptures did not occur. Accordingly, we speculate that death related to esophageal varices has little influence on the overall survival of the non-RT group.

In this study, the objective response rate of PVTT was higher in the RT group than in the non-RT group. In addition, 5FU/IFN with 3D-CRT did not worsen the hepatic reserve. However, the overall survival was similar in the two groups. In this study, we analyzed the objective response of both intrahepatic HCC and PVTT, not the objective response of PVTT alone, as contributing to overall survival. The objective response rate of both intrahepatic HCC and PVTT was similar between the two groups. We regarded this point as the reason for the similar survival rate in the two groups.

In conclusion, 5-FU/IFN combined with 3D-CRT attained a high response rate of PVTT by low RT dose. The combination therapy improved the response rate of PVTT and halted any deterioration of portal hypertension, resulting in reduced incidence of portal hypertension-related events, such as variceal rupture and uncontrollable ascites, and resulted in maintenance of PS and QOL compared with 5-FU/IFN alone. The results also showed that 5-FU/IFN combination therapy with 3D-CRT is well tolerated. However, any generalized statement on the results of this study should be guarded due to the small sample size. Further prospective studies are needed to investigate the factors involved in the survival benefits of 5-FU/IFN combination therapy with 3D-CRT.

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Case Report

The first Japanese case of COACH syndrome

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COACH syndrome is a disorder characterized by hypoplasia of cerebellar vermis, oligophrenia, congenital ataxia, coloboma and hepatic fibrosis, and 21 cases have been reported to date. Here we describe the first Japanese case of COACH syndrome, who was diagnosed at the age of 37 years and never progressed to liver failure. The patient was found to have delayed developmental milestones at the age of 5 months and mental retardation at the age of 7 years. She had been treated for hepatopathy of unknown origin from the age of 22 years. She was admitted to Hiroshima University Hospital at the age of 37 years after the identification of esophageal varices on a routine upper endoscopy. Computed tomography of the abdomen revealed portal hypertension and splenomegaly, and liver biopsy showed liver fibrosis. In addition, she had coordination disorder and dysarthria. Brain magnetic resonance images revealed hypoplasia of cerebellar vermis. The final diagnosis was COACH syndrome. She underwent endoscopic injection sclerotherapy for esophageal varices. From that point until her death from ovarian cancer at the age of 41 years, the liver function tests were stable without an episode of hematemesis. Physicians should be aware of COACH syndrome when they examine young patients who present with hepatopathy, portal hypertension of unknown origin and cerebellar ataxia.

INTRODUCTION

COACH SYNDROME IS one of the oculo-encephalo-hepato-renal syndromes and is a rare disorder with hypoplasia (or aplasia) of the cerebellar vermis, oligophrenia, congenital ataxia, coloboma and hepatic fibrosis. Verloes and Lambotte[1] were the first to describe this syndrome, and only 21 cases of COACH syndrome have been reported to date (Table 1). The term "congenital hepatic fibrosis" (CHF) was introduced by Kerr *et al.*[14], and a special subgroup of CHF is COACH syndrome. CHF is probably the most common cause of non-icteric hepatosplenomegaly and is encountered mainly in children and young adults.[8] Patients with CHF are rarely discovered after the age of 30 years.[15] In fact, only two of the 21 previously reported cases of COACH syndrome were diagnosed over the age of 30. Summerfield *et al.*[16] and Desmet[17] indicated that CHF resulted in hepatosplenomegaly and portal hypertension with normal liver function. However, five of the 21 previously reported cases progressed to liver failure and three of these five underwent liver transplantation. Here we describe the first Japanese case diagnosed as COACH syndrome at the age of 37 years who never progressed to liver failure until her death from ovarian cancer.

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CASE REPORT

OUR PATIENT WAS a 37-year-old Japanese female. She was born in 1966 as the youngest child of healthy unrelated parents after an uneventful pregnancy. Her older brother and sister were healthy. The family history was unremarkable. Her birth weight was 3 kg. She presented with delayed developmental milestones and was able to control her head at the age of 5 months. When she entered elementary school at the age of 7 years, she was found to have developmental delays. At the age of 22 years, she developed spontaneous pneumothorax and was found to have elevated liver transaminase levels. Since then, she had been treated with ursodeoxycholic acid for hepatopathy of unknown

Table 1 Comparison of the present patient with the previously reported cases of COACH syndrome

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Cerebellar vermis hypo/aplasia	+	+	NS	NS	+	+	NS	NS	+	NS	+	+	+	+	+	NS	NS	+	+	NS	+	+
Mental retardation	+	+	NS	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+
Ataxia	+	+	+	+	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+
Chorioretinal coloboma	+	+	NS	+	+	+	+	NS	+	+	+	+	+	–	–	+	+	+	+	+	+	–
Hepatic fibrosis	+	+	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal hypertension	–	NS	+	+	–	+	+	NS	NS	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal abnormalities	–	–	NS	+	NS	+	NS	+	+	+	+	NS	+	–	+	+	+	–	NS	+	–	–
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypotonia	+	NS	NS	+	NS	NS	NS	–	+	NS	+	NS	+	+	+	NS	+	+	+	+	NS	–
Ptosis	+	NS	NS	NS	+	+	+	+	NS	NS	+	–	+	–	+	–	+	NS	NS	–	NS	–
Nystagmus	–	NS	NS	+	+	+	+	+	+	NS	+	–	+	+	+	–	+	NS	NS	–	NS	–
Dysmorphic signs	+	+	NS	+	+	NS	+	+	NS	NS	+	+	+	+	+	+	+	+	+	+	+	+
Esophageal varices	–	NS	NS	+	NS	+	+	NS	NS	+	+	–	+	+	NS	NS	+	+	+	+	+	+
History of hematemesis	–	NS	NS	+	NS	–	+	NS	NS	+	+	+	–	+	NS	NS	+	+	–	+	+	+
Therapy	–	NS	NS	TIPS	NS	–	NS	NS	NS	NS	NS	–	–	NS	NS	NS	NS	NS	–	TIPS	NS	EIS
Gender	M	M	F	F	F	F	F	F	F	F	M	F	M	M	M	F	M	F	F	F	M	F
Age (years)† ‡	1.4†	34†	46‡	23†	6†	7†	14†	1.6‡	6‡	6‡	7†	11†	7†	15‡	2.6†	4†	1.2†	8†	11†	18†	7†	37†

