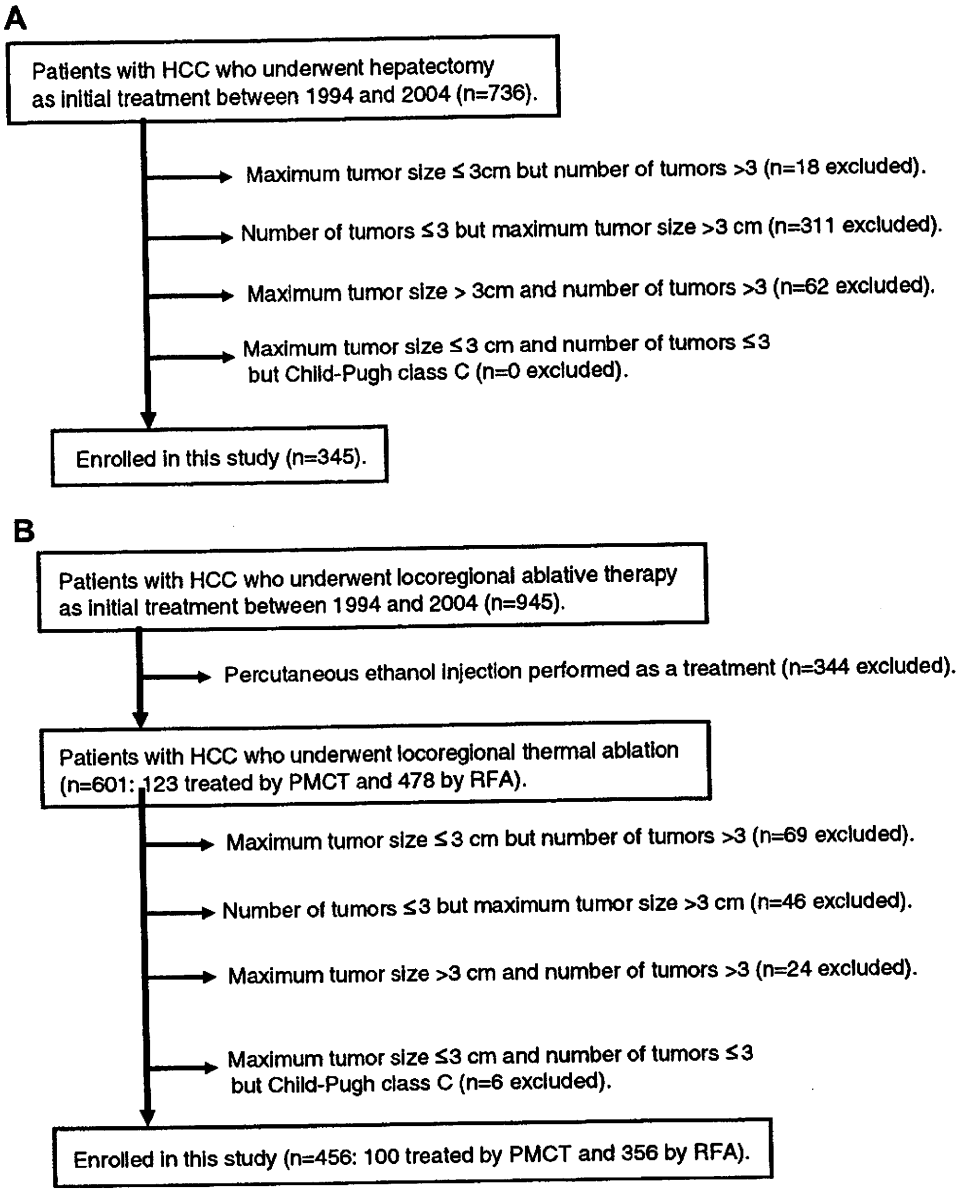

Fig. 1. Schematic representation of the enrolled patients treated by hepatectomy (A) and those treated by locoregional thermal ablation (LTA) (B). HCC, hepatocellular carcinoma; PMCT, percutaneous microwave thermocoagulation; RFA, radiofrequency ablation.

the cut-off level for each tumor marker as 400 ng/dL for AFP, 15% for AFP-L3, and 100 mAU/mL for DCP, respectively, based on our previous study [8]. Patients with pretreatment AFP of ⩾400 ng/dL, those with pretreatment AFP-L3 of ⩾15%, and those with pretreatment DCP of ⩾100 mAU/mL were considered as patients with pretreatment AFP elevation, those with pretreatment AFP-L3 elevation, and those with pretreatment DCP elevation, respectively. AFP-L3 of patients with AFP values not more than 10 ng/dL was decided as 0%, because AFP-L3 percent is normally quantifiable only in patients with AFP values above 10 ng/dL. Patients with AFP values not more than 10 ng/dL were, therefore, considered as those without pretreatment AFP-L3 elevation.

2.4. Statistical analyses

In both the patients who underwent hepatectomy and those who underwent LTA, we compared the survival and recurrence rates of patients stratified by the elevation of each tumor marker at the time of HCC diagnosis. The impact of each tumor marker on patient survival and HCC recurrence was assessed using univariate and multivariate analyses within each of the two groups. The date of HCC diagnosis was defined as time zero for calculations. Surviving patients and patients who died from causes other than liver disease, and patients without HCC recurrence were censored. Patients who died from HCC-related causes or HCC-related liver failure, and patients in whom recurrent HCC developed were not censored. Univariate survival and recurrence curves were calculated by the Kaplan-Meier method [18], and differences in survival and recurrence rates between groups were analyzed by a log-rank test [19]. To identify independent factors associated with the survival and recurrence rates, various likely predictors (p values ⩽0.1) associated with the survival or recurrence by univariate analysis were subjected to multivariate analysis. The Cox proportional hazards model [20] was used for multivariate analysis. The factors included for analyses were patient age, sex (female/male), etiology (hepatitis B virus [HBV]/hepatitis C virus [HCV]/non-HBV, non-HCV), Child-Pugh class (A/B), maximum tumor size (⩽2 cm/>2 cm), number of tumors (single/multiple), portal vein invasion (absent/present), pretreatment AFP level (<400 ng/dL/⩾400 ng/dL), pretreatment AFP-L3 level (<15%/⩾15%), and pretreat-

Table 1
Clinical characteristics of study patients who underwent hepatectomy ($n = 345$)

Age (median, years)	66 (range, 22–82)
Sex	
Male	195 (56.5%)
Female	150(43.5%)
Etiology of underlying liver disease	
HBV	55 (16.0%)
HCV	255 (73.9%)
HBV, HCV	6 (1.7%)
Non-HBV, non-HCV	29 (8.4%)
Child-Pugh class	
A	319 (92.5%)
B	26 (7.5%)
Albumin (mean ± S.D., g/dL)	3.81 ± 0.40
Total bilirubin (mean ± S.D., mg/dL)	0.87 ± 0.47
Tumor size	2.10 ± 0.33
⩽2 cm	173 (50.1%)
>2 cm	172 (49.9%)
Tumor number	1.25 ± 0.28
Single	273 (79.1%)
Multiple	72 (20.9%)
Portal vein invasion[a]	
Absent	338 (98.0%)
Present	7 (2.0%)
AFP (median, ng/dL)	19.1 (range, 0.8–5810)
<400 ng/dL	300 (87.0%)
⩾400 ng/dL	45 (13.0%)
AFP-L3 (median, %)	0.5 (range, 0–81.2)
<15%	282(81.7%)
⩾15%	63 (18.3%)
DCP (median, mAU/mL)	42.0 (range, 3.0–118000)
<100mAU/mL	234 (67.8%)
⩾100 mAU/mL	111 (32.2%)

Number of patients is shown unless otherwise indicated. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.
[a] Evaluated on imaging findings.

Table 2
Clinical characteristics of study patients who underwent locoregional thermal ablation ($n = 456$)

Age (median, years)	68 (range, 34–89)
Sex	
Male	298 (65.4%)
Female	158 (34.6%)
Etiology of underlying liver disease	
HBV	34 (7.5%)
HCV	381 (83.5%)
HBV, HCV	7 (1.5%)
Non-HBV, non-HCV	34 (7.5%)
Child-Pugh class	
A	319 (70.0%)
B	137 (30.0%)
Albumin (mean ± S.D., g/dL)	3.58 ± 0.51
Total bilirubin (mean ± S.D., mg/dL)	1.07 ± 0.69
Tumor size	1.92 ± 0.26
⩽2 cm	288 (63.2%)
>2 cm	168 (36.8%)
Tumor number	1.40 ± 0.31
Single	320 (70.2%)
Multiple	136 (29.8%)
Portal vein invasion*	
Absent	456 (100%)
Present	0
AFP (median, ng/dL)	19.4 (range, 0.8–3770)
<400 ng/dL	426 (93.4%)
⩾400 ng/dL	30 (6.6%)
AFP-L3 (median, %)	0.5 (range, 0–83.8)
<15%	406 (89.0%)
⩾15%	50 (11.0%)
DCP (median, mAU/mL)	30.0 (range, 5.0–27100)
<100 mAU/mL	348 (76.3%)
⩾100 mAU/mL	108 (23.7%)

Number of patients is shown unless otherwise indicated. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin. *Evaluated on imaging findings.

ment DCP level (<100 mAU/mL/⩾100 mAU/mL). (Portal vein invasion was withdrawn from the factors in multivariate analysis of patients who underwent LTA.) All analyses were performed using SAS statistical software (version 8.2; SAS Institute, Cary, NC) or the SPSS Medical Pack for Windows (version 10.0; SPSS, Inc., Chicago, IL). All p values were derived from two-tailed tests, and p values <0.05 were considered statistically significant.

3. Results

3.1. Clinical backgrounds and survival rates of patients who underwent hepatectomy or locoregional thermal ablation

The clinical backgrounds of patients who underwent hepatectomy and LTA are shown in Tables 1 and 2, respectively. More than 90% of patients who underwent hepatectomy had Child-Pugh class A liver function, and the tumor size of half of the patients was greater than 2 cm. In patients who underwent LTA, 30% had Child-Pugh class B liver function, and the tumor size was not greater than 2 cm in more than 60% of patients. No patients who underwent LTA exhibited portal vein invasion of the tumor on imaging findings. AFP, AFP-L3, and DCP were equal to or above the cut-off levels in 13.0%, 18.3%, and 32.2% of hepatectomy patients, respectively, while they were equal to or above the cut-off levels in 6.4%, 11.0%, and 23.4% of LTA patients, respectively.

When focusing on patients with pretreatment AFP-L3 elevation, no significant difference was observed between patients who underwent hepatectomy and those who underwent LTA except for higher rate of Child-Pugh class A in patients treated by hepatectomy. When focusing on patients with pretreatment DCP elevation, in contrast, younger age, lower rate of HCV infection, greater tumor size, smaller tumor number were observed, in addition to higher rate of Child-Pugh class A, in patients treated by hepatectomy (data not shown).

During the follow-up period, 168 patients died, 64 patients lost for follow-up. Twenty patients died from causes other than liver disease, including pneumonia, brain hemorrhage, perforation of duodenal ulcer, acute myocardial infarction, and sepsis caused by cholangitis with common bile duct stone. The 3-year and 5-year survival rates were 80.0% and 66.3%, respectively, in patients who underwent hepatectomy, and they were 76.2% and 61.3%, respectively, in patients who underwent LTA. The recurrence of HCC was observed in 163 of 345 patients (47.2%) who underwent hepatectomy and 292 of 465 patients (62.8%) who underwent LTA. The 1-year and 3-year recurrence rates were 16.8% and 54.8%, respectively, in patients who underwent hepatectomy, and they were 27.9% and 72.6%, respectively, in patients who underwent LTA.

3.2. Impact of tumor markers on the survival of patients who underwent hepatectomy and of patients who underwent locoregional thermal ablation

In patients who underwent hepatectomy, 302 of 345 patients (87.5%) had a complete response and the other 43 patients did not. We found no correlation between pretreatment elevation of tumor markers and the response of hepatectomy. By univariate analysis, we found a significant difference in survival rates by pretreatment AFP-L3 elevation ($p = 0.0264$), as well as Child-Pugh class ($p = 0.0010$) and portal vein invasion of the tumor ($p = 0.0068$, left column of Table 3). In contrast, we found no difference by pretreatment AFP ($p = 0.8151$) or DCP ($p = 0.1919$) elevation (Fig. 2). According to multivariate analysis for the factors that could influence patient survival, Child-Pugh class B ($p = 0.0103$) was selected as a

Table 3
Univariate analysis for factors that influenced survival after treatment in patients who underwent hepatectomy ($n = 345$) and those who underwent locoregional thermal ablation ($n = 456$)

	Patients who underwent Hepatectomy ($n = 345$)			Patients who underwent locoregional thermal ablation ($n = 456$)		
	3-year[a]	5-year[a]	*p*-value	3-year[a]	5-year[a]	*p*-value
Age (years)						
<65	80.7	63.1	0.8459	74.7	58.2	0.5944
⩾65	79.7	70.1		77.1	63.4	
Sex						
Male	79.7	70.1	0.8459	77.1	63.4	0.5944
Female	80.7	63.1		74.4	58.2	
Etiology						
HBV	86.4	65.6	0.4027	86.7	74.3	0.1949
HCV	77.7	65.2		73.8	58.4	
Non-HBV, non-HCV	83.8	83.8		90.0	83.1	
Child-Pugh class						
A	81.9	69.8	0.0010	81.3	68.0	0.0011
B	60.9	35.5		64.7	44.9	
Tumor size						
⩽2 cm	81.9	66.1	0.5985	79.5	65.4	0.0160
>2 cm	78.1	66.5		70.0	54.4	
Tumor number						
Single	78.7	68.4	0.2557	80.7	67.7	0.0002
Multiple	84.3	59.0		64.9	45.1	
Portal vein invasion[b]						
Absent	80.4	66.6	0.0068			
Present	–	–				
AFP						
<400 ng/dL	80.5	66.2	0.8151	76.6	60.9	0.7702
⩾400 ng/dL	76.9	68.4		70.9	70.9	
AFP-L3						
<15%	81.4	66.9	0.0264	78.8	62.9	0.0005
⩾15%	73.8	58.4		50.0	50.0	
DCP						
<100 mAU/mL	81.8	68.6	0.1919	82.0	67.6	<0.0001
⩾100 mAU/mL	76.2	61.5		58.3	40.3	

HBV, hepatitis C virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

[a] 3-year and 5-year survival rates.

[b] No patients with portal vein invasion who underwent hepatectomy were observed more than 3 years.