1, Foell *et al.*;[2] 2, 3, Wiesner *et al.*;[3] 4, 5, Kumar and Rankin;[4] 6, Barzilai *et al.*;[5] 7, 8, Thompson and Baraitser;[6] 9, 10, Dietrich and Straub;[7] 11–13, Verloes and Lambotte;[1] 14, 15, Gentile *et al.*;[8] 16, 17, Hunter *et al.*;[9] 18, Herzog *et al.*;[10] 19 Kirchner *et al.*;[11] 20, Uemura *et al.*;[12] 21, Gleeson *et al.*;[13] 22, our patient.

†Age at liver biopsy or when diagnosed as COACH syndrome. ‡Age at death. + present. – absent.

EIS, endoscopic injection sclerotherapy; F, female; M, male; NS, not specified; TIPS, transjugular intrahepatic portosystemic shunt.

Table 2 Laboratory data on admission

Complete blood count			LDH	172	IU/L	Tumor marker		
WBC	4690	/μL	ALP	466	IU/L	AFP	<5.0	ng/mL
RBC	4.12×10^6	/μL	γGTP	83	IU/L			
Hb	12.7	g/dL	TP	7.5	g/dL	Virus markers		
Ht	37.8	%	Alb	4.2	g/dL	HBsAg	(−)	
Plt	134×10^3	/μL	TC	161	mg/dL	HCVAb	(−)	
			TTT	5	U			
Blood coagulation test			ZTT	12	U	Autoantibodies		
PT	90	%	BUN	15	mg/dL	ANA	(−)	
			Cr	0.48	mg/dL	AMA(M2)	(−)	
Blood chemistry			CRP	<0.3	mg/dL			
TBil	0.9	mg/dL	FBS	79	mg/dL			
AST	35	IU/L	HbA_{1c}	3.9	%			
ALT	38	IU/L	NH_3	47	μg/mL			

AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; AST, aspartate aminotransferase; Cr, creatinine; CRP, c-reactive protein; FBS, fasting blood sugar level; Hb, hemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C antibody; LDH, lactate dehydrogenase; Plt, platelets; PT, prothrombin time; RBC, red blood cells; TBil, total bilirubin; TC, total cholesterol; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

origin at a local clinic. At the age of 37 years, an upper endoscopy was performed as part of a routine examination and found esophageal varices (Lm, F2, Cb, RC1, Te).[18] She was referred to our hospital for admission and treatment of esophageal varices.

Physical examination showed micrognathia, saccadic eye movement, hepatosplenomegaly and varicose veins on both lower legs. No coloboma or hypotonia were present. Neurological examination showed mental delayed development and cerebellar ataxia. Based on the Wechsler Adult Intelligence Scale – Revised (WAIS-R), her intelligence quotient (IQ) was evaluated as verbal IQ 69, performance IQ 61 and full-scale IQ 61. She presented with scanning speech. She was unable to walk with tandem gait or to stand on one leg. She was poor at the nose–finger–nose test and had an intention tremor.

Laboratory tests (Table 2) showed aminotransferases within the normal range, but the cholestatic parameters were increased (alkaline phosphatase 466 IU/L, γ-glutaryltransferase 83 IU/L). Platelet count was low (134×10^3/μL). Viral hepatitis and autoimmune hepatitis were excluded. Renal function tests were normal and banded karyotyping at the 400–550 band level of resolution was normal (46, XX).

Ultrasound examination and computed tomography (CT) (Fig. 1a,b) of the abdomen showed hepatomegaly, large collateral vessels and splenomegaly associated with portal hypertension. Both kidneys were normal. Ultrasound-guided liver biopsy (Fig. 2a,b) was performed and showed hepatic fibrosis, containing bile ductular proliferation and mild inflammatory infiltrate of lymphocytes and neutrophils. This pathology was diagnostic for congenital hepatic fibrosis.

Magnetic resonance imaging (MRI) of the brain (Fig. 3) showed hypoplasia of the inferior part of the cerebellar vermis, so-called the Molar Tooth Sign. The electroencephalogram was normal. Based on the

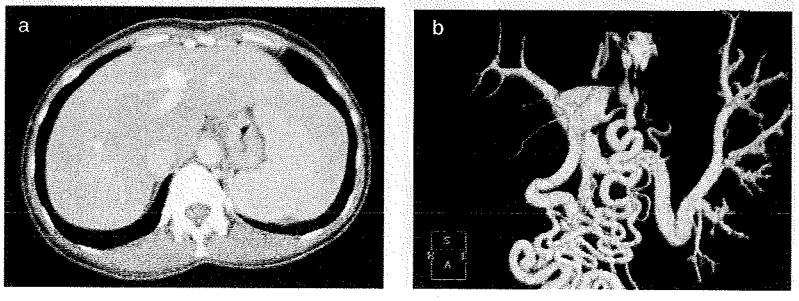

Figure 1 Computed tomography of the abdomen. (a) Axial image shows hepatosplenomegaly. (b) Multiplanner reconstruction image of the portal vein shows portosystemic collaterals associated with portal hypertension.

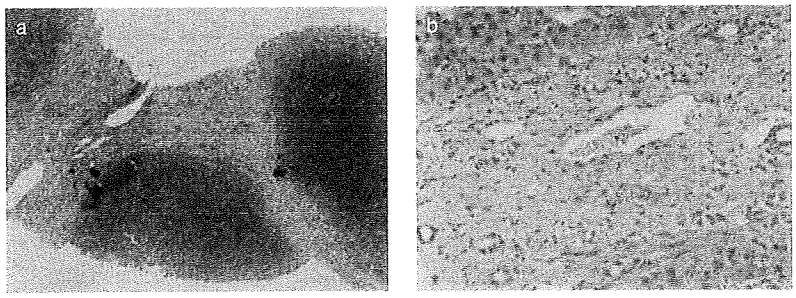

Figure 2 Liver biopsy. Hepatic fibrosis with bile duct proliferation and with mild inflammation including lymphocytes and neutrophils. There are no findings of narrowing or occlusion of the portal vein. (a) Azan, magnification × 40. (b) Hematoxylin and eosin, magnification × 400.

previously reported cases, physical, laboratory and imaging findings, these histopathological findings established the diagnosis of COACH syndrome.