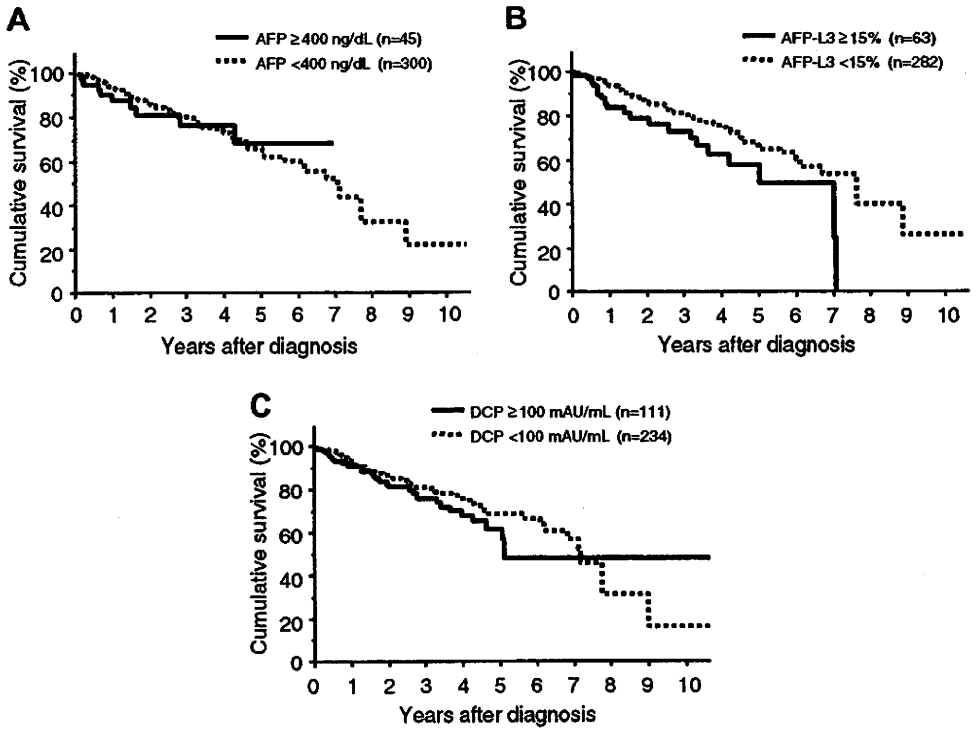

Fig. 2. Cumulative survival rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients treated by hepatectomy. No significant difference was found between patients with or without elevated pretreatment AFP or DCP (AFP, $p = 0.8151$; DCP, $p = 0.1919$). The survival rate of patients with elevated pretreatment AFP-L3 was significantly lower than that of patients without the elevation ($p = 0.0264$).

factor that significantly affected decreased survival rate. Portal vein invasion of the tumor ($p = 0.0732$) and AFP-L3 elevation ($p = 0.0657$) also tended to decrease survival rate (Table 4).

In patients who underwent LTA, all patients had complete response on the basis of the imaging evaluation, because patients received additional sessions of LTA until complete ablation was confirmed. According to univariate analysis, we found a significant difference in survival rates by pretreatment AFP-L3 ($p = 0.0005$) and DCP ($p < 0.0001$) elevation, as well as Child-Pugh class ($p = 0.0011$), tumor size ($p = 0.0160$), and tumor number ($p = 0.0002$, right column of Table 3). We found no difference by pretreatment AFP elevation ($p = 0.7702$, Fig. 3). According to multivariate analysis, Child-Pugh class B ($p = 0.0097$), multiple tumors ($p = 0.0049$), AFP-L3 elevation ($p = 0.0171$), and DCP elevation ($p = 0.0004$) were selected as factors that significantly affected decreased survival rate and tumor diameter >2 cm ($p = 0.0503$) tended to decrease survival rate (Table 5). The elevation of pretreatment DCP level had the strongest effect on decreased survival rate of patients who underwent LTA.

Table 4
Multivariate analysis for factors that influenced survival of patients who underwent hepatectomy ($n = 345$)

Factor	Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*p* value
Child-Pugh class					
1: Class A				1	
2: Class B	0.410	0.147	6.589	1.5069 (1.1090–1.9827)	0.0103
Portal vein invasion					
1: Absent				1	
2: Present	0.817	0.368	3.211	2.2632 (0.9073–4.1450)	0.0732
AFP-L3					
1: <15%				1	
2: ⩾15%	0.252	0.131	3.386	1.2866 (0.9829–1.6495)	0.0657

AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein.

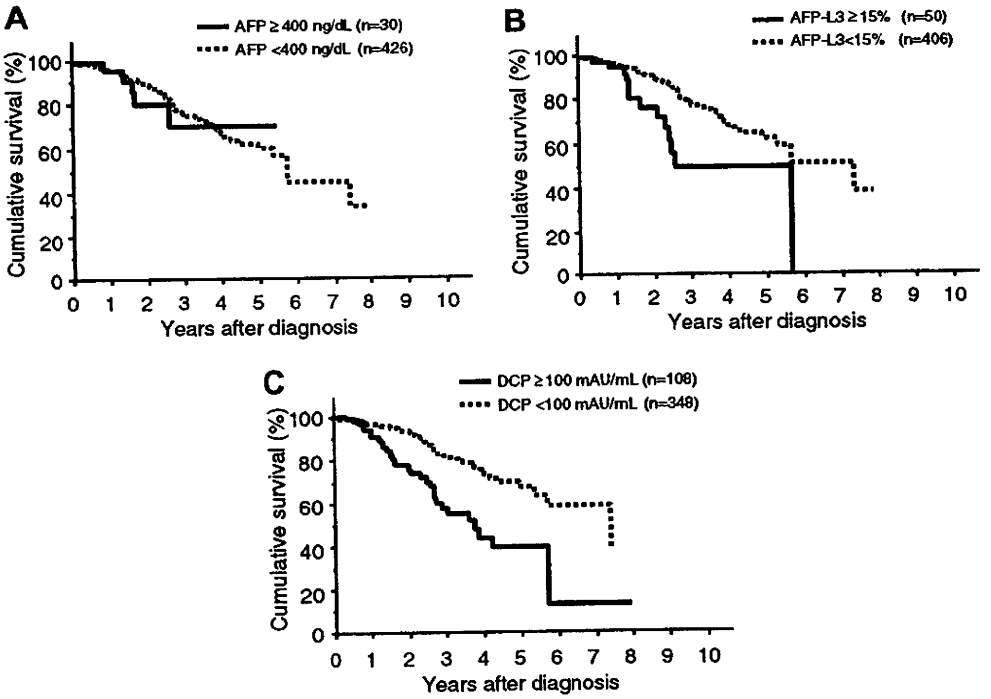

Fig. 3. Cumulative survival rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients who underwent LTA. No significant difference was found between patients with or without elevated pretreatment AFP (AFP, $p = 0.7702$). The survival rate of patients with elevated pretreatment AFP-L3 or DCP was significantly lower than that of patients without the elevation (AFP-L3, $p = 0.0005$; DCP, $p < 0.0001$).

3.3. Impact of tumor markers on the recurrence of HCC in patients who underwent hepatectomy and in patients who underwent locoregional thermal ablation

In patients who underwent hepatectomy, only multiple tumors affected increased recurrence rate in both univariate ($p = 0.0236$) and multivariate ($p = 0.0298$) analyses (data not shown). Pretreatment elevation of AFP, AFP-L3, and DCP did not affect recurrence rate (Fig. 4).