The patient underwent endoscopic injection sclerotherapy (EIS) for esophageal varices in order to prevent potential rupture. Esophageal varices improved to Lm, F0, Cw, RC0, UI[18] two weeks after the treatment. Subsequently, she was treated with protirelin. Ataxia did not improve, but liver function tests stabilized. At the age of 40 years, she presented with lumbago. Detailed examination showed an ovarian cancer. At the age of 41 years, she died from ovarian cancer. Until her death, hematemesis and/or liver failure never occurred.

DISCUSSION

WE OFTEN EXPERIENCE patients with liver disorders of unknown origin. This case was followed as hepatopathy of unknown origin from the age of 22 years. A routine medical check up identified esophageal varices requiring treatment. CT, ultrasound, MRI, liver biopsy and physical examination revealed that she suffered from COACH syndrome. COACH syndrome is a very rare disorder consisting of cerebellar vermis hypoplasia (or aplasia), oligophrenia, congenital ataxia, coloboma and hepatic fibrosis. An autosomal recessive mode of inheritance was suggested,[16] but the primary cause of this disease remains unknown.

Liver dysfunction and portal hypertension-related COACH syndrome is due to CHF. Kerr *et al.*[14] were the first to describe CHF as a distinct entity from cirrhosis. CHF is an inherited malformation, defined pathologically by bands of fibrous tissue within the liver, linking portal tracts and containing multiple bile ductules.[14,17] The main characteristics of CHF are normal liver parenchyma, fibrosis of the portal spaces and ductal changes.[19] The lack of inflammatory infiltrates in the connective tissue differentiates the congenital form of fibrosis from the acquired form.[8]

The clinical picture of CHF includes hepatosplenomegaly and portal hypertension with normal liver function.[16] Complications of portal hypertension, including hematemesis due to esophageal varices, gastrointestinal hemorrhage and hypersplenism, are frequently observed.[20] Our case had esophageal varices due to

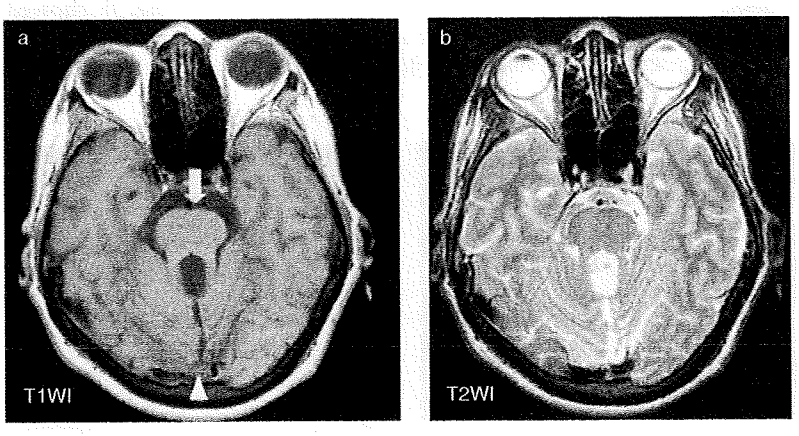

Figure 3 Axial magnetic resonance images show the molar tooth sign (arrow) and cerebellar vermis hypoplasia (arrowhead). (a) T1-weighted image. (b) T2-weighted image.

portal hypertension and underwent EIS. Since then, she did not develop hematemesis. Twelve of the previously reported 21 cases had esophageal varices and two cases underwent transjugular intrahepatic portosystemic shunt (TIPS) (Table 1).

Many cases underwent close investigation to diagnose the presence of oligophrenia and were diagnosed as COACH syndrome. Although the past history in our case also indicated delayed developmental milestones in early childhood, like other cases, close examination was not made. Therefore, the diagnosis of COACH syndrome was made at a relatively advanced age. The late appearance of symptoms and their clinical evolution suggest that CHF is a dynamic and progressive condition. Some studies indicate that there is a progressive build-up of liver fibrosis over the years.[21,22] Five of the previously reported 21 cases progressed to liver failure and three of these five underwent liver transplantation. In comparison, our case never progressed to liver failure in her lifetime, although she had esophageal varices due to portal hypertension.

Five of the previously reported 21 cases with COACH syndrome died. One patient died from liver failure without liver transplantation, one died from acute hepatitis C after liver transplantation, one died from hematemesis from esophageal varices, one died from renal failure and one died from aspiration pneumonia. Complications of hepatopathy are major contributing factors to morbidity and mortality during the course of the disease. Most reported cases died from COACH syndrome-related disorders. However, our patient died from ovarian cancer after a clinical course free of liver failure or hematemesis. Whether COACH syndrome is related to ovarian cancer or not is not clear at this stage. To date, 10 CHF cases with liver tumor have been reported,[11] five patients with cholangiocellular carcinoma, two patients with hepatocellular carcinoma and three patients with benign liver tumor. However, none of the 21 patients with COACH syndrome was reported to have malignant tumors beyond the hepatobiliary system.

In summary, we experienced the first Japanese case of COACH syndrome. Physicians should be aware of COACH syndrome when they examine young patients who present with hepatopathy, portal hypertension of unknown origin and cerebellar ataxia.

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Original Article

Predicting the response to 48-week combination therapy with peginterferon α-2b plus ribavirin from the estimated HCV RNA load index after negative serum change in genotype 1b hepatitis C patients

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Aim: We estimated viral dynamics after serum hepatitis C virus (HCV) RNA became negative and assessed the relation between the estimated viral load at the end of treatment (EVE) index and the response to the combination therapy with peginterferon α-2b plus ribavirin.

Methods: Patients with chronic HCV, genotype 1b, and a high viral load were treated with this combination therapy for 48 weeks, and serum HCV RNA was measured frequently during the treatment period. In the patients showing an end-of-treatment response (ETR), the viral load profile from the start of treatment until serum HCV RNA became negative was expressed by an approximate curve. Then the EVE index was calculated by using the expression obtained from the curve, and differences between the sustained virologic response (SVR) and relapse groups were investigated. Results: The SVR rate increased as the EVE index became lower, and the EVE index was significantly lower in the SVR group than in the relapse group. The SVR rate was higher for those in whom the EVE index was below the cut-off point.

Conclusion: Prediction of SVR and relapse from the EVE index is more useful than prediction from viral dynamics at the time when HCV RNA becomes negative or when HCV RNA shows a decrease of 2-log or more.

Key words: hepatitis C virus, peginterferon, virologic response

INTRODUCTION

COMBINATION THERAPY WITH peginterferon (Peg-IFN) plus ribavirin (RBV) is a standard treatment for chronic hepatitis C around the world. However, the virologic efficacy of this combination is lower for chronic hepatitis C virus (HCV) patients with genotype 1 and a high viral load than for other patients. The sustained virologic response (SVR) rate after 48 weeks of treatment at a standard dose is approximately 40–50%, which is not satisfactory.[1–4] The virologic response soon after the start of treatment is classified as no response (NR: HCV RNA is detected throughout the treatment period), rapid virologic response (RVR: HCV RNA is not detected after 4 weeks of treatment), complete early virologic response (cEVR: not an RVR, but HCV RNA is not detected after 12 weeks of treatment), partial virologic response (pEVR: HCV RNA is detected after 12 weeks of treatment, but the load is reduced by 2 logs or more compared with before treatment), and late virologic response (LVR: HCV RNA is not detected after 12 weeks or more of treatment).

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According to previous reports, treatment was discontinued early in patients in whom SVR could not be expected with interferon therapy,[5,6] while an appropriate treatment period was selected so that relapse was decreased and the SVR rate was increased when interferon therapy was promising.[6–12] It has also been reported that the SVR rate was higher at 72 weeks than at 48 weeks after starting treatment in patients in whom RVR was not noted at the time when HCV RNA became negative.[7,11,12]

According to the 2007 Japanese guidelines, it is recommended that combined therapy with Peg-IFN plus RBV for chronic HCV with genotype 1 and a high viral load should be continued for 48 weeks in the case of cEVR and for 72 weeks in the case of LVR. It has also been reported that a 24-week treatment period may possibly be appropriate for the RVR group, in whom HCV RNA becomes negative within 4 weeks of starting treatment.[13,14] Since the optimum treatment period differs for each patient, it is useful for improving patient motivation to clarify the SVR rate at the end of each treatment period and also the treatment period that is sufficient to attain SVR in each patient. However, it is actually impossible to measure the residual viral load and the extent of its decrease, especially in the liver, when serum HCV RNA becomes undetectable soon after the start of interferon therapy. If there is an index that estimates the viral load at the end of treatment (EVE index), which is calculated from the changes of viral load between the start of therapy and the time when serum HCV RNA becomes undetectable, it would become possible to predict the SVR rate during shorter treatment periods and select the optimum treatment period to prevent relapse for each patient. However, such estimation has not been attempted on the basis of viral dynamics after serum HCV RNA becomes undetectable.

The objective of the present study was to investigate whether SVR and relapse could be predicted by the EVE index.

MATERIALS AND METHODS

Patients

COMBINATION THERAPY WITH Peg-IFNα2b plus RBV was performed in 106 patients with chronic HCV who were enrolled by the Hiroshima Liver Disease Study Group from January to December 2005. All of them met the following criteria: greater than 18 years of age, a persistent increase of serum alanine transaminase for the past 6 months, positive for anti-HCV antibody by a third-generation enzyme immunoassay (Chiron, Emerville, CA), and HCV genotype 1b. Each patient also had a high viral load ($\geq 1.0 \times 10^5$ IU/mL) on quantitative analysis of HCV RNA by PCR with Cobas Amplicore HCV monitor, version 2.0, using the 10-fold dilution method (Roche Diagostic Systems, Tokyo) at the start of treatment, and histologic evidence of chronic hepatitis on examination of a liver biopsy specimen obtained within the previous 24 months. Exclusion criteria included decompensation of liver function, co-existing serious medical or psychiatric illness, liver disease other than that caused by HCV infection, a neutrophil count less than 1.5×10^9/L, a platelet count less than 80×10^9/L, a hemoglobin less than 12 g/dL, a serum creatinine greater than 1.5 times the upper limit of the normal range, and co-infection with hepatitis A virus, hepatitis B virus, or human immunodeficiency virus. Patients were also excluded if they had received any systemic antiviral, antineoplastic, or immunomodulatory therapy within 6 months before the study. Pregnant and breast-feeding women and male partners of pregnant women were also excluded. All participants had to use two forms of effective contraception during treatment and throughout the 24-week follow-up phase of the study.

Study design

All patients were treated with Peg-IFNα2b (1.5 μg/kg subcutaneously) once weekly for a 48-week period, plus RBV at a dose adjusted for body weight (patients over 80 kg in weight received 1000 mg, those weighing from 60–80 kg received 800 mg, and those under 60 kg received 600 mg). A post-treatment follow-up period of 24 weeks was also included in the study.