Pretreatment DCP elevation significantly affected increased recurrence rate ($p < 0.0001$, Fig. 5), as well as male sex ($p = 0.0131$) and multiple tumors ($p < 0.0001$), in univariate analysis of patients who underwent LTA. These 3 factors significantly affected increased recurrence rate also in multivariate analyses

Table 5
Multivariate analysis for factors that influenced survival of patients who underwent locoregional thermal ablation ($n = 456$)

Factor	Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*p* value
Child-Pugh class					
1: Class A				1	
2: Class B	0.286	0.108	6.691	1.3310 (1.0731–1.6442)	0.0097
Tumor size					
1: ≤2 cm				1	
2: > 2 cm	0.224	0.113	3.832	1.2508 (0.9997–1.5583)	0.0503
Tumor number					
1: Single				1	
2: Multiple	0.314	0.109	7.906	1.3687 (1.1016–1.6942)	0.0049
AFP-L3					
1: <15%				1	
2: ≥ 15%	0.388	0.150	5.682	1.4743 (1.0768–1.9488)	0.0171
DCP					
1: < 100 mAU/mL				1	
2: ≥ 100 mAU/mL	0.405	0.111	12.468	1.4992 (1.2022–1.8595)	0.0004

AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

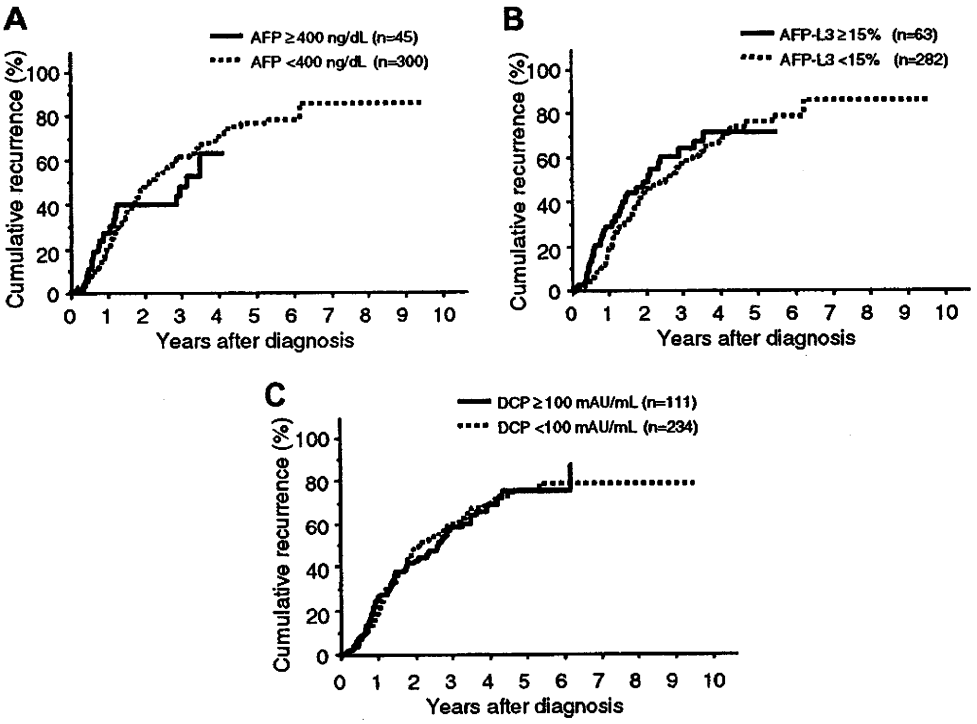

Fig. 4. Cumulative recurrence rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients treated by hepatectomy. No significant difference was found between patients with or without elevated pretreatment AFP, AFP-L3, or DCP (AFP, $p = 0.6924$; AFP-L3, $p = 0.2889$; DCP, $p = 0.8992$).

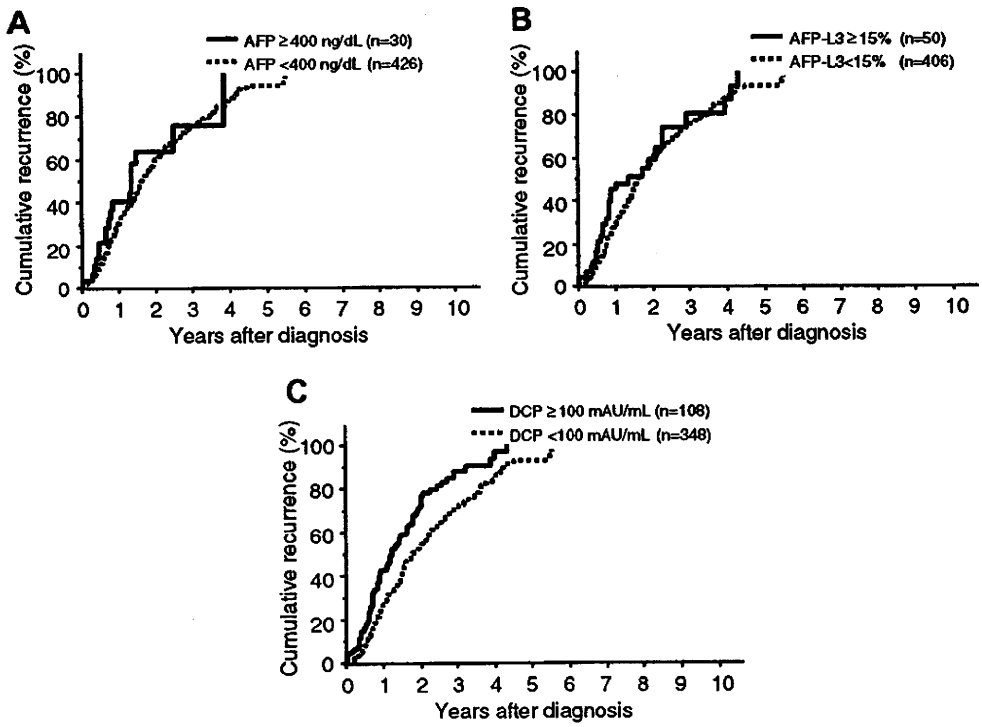

Fig. 5. Cumulative recurrence rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients who underwent LTA. No significant difference was found between patients with or without elevated pretreatment AFP or AFP-L3 (AFP, $p = 0.2323$; AFP-L3, $p = 0.2779$). The recurrence rate of patients with elevated pretreatment DCP was significantly higher than that of patients without the elevation ($p < 0.0001$).

($p = 0.0027$, $p = 0.0352$, and $p < 0.0001$, respectively, data not shown).

4. Discussion

In the present study, we evaluated the prognostic value of the pretreatment levels of three tumor markers for HCC (AFP, AFP-L3, and DCP) in patients who undergo treatments for HCC with curative intent, i.e., hepatectomy and LTA. We excluded patients treated by ethanol injections, which had been widely performed as a locoregional curative treatment for HCC before the emergence of thermal ablation, because a lower survival rate and a higher recurrence rate were reported in patients who underwent ethanol injection relative to those treated by LTA [9].

The survival rate was comparable between patients who underwent hepatectomy and those who underwent LTA. However, we cannot make any conclusions as to the differential benefits of these two treatment modalities. The backgrounds of patients were largely different between the two study populations. The remnant liver function was significantly better in patients who underwent hepatectomy than in those who underwent LTA, with a higher rate of Child-Pugh class A patients, higher serum albumin levels, and lower serum total bilirubin levels (all, $p < 0.0001$). In addition, the tumor size (of maximum tumor) was significantly larger in patients who underwent hepatectomy than in those who underwent LTA ($p < 0.0001$). The purpose of the present study is not to compare the impact of treatment modalities on patient survival, but rather to compare the prognostic value of the pretreatment elevation of tumor markers for HCC in different patient subgroups that underwent different treatments.