HCV RNA detection and viral kinetic studies

Serum samples were frozen at −80°C within 4 h of collection and then were thawed at the time of measurement. The HCV genotype was determined by the polymerase chain reaction (PCR) using a mixed primer set based on the nucleotide sequence of the NS5 region.[15] The serum HCV RNA level was measured with a quantitative HCV RNA assay (Cobas Amplicore HCV monitor ver 2.0; Roche Diagostic Systems, Tokyo, Japan) using the 10-fold dilution method before, during, and after therapy. The range of the assay was 5.0×10^3 to 5.0×10^6 IU/mL. When the measured

serum HCV RNA level was 5.0×10^3 IU/mL, HCV RNA was also determined by a quantitative PCR assay (Amplicor HCV v2.0®, Roche Diagnostic Systems, Tokyo, Japan), which had a detection limit of 50 IU/mL. The response of the patients was classified as follows: end-of-treatment response (ETR: quantitative HCV RNA assay showed that HCV RNA was below the detection limit of 50 IU/mL at the end of treatment), SVR (quantitative HCV RNA assay showed that serum HCV RNA was still below the detection limit at 24 weeks after the end of treatment), relapse (serum HCV RNA was below the detection limit at the end of treatment, but was detected again by 24 weeks after the end of treatment), and no response (NR) (serum HCV RNA was not below the detection limit at the end of treatment). Routine laboratory tests, including HCV RNA determinations, were performed at each participating institution.

Viral dynamics were assessed by measuring the serum HCV RNA level before the initial dose (0 W), after 2 and 4 weeks of treatment, and then at least every 4 weeks during the remainder of the study period. When ETR was noted, the viral load until serum HCV RNA became negative was expressed in units of log IU/mL, and an approximate exponential equation was generated from the weekly changes by using Microsoft Office Excel 2003 for Windows (Microsoft Corporation, Washington, DC; Fig. 1a). Then, assuming that the HCV RNA load continued to decrease in a similar manner after it became negative, the load after 48 weeks of treatment was estimated by extrapolation with this equation (Fig. 1b). The estimated viral load at the end of treatment was defined as the EVE value. When the viral load profile shown in Figure 1a was plotted as logarithmic (log) values on the ordinate for simplicity, the EVE value was 4.6×10^{-3} (Fig. 1c). Differences between the SVR group and the relapse group were investigated. When calculations were done, all HCV RNA levels exceeding 5.0×10^6 IU/mL were regarded as equal to 5.0×10^6 IU/mL (6.7 log IU/mL) for convenience, while HCV RNA levels under 5.0×10^3 IU/mL were regarded as equal to 5.0×10^3 IU/mL (3.7 log IU/mL) when HCV RNA was first found to be below 5.0×10^3 IU/mL. Also, the HCV RNA level was regarded as being equal to 50 IU/mL (1.7 log IU/mL) when a level below the detection limit was first noted. Liver biopsy specimens were examined by specialist pathologists, who assigned a numerical score for necroinflammatory activity (Grades 0 to 3) and another score for fibrosis and architectural distortion (stages 0 to 4) according to the method of Desmet *et al.*[16]

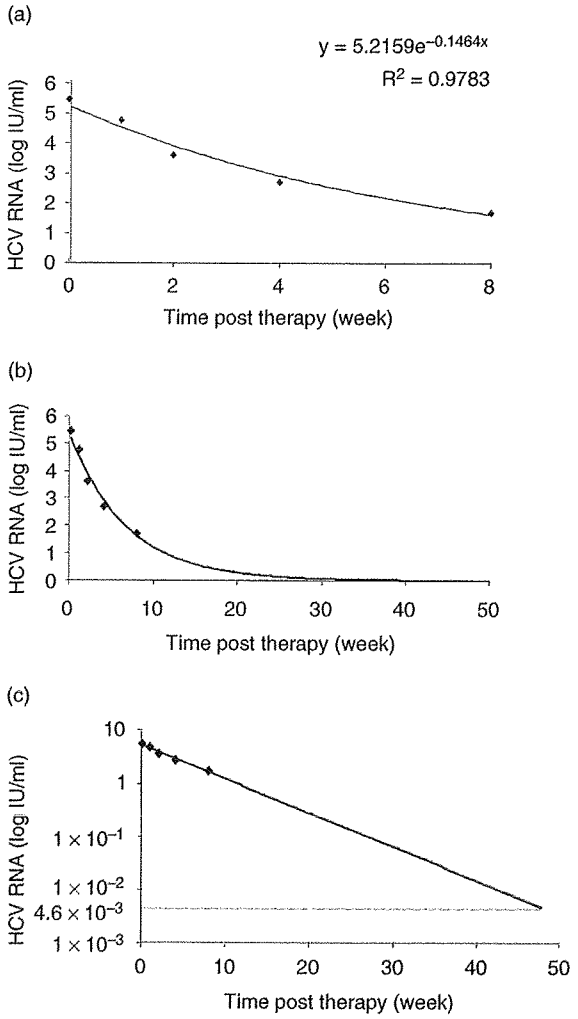

Figure 1 (a) The curve drawn from the equation shows the profile hepatitis C virus (HCV) RNA load. (b) Viral load profile estimated extrapolation for 48 weeks from the curve shown in Figure 1a. (c) The EVE index profile plotted on the ordinate in Figure 1b is express as log values.

Statistical analysis

Differences between groups were examined for statistical significance by Wilcoxon's test and the χ^2-test as appropriate. A P value of less than 0.05 (two-tailed) was considered significant. Statistical analysis was performed with JMP software (JMP USA, Cary, NC). The sensitivity, specificity, positive predictive value, and negative predictive value were also calculated. Receiver operating characteristics curves were generated for each cut-off point of the EVE index, and the cut-off points

Table 1 Baseline characteristics of patients receiving peginterferon α-2b plus ribavirin for genotype Ib with a high viral load

	Sustained virologic responders ($n = 63$)	Relapsers ($n = 22$)	P
Age (years)	55 ± 12	61 ± 6	0.027
Gender, n (%)			
Female	22 (35)	10 (45)	0.38
Male	41 (65)	12 (55)	
Virus loa (Log IU/mL)	5.8 ± 0.6	6.0 ± 0.3	0.23
Treated, n (%)	23 (37)	8 (36)	0.99
Body weight (kg)	60.6 ± 14.5	60.2 ± 10.4	0.84
Body mass index (kg/m^2)	23.0 ± 3.1	23.6 ± 3.3	0.49
Hemoglobin (g/dL)	14.2 ± 1.3	14.1 ± 1.2	0.57
Platelets (×10^4/μL)	16.8 ± 4.5	14.9 ± 3.2	0.07
ALT (IU/mL)	84 ± 82	71 ± 84	0.12
Creatinine level (mg/dL)	0.66 ± 0.20	0.60 ± 0.27	0.52
Fibrosis stage, n (%)			
F0F1	23	1	0.0045
F2F3	32	17	
Unknown	8	4	

Results are presented as mean ± standard deviation. ALT, alaizine aininotransferase.

were set so that SVR had a probability of 95%. The area under each receiver operating characteristics curve was calculated to assess the extent of discrimination provided by these parameters.

This study was carried out in accordance with the Helsinki Declaration, and was approved by the Human Ethics Review Committee of Hiroshima University and the ethics committees of each center. All patients provided written informed consent.

RESULTS

STANDARD THERAPY WITH Peg-IFNα2b plus RBV was performed in 106 chronic HCV patients with genotype 1b and a high viral load between January and December 2005. After excluding the subjects who were administered less than 80% of the scheduled combination dose of Peg-IFNα2b and RBV, there were 96 patients (90.6%) who completed the 48-week treatment period. Among them, 85 patients (80.2%) and 63 patients (59.4%) showed ETR and SVR, respectively, while 22 patients (20.8%) suffered from relapse and became positive for HCV RNA again within 24 weeks after the end of treatment.

Baseline characteristics of the SVR group and relapse group

To predict SVR and relapse, comparison of the two groups was performed with respect to various baseline characteristics, including age, sex, viral load, prior IFN therapy, body weight, body mass index, hemoglobin, platelet count, alanine aminotransferase, creatinine, and liver histology (F0 or F1). Patients were younger in the SVR group than in the relapse group ($P = 0.027$), and the incidence of F0 or F1 liver fibrosis was higher in the SVR group ($P = 0.0045$) (Table 1).

EVE index in the SVR and relapse groups

The mean R^2 value (coefficient of determination) was 0.97, when changes in the viral load of each patient until HCV became negative were described by an approximate exponential curve to calculate the EVE index.

Figure 2 displays the EVE index in each group using dots and box-and-whisker plots. The EVE index was significantly lower in the SVR group than in the relapse group (0.022 ± 0.048 vs. 0.30 ± 0.29, $P < 0.0001$).

SVR was noted 0% ($n = 0$) of patients when the EVE index was 1 to less than 10 ($n = 1$), 16.7% ($n = 3$) when it was 10^{-1} to less than 1 ($n = 18$), 73.7% ($n = 14$) when it was 10^{-2} to less than 10^{-1} ($n = 19$), 96.4% ($n = 27$) when it was 10^{-3} to less than 10^{-2} ($n = 28$), and 100% ($n = 19$) when it was 10^{-3} or less ($n = 19$). Receiver operating characteristics (ROC) analysis showed that the AUC value discriminating SVR from relapse based on the EVE index was 0.94. When the cut-off point of the EVE index was set at

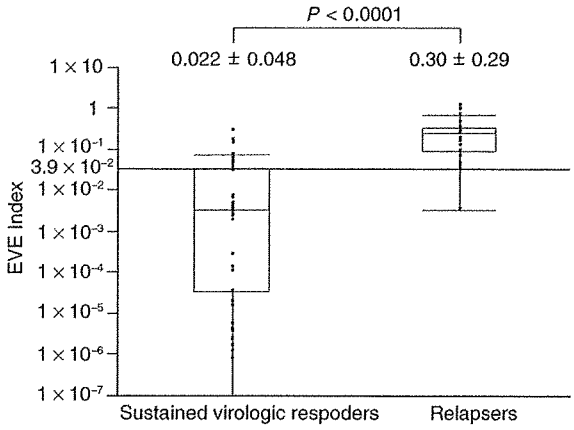

Figure 2 Box plot of the end of treatment (EVE) index in the sustained virologic response and relapse groups.

3.9×10^{-2} or less (Fig. 2), the positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and accuracy for SVR were 96.2%, 60.6%, 79.3%, 90.9%, and 82.3%, respectively. On the other hand, SVR was only noted in 39.4% of patients in whom the predicted load was above the cut-off point. When SVR was estimated from viral dynamics at the time when serum HCV RNA became negative after 12 weeks of treatment, the PPV, NPV, sensitivity, specificity, and accuracy for SVR were 92.2%, 81.0%, 93.7%, 77.3%, and 79.0%, respectively. When SVR was estimated from viral dynamics at the time of a decrease of serum HCV RNA by at least 2 log units (4 weeks/0 weeks), the PPV, NPV, sensitivity, specificity, and accuracy were 82.5%, 50.0%, 82.5%, 50.0%, and 64.9%, respectively (Table 2).

Relationship between the cut-off point and SVR stratified by the timing of HCV RNA negativity in each group

All of the patients showing RVR ($n = 14$) also had SVR, and the EVE index was below the cut-off point in all 14 patients. Among the patients with cEVR ($n = 50$), SVR was noted in 90.0%. In this group, SVR was achieved in 75.0% of patients with an EVE index above the cut-off point, while it was achieved in 94.7% of patients with an index below the cut-off point ($P = 0.047$). Among the patients showing LVR ($n = 21$), SVR was achieved in 19.0%, and no patient had an EVE index that was below the cut-off point (Fig. 3).

Relationship between the cut-off point and SVR stratified by the time when HCV RNA showed a 2-log or more decrease in each group

When the HCV RNA load showed a 2-log or more decrease after 1 or 2 weeks of treatment (1 week: $n = 5$, 2 weeks: $n = 10$), SVR was noted in all 15 patients and the EVE index was below the cut-off point in all of them. When the HCV RNA load showed a 2-log or more decrease after 4 weeks of treatment, SVR was achieved in 77.1%. It was noted in 43.8% of patients in whom the EVE index was above the cut-off point, while it was seen in 93.8% of those in whom the index was below the cut-off point ($P < 0.0001$). When the HCV RNA load showed a 2-log or more decrease after 8 weeks of treatment, SVR was noted in 52.6%. It was seen in 25.0% of patients in whom the EVE index was above the cut-off point, while it was achieved in all of the patients in whom the index was below the cut-off point ($P = 0.002$). When the HCV RNA load showed a 2-log or more decrease after 12 weeks of treatment, SVR was noted in 33.3%, and there was no patient in whom the EVE index was below the cut-off point (Fig. 4).