In univariate and multivariate analyses of patient survival, we found no difference in survival rates between patients with and without elevated serum AFP, in both patients treated by hepatectomy and those treated by LTA. AFP is currently the most widely used marker for monitoring the development of HCC [3,21,22]. However, AFP also increases in association with hepatocyte regeneration and is associated with serum alanine aminotransferase activity; AFP values above the normal limit (>20 ng) are observed in up to 20% of patients with chronic hepatitis and in 20% to 60% of patients with cirrhosis, even in the absence of HCC [6]. In addition, a recent study revealed the significance of AFP as a marker for liver fibrosis [23]. AFP, therefore, does not always directly reflect the development or progression of HCC. In our previous studies [7,8], the elevation of AFP was not associated with the progression of HCC, although its elevation predicted lower survival rates. These characteristics of AFP probably accounted for the lack of influence of AFP elevation on the survival of patients who underwent hepatectomy or LTA.

The elevation of pretreatment AFP-L3 levels significantly influenced decreased survival rate of patients who underwent LTA, and it also had a tendency to decrease survival rate of patients who underwent hepatectomy in multivariate analyses. In univariate analyses of hepatectomy patients, the survival rate of patients with elevated serum AFP-L3 was significantly lower than that in patients without AFP-L3 elevation. Our previous study revealed that pretreatment AFP-L3 elevation reflects greater number of tumors as well as larger HCC in size [7]. The pretreatment elevation of AFP-L3 might reflect the presence of small HCC tumors that cannot be detected in the liver by currently available imaging technologies, such as intrahepatic microscopic metastases of HCC, resulting in decreased survival rates in patients with elevated pretreatment AFP-L3 after the initial treatment of both hepatectomy and LTA. However, we found no difference in recurrence rate of HCC in both patient subgroups. Further study will be needed for the effect of pretreatment AFP-L3 elevation on patient survival and HCC recurrence in patients treated by curative therapies.

The elevation of pretreatment DCP level exhibited the most significant impact on decreased survival rate of patients who underwent LTA, whereas it did not affect the survival rate of hepatectomy patients. DCP elevation is reportedly an indicator of portal vein invasion of HCC [24]. Our previous study also revealed higher rates of HCCs with portal vein invasion as well as larger HCC in size in patients with elevated pretreatment DCP [7]. The presence of microscopic invasions of HCC tumors into the portal vein that was not detected by imaging modalities might be present more likely in patients with elevated pretreatment DCP. The presence of such invasions might affect decreased survival rate of patients who underwent LTA with increased recurrence rate, whereas hepatectomy might be able to overcome this microscopic invasion.

In summary, tumor markers for HCC had different impacts on the survival of HCC patients who underwent hepatectomy or LTA. Elevated pretreatment AFP-L3 levels affect decreased survival of patients who underwent hepatectomy and LTA but without increased recurrence. Elevated levels of pretreatment DCP had a significant impact on decreased survival only of patients who underwent LTA with increased recurrence rate. These data should be taken into consideration when selecting treatment options. In addition, further studies will be needed to evaluate the impact of other potential tumor markers for HCC, such as glypican-3, Golgi protein 73, hepatocyte growth factor, insulin growth factor 1, vascular endothelial growth factor, transforming growth factor-beta1, and alpha-L-fucosidase [25], on the survival of patients treated by different therapies.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2008.04.013.

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Mutations in the Interferon Sensitivity-Determining Region of Hepatitis C Virus Genotype 2a Correlate With Response to Pegylated-Interferon-Alpha 2a Monotherapy

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The interferon sensitivity-determining region (ISDR) is thought to be inhibited by the double-stranded RNA-dependent protein kinase (PKR). Several studies have reported a relationship between the ISDR and interferon (IFN) responsiveness. However, this relationship is controversial. The aim of this study was to investigate whether genomic heterogeneity of the ISDR among patients with hepatitis C virus (HCV) genotype 2a affects the response to pegylated-IFN-alpha 2a monotherapy. Eighty patients (47 men, 33 women; mean age: 54.2 ± 12.9 years) infected with HCV genotype 2a were evaluated. HCV viral loads were determined by real-time PCR. The ISDR (amino acids 2193–2228) was examined by direct sequencing. Thirty-one patients received subcutaneous injections of pegylated-IFN-alpha 2a (180 μg) once weekly for 24 weeks, and 35 patients received injections for 48 weeks. Fourteen patients withdrew from treatment. Of the remaining 66 patients, 51 (77.3%) showed a sustained virologic response. Factors related to sustained virologic response on multivariate analysis were rapid virologic response (negative HCV at 4 weeks; odds ratio: 0.033; 95% confidence interval (95% CI) 0.003–0.363; $P = 0.0052$) and the number of mutations in the ISDR (odds ratio: 0.025; 95% CI 0.001–0.476; $P = 0.0141$). There were no significant differences in other factors, including sex, age, aspartate aminotransferase, alanine aminotransferase, platelet count, duration of treatment, and HCV viral load. Rapid virologic response and the ISDR sequence variations are significantly associated with response to pegylated-IFN-alpha 2a monotherapy in Japanese patients with HCV genotype 2a. ***J. Med. Virol. 81:459–466, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: sustained virologic response; rapid virologic response; chronic hepatitis C

INTRODUCTION

Hepatitis C virus (HCV) is a member of the *Flaviviridae* family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) [Seeff, 2002]. HCV infection is a significant global health problem, affecting 170 million individuals worldwide. HCV consists of three structural proteins (core, envelope 1, and envelope 2) and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). HCV NS5A protein was reported to have a domain associated with interferon (IFN) response. This domain, located in the NS5A region of HCV, is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996; Murakami et al., 1999; Nakano et al., 1999; Pascu et al., 2004]. There are several modes of IFN action

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against HCV infection, and the final mode is still under debate. However, one mechanism of IFN action involves inhibition of viral replication by inducing the double-stranded RNA-dependent protein kinase (PKR). The ISDR is located in the 5′ end of the PKR-binding domain and is inhibited by PKR in vitro [Gale et al., 1998]. Therefore, ISDR heterogeneity is an important factor that may affect response to IFN. The utility of ISDR sequences for predicting IFN responsiveness has been investigated for HCV genotype 1b, as well as for genotypes 2 and 3, because HCV genotypes, which vary in prevalence around the world, influence IFN responsiveness [Manns et al., 2001; Fried et al., 2002; Simmonds et al., 2005]. HCV genotype 2a is relatively common in Japan [Enomoto et al., 1990; Hayashi et al., 2003]. However, there are few reports regarding the ISDR and IFN responsiveness in HCV genotype 2a [Murakami et al., 1999; Kobayashi et al., 2002; Akuta et al., 2005], and the association of mutations in the ISDR and response to IFN therapy among patients with HCV genotype 2a is not well understood. The aim of the present study was to determine whether genomic heterogeneity of the ISDR among patients with HCV genotype 2a affects the response to pegylated-IFN-alpha 2a monotherapy.

MATERIALS AND METHODS

This prospective analysis involved 80 patients with chronic hepatitis C who received pegylated-IFN-alpha 2a monotherapy between January 2004 and December 2005. Patients who were previously treated with IFN were excluded. All patients were positive for serum anti-HCV antibody on a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV-RNA on a commercial polymerase chain reaction (PCR) test (Roche Diagnostic Systems, Tokyo, Japan). No patients had hepatitis B surface antigen, coinfection with human immunodeficiency virus, autoimmune disease, or chronic alcohol abuse.