DISCUSSION

THE PRESENT STUDY revealed that when combination therapy with Peg-IFNα2b plus RBV is given to HCV patients with genotype 1b and a high viral load, it

Table 2 Predicting sustained virologic response (SVR) using end of treatment (EVE) index

Criteria for SVR prediction	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	accuracy (%)
EVE index ≤ 3.9×10^{-2}	96.2	60.6	79.3	90.9	82.3
Negative for serum HCV RNA after 12 weeks of treatment	92.2	81.0	93.7	77.3	79.0
The ratio of decrease in serum HCV RNA (4 week/0 week) >2 logs	82.5	50.0	82.5	50.0	64.9

HCV, hepatitis C virus; NPV, negative predictive value (% not meeting the criteria for SVR prediction that were relapse); PPV, positive predictive value (% meeting the criteria for SVR prediction that were SVR).

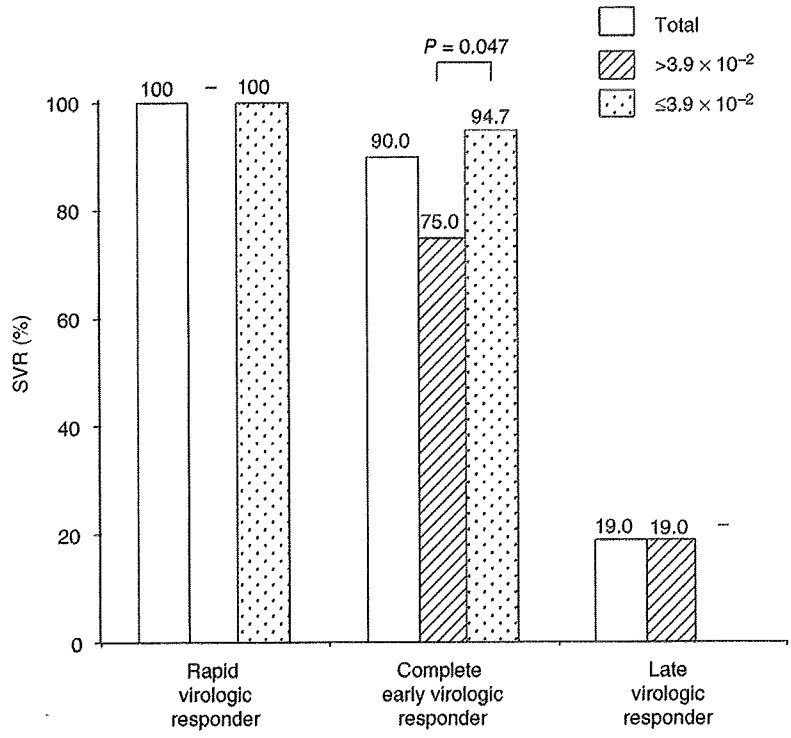

Figure 3 Sustained virologic response (SVR) rate stratified by the time when serum hepatitis C virus RNA became negate whom the end of treatment index was lower or higher than the cut-off point ($\leq 3.9 \times 10^{-2}$).

is possible to predict SVR or relapse by using the EVE index.

In recent years, several reports have been published concerning prediction of the efficacy of combination therapy with Peg-IFN plus RBV. In some studies, treatment was discontinued if the early virologic response suggested that interferon therapy would not achieve SVR, while an attempt was made to select an appropriate

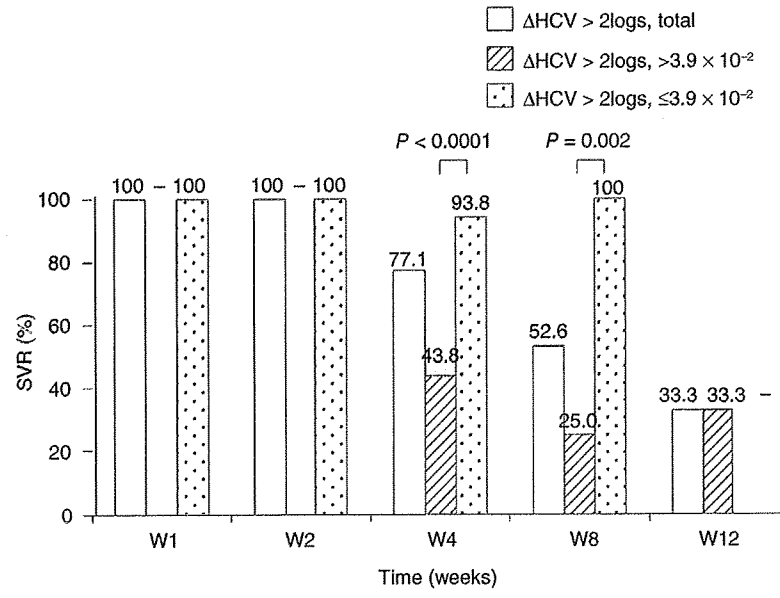

Figure 4 Relationship between the cut-off point ($\leq 3.9 \times 10^{-2}$) and the sustained virologic response rate when serum hepatitis C virus (HCV) RNA showed a 2-log or more decrease in each group.

treatment period for prevention of relapse and to increase the SVR rate if interferon was considered likely to achieve SVR.[4,6–12] Attempts have also been made to shorten the treatment period to 24 weeks in RVR patients showing a very good early virologic response.[4,12–14]