Schedule of IFN Therapy

Patients received pegylated-IFN-alpha 2a (Pegasys Roche, Tokyo, Japan) at a dose of 180 μg injected subcutaneously once weekly for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluation of adverse events were performed once weekly during treatment. The pegylated-IFN-alpha 2a dose was dropped to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50,000 cells/mm^3) occurred. Pegylated-IFN-alpha 2a was discontinued when neutropenia (<250 cells/mm^3) or a platelet count below 25,000 cells/mm^3 was observed. Patients who did not receive 80% of the ideal total dose of IFN were defined as the reduced-dose group. Serum HCV-RNA levels were examined at 4, 12 weeks, at the end of IFN therapy, and 6 months after the end of treatment. Serum was stored at −80°C for virologic examination. Patients who were persistently negative for serum HCV-RNA and who had a normal serum alanine aminotransferase (ALT) level 24 weeks after withdrawal of IFN treatment were considered to have a sustained virologic response. Patients who were HCV-negative at the end of the treatment but returned to HCV-positive status after withdrawal of IFN were defined as virologic relapsers. Patients who did not become HCV-negative with IFN therapy were defined as virologic non-responders. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virologic Tests

The HCV-RNA quantitative viremia load was determined using real-time PCR [Takeuchi et al., 1999]. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously [Otagiri et al., 2002; Hayashi et al., 2003]. The genotypes were classified according to the nomenclature proposed by a previous report [Simmonds et al., 2005]. Direct sequencing of the ISDR region was performed using serum samples taken within 2 days before the first administration of pegylated-IFN-alpha 2a. In brief, RNA was extracted from 140 μl of sera with a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. Ten nanograms of the RNA was used for reverse transcription using the oligo and random hexamer primers of a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA). The ISDR was amplified by hemi-nested PCR. In brief, each 50-μl PCR reaction contained 100 nM of each primer, 1 ng template cDNA, 5 μl of GeneAmp 10× PCR buffer, 2 μl of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primer sequences were sense, 5′-ACGTCCATGCTAACAGACCC-3′ and antisense, 5′-GGGAATCTCTTCTTGGGGAG-3′. Amplification conditions consisted of 10 min at 94°C followed by 40 cycles of 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was done in the same reaction buffer with the first-round PCR product as the template, the sense primer from the first-round PCR, and a new antisense primer, 5′-CGAGAGAGTC-CAGAACGACC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers using a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). The neighbor-joining method was used for phylogenetic analysis of the ISDR (amino acids 2193–2228) in the NS5A region [Saitou and Nei, 1987], and bootstrap analysis (1,000 replicates) was performed [Felsenstein, 1985].

Statistical Analysis

The data are expressed as mean ± standard deviation (SD). The paired *t*-test was used to analyze differences in the variables. A *P*-value of <0.05 was considered statistically significant. Multiple logistic regression models were used to identify factors predictive of sustained virologic response. Statview 5.0 software (SAS Institute Inc., Cary, NC) was used for all analyses.

RESULTS

The patients' clinical characteristics are summarized in Table I. All patients were infected with HCV genotype 2a, and 27 of 80 (33.8%) patients had a serum HCV-RNA level higher than 1 million copies/ml. Eighty patients were initially entered, but 14 patients withdrew from IFN therapy, and 4 of these 14 patients could not be followed-up. The remaining 66 patients were followed-up for 6 months after the end of treatment. The completion rate was 82.5% (66/80). Thirty-one patients were treated with pegylated-IFN-alpha 2a for 24 weeks, and 35 patients were treated for 48 weeks. Virologic response is shown in Table II. The rapid virologic response rate, which was defined as negativity for HCV after 4 weeks of treatment, was 74.2% (49/66). The early virologic response rate, which was characterized by undetectable HCV at 12 weeks, was 92.4% (61/66). The virologic response rate at the end of the treatment was 97.0% (64/66). Finally, 51 of 66 (77.3%) patients achieved sustained virologic response. There were no significant differences in clinical characteristics and virologic response between patients treated for 24 weeks and those treated for 48 weeks. ISDR sequences were obtained in 62 patients, and the sequence alignments of the ISDR according to virologic response are shown in Figure 1. The mean number of ISDR mutations in patients with non-sustained virologic response was 1.2 ± 0.6, and that in patients with sustained virologic response was 2.8 ± 2.1. Patients with sustained virologic response had a significantly higher number of mutations in the ISDR than did patients with non-sustained virologic response ($P = 0.0090$). Codon 2205 was frequently changed. The association of this single mutation with sustained virologic response was examined; however, there was no significant relationship between a single mutation at codon 2205 and sustained virologic response. Sequences of the HCJ6 strain and the HCJ6 strain with all nucleotide substitutions in codon 2205 were defined as the wild type, and ISDR sequences that deviated from these strains were defined as mutant type. A rapid virologic response was achieved in 7 of 33 patients with wild-type ISDR and 5 of 41 patients with mutant-type ISDR. There were no correlations between rapid virologic response and ISDR sequence. Mutant-type ISDR was detected more frequently in sustained virologic response patients (66.7%) than in non-SVR patients (28.6%) (odds ratio: 0.200; 95% confidence interval (95% CI) 0.054–0.738; $P = 0.015$). Phylogenetic analyses of the ISDR (amino acids 2193–2228) of the 62 patients were performed, and the results are shown in Figure 2. There were differences in distinctive clustering between the wild type and the mutant type defined by counting the number of substitutions in the ISDR, but no distinctive clustering was observed in wild types with A2205 and with T2205 and with V2205. The phylogenetic analyses did not show a significant relationship between the ISDR sequences and sustained virologic response. The clinical characteristics of the patients who achieved sustained virologic response are compared to those without sustained virologic response in Table III. There were significant differences in four factors (age, HCV-RNA level, the number of mutations in the ISDR, and rapid virologic response) between the sustained virologic response group and the non-sustained virologic response group on univariate analysis. The results of the multivariate analyses of factors predictive of sustained virologic response are shown in Table IV. The variables were recorded categorically as ordinal data. The background factors were: age (<60 years vs. ≥60 years); sex (male vs. female); platelet count ($<15 \times 10^4/mm^3$ vs. $\geq 15 \times 10^4/mm^3$); HCV-RNA level ($<10^6$ copies/ml vs. $\geq 10^6$ copies/ml); ALT levels (<70 IU/L vs. ≥70 IU/L); AST levels (<60 IU/L vs. ≥60 IU/L); length of IFN therapy (24 weeks vs. 48 weeks); reduction of IFN dose (yes or no); ISDR (wild type vs. mutant type); and rapid virologic response (yes or no). Rapid virologic response at 4 weeks was the most influential factor ($P = 0.0052$), followed by mutations in the ISDR ($P = 0.0141$). No other factors achieved statistical significance. Analysis of rapid virologic response in combination with the ISDR revealed that 28 of 29 patients with mutant-type ISDR and rapid virologic response achieved sustained virologic response. The positive predictive value for sustained virologic response was 96.6% (28/29). IFN therapy was withdrawn from 14 patients. The reasons for discontinuing therapy, length of IFN therapy, ISDR sequences, rapid virologic response, and outcomes are shown in Figure 3. Ten patients discontinued therapy within 16 weeks, but 4 of the 10 patients achieved sustained virologic response. All sustained virologic response patients who withdrew from therapy within 16 weeks had at least three ISDR mutations.

TABLE I. Clinical Characteristics

	N = 80
Age (y.o.)	54.2 ± 12.9
Sex: male/female	47/33
AST (IU/L)	57.9 ± 37.5
ALT (IU/L)	81.1 ± 65.3
Platelet count ($10^4/\mu l$)	20.7 ± 22.2
HCV-RNA level (copies/ml)	360,000 (540–63,000,000)
Body weight (kg)	60.8 ± 9.8

Data are expressed as mean ± standard deviation. HCV-RNA level was shown by median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

TABLE II. Virologic Response Rates

	All (n = 66)	24 weeks (n = 31)	48 weeks (n = 35)
Rapid virologic response	74.2% (n = 49)	77.4% (n = 24)	71.4% (n = 25)
Early virologic response	92.4% (n = 61)	96.8% (n = 30)	88.6% (n = 31)
End of treatment response	97.0% (n = 64)	96.8% (n = 30)	97.1% (n = 34)
Sustained virologic response	77.3% (n = 51)	77.4% (n = 24)	77.1% (n = 27)

Rapid virologic response as HCV-negative at 4 weeks. Early virologic response as HCV-negative at 12 weeks. End of treatment response as HCV-negative at the end of the treatment. Sustained virologic response as HCV-negative at 24 weeks after withdrawn of treatment.

DISCUSSION

HCV genotype is one of the most important factors that predict response to IFN therapy. Genotypes 1 and 4 respond poorly to IFN therapy, whereas genotypes 2 and 3 show a sustained virologic response to IFN therapy. However, patients infected with HCV genotype 2 respond differently to IFN therapy, suggesting that an additional viral factor associated with resistance to IFN exists. The ISDR sequence in the HCV NS5A region may influence the IFN response of patients with HCV genotype 1b [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004]. The influence of the ISDR sequence in response to IFN has been investigated in patients with HCV genotypes 2a and 2b [Murakami et al., 1999; Kobayashi et al., 2002; Akuta et al., 2005]. In the present study, it was hypothesized that the amino acid variations in ISDR would explain differences in IFN resistance in patients infected with HCV genotype 2a. Multivariate analyses showed that mutation of the ISDR is one of the most influential factors for sustained virologic response (odds ratio: 0.025; 95% CI 0.001–0.476; $P = 0.0141$). The sustained virologic response rate of patients with more than three mutations in the ISDR was 100% (23/23) in the present study. The results confirmed that the number of mutations in the ISDR is an important determinant of the effectiveness of pegylated-IFN-alpha 2a monotherapy in patients with

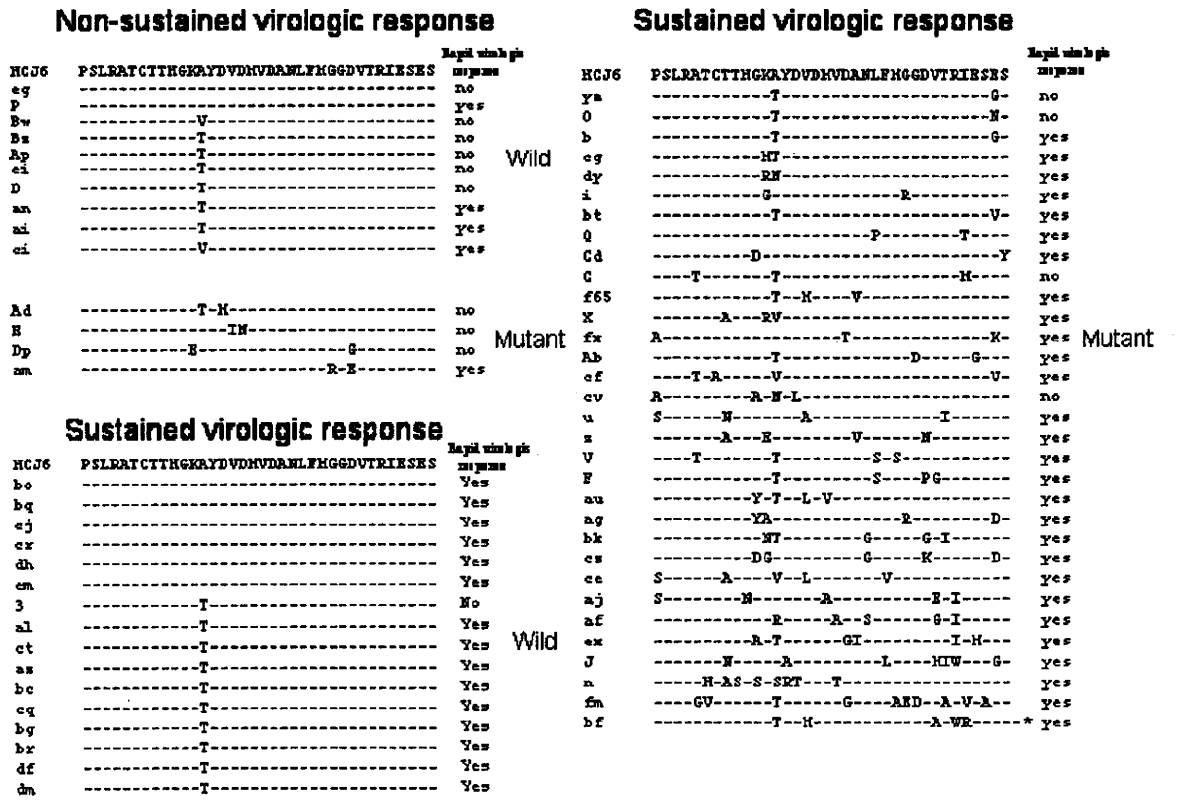

Fig. 1. Alignment of the amino acid sequence of the ISDR and response to pegylated-interferon-alpha 2a therapy. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCJ6. Sequences of the HCJ6 strain and the HCJ6 strain with all nucleotide substitutions in codon 2205 were defined as wild-type ISDR, and the other strains were defined as mutant-type ISDR. The strain marked with an asterisk had an insertion mutation. ISDR, interferon sensitivity-determining region.

Fig. 2. Results of phylogenetic analysis of 62 sequences from the interferon sensitivity-determining region (amino acids 2193–2228) and relationship with the response to pegylated-interferon-alpha 2a therapy. Phylogenetic analysis was performed by the neighbor-joining method. HCVJ, which is the prototype of genotype 1b, was used as the outer group. The scale bar indicates genetic distance. Each strain from the present study is shown with original code followed by the virologic response. All strains without description of virologic response were rapid virologic response and sustained virologic response. Definition of wild type was counting the number of substitution in the ISDR.