However, the above studies did not consider differences in the baseline viral load between individual patients because the efficacy of interferon therapy was estimated from viral dynamics at the time when serum HCV RNA became negative or the time when serum HCV RNA showed a 2-log or more decrease. Moreover, comparison was only done between two points (i.e. before treatment and at each time of testing). On the other hand, the accuracy of estimation was higher in the present study, because the response was predicted from the viral load profile obtained by frequent measurement from before treatment until the time when HCV RNA became negative. Frequent measurement was performed because even among patients who show a negative result for HCV-RNA or a 2-log or more decrease after the same period, the slope of the decrease of the viral load until it becomes negative may vary due to differences of the load before or during treatment, and this might lead to differences of the HCV RNA load at the end of treatment. Time and the viral load were plotted on the abscissa and ordinate, respectively, and the decrease of viral load until HCV RNA became negative was approximated by an exponential curve, which was then extended to estimate the viral load after serum HCV RNA became negative and also the slope of its decrease. According to our results, the SVR rate showed a significant difference in relation to whether or not the EVE index was below the cut-off point among patients showing EVR and also among patients in whom the HCV RNA load first showed a 2-log or more decrease after 4 or 8 weeks of treatment (Figs 3,4). These results suggested that the accuracy of predicting the response from the EVE index is higher than the conventional method based on the time when serum HCV RNA becomes negative or the time when HCV RNA shows a 2-log or more decrease. Another advantage of our method is that the SVR is not only predictable at a limited number of times (such as 24 and 48 weeks) after HCV RNA becomes negative, but also at the end of any specified treatment period, because the viral load can be calculated for any duration of treatment after serum HCV RNA becomes negative.

The main limitation of our method is difficulty in predicting efficacy because viral kinetics would deviate from the decay curve for the EVE index if a marked change is made to the dose of Peg-IFN and/or RBV after the start of treatment. This is also a problem when predicting the therapeutic efficacy at the time of viral negativity and the period when the viral load is reduced to 2 log or less. Prediction of efficacy would become further difficult depending on the changes in the amount or timing of drug administration after determining the predicted dose. None of the subjects had marked changes of the dosage in this study because subjects with <80% adherence to the Peg-IFN and RBV regimen were excluded. The EVE index is a useful method for predicting efficacy when administration of the standard dose is continued.

The viral dynamics during combination therapy with Peg-IFN plus RBV in chronic hepatitis C patients can be divided into 3 phases, which are the first phase (1–2 days) with a rapid decline of the viral load, followed by the "shoulder phase" (4–28 days) in which viral load declines slowly or remains constant, and the third phase of a further decrease in the load. In recent years, each of these 3 phases of viral dynamics has been reported to be useful for predicting the response to IFN therapy in patients with hepatitis C.[17–22] In the model of Herrmann *et al.*, the slope of the "shoulder phase" in patients with triphasic viral decay represents the pretreatment death rate of infected cells and the slope during the third phase represents the treatment-enhanced death rate of infected cells due to the immunomodulatory effect of RBV.[17] In contrast, Dahari *et al.* stated that the shoulder phase does not represent the intrinsic death rate of infected cells, and they considered the third-phase slope to be close to the intrinsic death rate of these cells.[18] The curves generated in the present study represent viral dynamics from the first to third phases as a continuous curve based on exponential approximation (indefinite phase) rather than as three lines (Fig. 1b). For easier clinical use, index approximation was applied. As a result, the mean R^2 value (coefficient of determination) calculated for our approximate exponential curve was 0.97, which indicated a strong correlation.

In conclusion, predicting the response from the EVE index is more useful than conventional estimation from the viral load profile at the time when serum HCV RNA becomes negative or when serum HCV RNA shows a 2-log or more decrease. Using our method, it is possible to calculate the EVE index any time during treatment or for any treatment period. This may help to improve the motivation of patients who have been treated for a long period while suffering from adverse reactions.

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Randomized Trial of High-Dose Interferon-α-2b Combined With Ribavirin in Patients With Chronic Hepatitis C: Correlation Between Amino Acid Substitutions in the Core/NS5A Region and Virological Response to Interferon Therapy

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The aim of this study was to compare the efficacy of high-dose interferon (IFN)-α-2b with standard dose of IFN-α-2b in combination with ribavirin (RBV) for patients with chronic hepatitis C virus (HCV) infection, and to investigate the predictive factors associated with virological response. Two hundred Japanese patients with high HCV viral load (>100 KIU/ml) were randomized to 6 or 10 mega units (MU) of 24-week IFN-α-2b with RBV. Predictive factors were investigated; including pretreatment amino acid (aa) sequences of the core region and the IFN-sensitive determining region (ISDR). The sustained virological response rate was not different in the two groups (24% vs. 30%) but the incidence of depression was significantly higher in the 10 MU group than 6 MU group (7% vs. 0%, $P=0.02$). Younger age (<60) and HCV genotype (2a/b) were significant predictors of sustained virological response. In patients infected with genotype 1b, substitutions of core aa 70 and/or 91 were predictive for non-virological response ($P<0.001$), and substitutions in the ISDR was observed frequently in virological responders. Early viral kinetics study showed that serum HCV core antigen decreased more slowly in both patients with aa 70 and/or 91 substitutions in the core and with absence of substitutions in the ISDR. In conclusion, the use of a higher dose of IFN-α-2b in combination with RBV did not improve virological response but resulted in higher incidence of depression. Amino acid substitutions in the core and ISDR are predictive of virological response to the therapy in patients with genotype 1b and high viral load. ***J. Med. Virol. 81:640–649, 2009.***

KEY WORDS: HCV; interferon; ribavirin; core region; ISDR

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is the leading cause of cirrhosis, liver failure, and hepatocellular carcinoma [Kiyosawa et al., 1990; Niederau et al., 1998]. Interferon (IFN) is an essential component of therapy for patients with chronic HCV infection. The most effective therapy available at present is the combination therapy of pegylated (PEG)-IFN and ribavirin (RBV) [Manns et al., 2001; Fried et al., 2002; Hoofnagle et al., 2003]. Among HCV genotypes, genotype 1b is the most resistant genotype to IFN therapy [Fried et al., 2002]. The limitation of use of the combination therapy for HCV infection with genotype 1b is due to the low response rate during therapy and high relapse rate after the therapy [McHutchison et al., 1998]. Several studies have evaluated the potential benefits of a larger dose of IFN with varying results [Lindsay et al., 1996; Fried et al., 2000; Ferenci et al., 2001; Hadziyannis et al., 2001; Di Marco et al., 2002; Brouwer et al., 2004]. Although treatment has been

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