TABLE III. Clinical Characteristics of Patients With or Without Sustained Virologic Response

Factors	Sustained virologic response (n=51)	Non-sustained virologic response (n=15)	*P*-value
Age (y.o.)	52.7 ± 13.1	60.3 ± 6.8	0.0356
Gender: male/female	33/18	6/9	0.1346
ALT (IU/L)	75.6 ± 57.7	66.8 ± 14.1	0.6002
AST (IU/L)	51.8 ± 29.4	56.5 ± 40.1	0.6218
PLT ($\times 10^4$/mm^3)	18.5 ± 6.0	15.5 ± 5.4	0.0866
HCV-RNA level (copies/ml)	340,000 (2,600–63,000,000)	1,400,000 (50,000–22,000,000)	0.0067
Reduction: yes/no	8/43	6/9	0.0691
Duration: 24 weeks/48 weeks	24/27	7/8	0.9999
Mutations in the ISDR	2.8 ± 2.1	1.2 ± 0.6	0.0090
Rapid virologic response: yes/no	44/7	5/10	0.0001

Data are expressed as mean ± standard deviation. HCV-RNA level was shown by median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

HCV genotype 2a. In addition, 10 patients discontinued IFN therapy within 16 weeks in the present study; 4 of these 10 patients achieved sustained virologic response. All sustained virologic response patients who discontinued IFN therapy were infected with mutant-type ISDR. Thus, the mutant-type ISDR appears to be associated with good response to IFN. The ISDR sequence variation of HCV genotype 2a may also play an important role as a predictor of IFN responsiveness. However, most Western reports have not confirmed the clinical usefulness of ISDR analysis for predicting response to IFN therapy [Zeuzem et al., 1997; Chung et al., 1999; Squadrito et al., 2002]. Bias relating to the IFN therapy regimens, racial differences, and HCV strains may have produced this conflicting result. To investigate the role of the ISDR while avoiding bias, all of the patients in the present study were infected with genotype 2a and received pegylated-IFN-alpha 2a monotherapy. Most studies that did not find ISDR analysis useful had a lower dose of IFN than those that reported that ISDR analysis was useful (3 million units vs. 6–10 million units). A low IFN dose was associated with a low sustained virological response rate. The present study and the studies that confirmed the usefulness of ISDR analysis had a higher sustained virological response rate (mean 50.5%) than those that did not confirm the usefulness of ISDR analysis (mean 9.6%) [Enomoto et al., 1996; Zeuzem et al., 1997; Chung et al., 1999; Murakami et al., 1999; Nakano et al., 1999; Squadrito et al., 2002]. The low sustained virological response rate, as well as the low IFN dose, would not favor the use of ISDR analysis for predicting IFN responsiveness. The number of substitutions in the ISDR in reports with negative results was significantly smaller than in studies that confirmed the correlation between ISDR mutations and IFN responsiveness [Herion and Hoofnagle, 1997]. The present study and other studies that confirmed the association between ISDR mutations and IFN sensitivity frequently found that the patients had ISDR mutant type [Saiz et al., 1988; Murakami et al., 1999; Nakano et al., 1999]. The prevalence of patients infected with ISDR mutant type would affect the association between ISDR sequence and IFN responsiveness. Thus, a study including a large number of patients with two or more amino acid substitutions in the ISDR would be suitable for using the ISDR system to predict sustained virologic response. The original classification for the ISDR sequence of genotype 1b included three categories (wild, intermediate, and mutant) according to the number of amino acid substitutions compared to the HCVJ strain. In the present study, sequences of the HCJ6 strain and the HCJ6 strain with all amino acid substitutions in codon 2205 were defined as the wild type, and the other strains were mutant type. The classification for the ISDR sequence was minimally modified for ease of analysis

TABLE IV. Multivariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.0554	8.306	0.952	72.486
Sex: male	0.8270	1.228	0.194	7.778
ALT: <70 IU/L	0.5065	0.227	0.003	17.976
AST: <60 IU/L	0.9923	1.020	0.018	58.089
PLT: $<15 \times 10^4$/mm^3	0.1528	0.154	0.012	2.001
HCV-RNA level: $<10^6$ copies/ml	0.4830	0.437	0.043	4.425
Reduction: yes	0.2242	0.187	0.013	2.790
Duration: 48 weeks	0.1016	8.100	0.662	99.135
ISDR: wild	0.0141	0.025	0.001	0.476
Rapid virologic response: no	0.0052	0.033	0.003	0.363

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

Characteristics of the 14 patients withdrawing the treatment

length	reasons	HCJ6	PSLRATCTTHGKAYDVDHVDANLFHGGDVTRIESES	Rapid virologic response	effect
3wks	economic	none	////////////////////////////////////	no	relapse
4wks	fatigue	none	////////////////////////////////////	yes	Sustained virologic response
5wks	economic	ah	----------T----------	no	No virologic response
9wks	rash	bu	----------D----------R----W-----	yes	Sustained virologic response
10wks	ALT elevate	G	S------A----T----LT----------I------	yes	Sustained virologic response
10wks	moving	ca	----------	yes	dropout
11wks	ALT elevate	ec	----------R----------	no	relapse
12wks	unknown	fv	----------	yes	dropout
13wks	fatigue	aq	----------M----S--S-------G-	yes	Sustained virologic response
15wks	ineffective	Y	----T-------T----------	no	No virologic response
20wks	unknown	cs	----T--A--------A----------I------	yes	dropout
27wks	depression	L	A----------V----------G-V------	yes	Sustained virologic response
28wks	moving	h	----------YC----------	yes	dropout
40wks	pneumonia	r	--------YG---H-M----------	yes	Sustained virologic response

Fig. 3. Clinical characteristics of the 14 patients who withdrew from pegylated-interferon-alpha 2a therapy. Reasons for discontinuing therapy, length of therapy, alignment of the amino acid sequence of the ISDR, rapid virologic response, and response to IFN therapy are shown. ISDR, interferon sensitivity-determining region.

and adjusted for genotype and IFN protocol. Adjustment for racial differences, diversity between the HCV strains with respect to genotype and ISDR sequence, and IFN regimen would be needed to use the ISDR as a simple diagnostic tool to predict sustained virologic response. Nevertheless, the present study had a few limitations. Only the correlation between mutations within the ISDR and sustained virologic response was analyzed, although other parts of NS5A have been reported to be associated with IFN response [Nousbaum et al., 2000; Murphy et al., 2002]. The approach of counting the number of mutations to the chosen consensus sequence in the ISDR, originally reported by Enomoto, was used for the present analysis; however, this method may not be the best way to measure sequence variation. Phylogenetic analyses of the ISDR were used to evaluate the diversity of the ISDR sequence, but distinctive clustering was not found in the wild types with A2205 and with T2205 and with V2205. The ISDR interacts with PKR and inactivates replication of HCV in vitro [Gale et al., 1998]. However, some reports have not confirmed the interaction between PKR and NS5A [Podevin et al., 2001; Tan and Katze, 2001]. PKR-independent effects of NS5A have been reported [Polyak et al., 2001; Evans et al., 2004]. Although the effect of amino acid substitutions of the ISDR was unclear, the ISDR system could be used clinically as a simple diagnostic tool to predict sustained virologic response in patients infected with genotype 2a who received pegylated-IFN-alpha 2a monotherapy.

The current recommended therapy for patients with HCV genotype 2 is a combination of pegylated-IFN and ribavirin for 24 weeks [Strader et al., 2004]. However, pegylated-IFN-alpha 2a monotherapy in patients with HCV genotype 2a resulted in a high sustained virologic response rate (77.3%). Most reports dealing with pegylated-IFN-alpha and ribavirin combination therapy did not differentiate between HCV genotypes 2 and 3 or did not classify subgenotypes 2a and 2b [Zeuzem et al., 2004; Mangia et al., 2005; von Wagner et al., 2005; Shiffman et al., 2007]. There is also limited information regarding sustained virologic response in patients with HCV genotype 2a treated with pegylated-IFN-alpha and ribavirin combination therapy. Thus, it is difficult to compare the present results to those obtained with pegylated-IFN-alpha and ribavirin combination therapy. Large, randomized, prospective studies of pegylated-IFN-alpha with or without ribavirin for patients with genotype 2a, especially ISDR mutant, are needed to clarify these issues. The present study combined two predictive factors: rapid virologic response and the amino acid variations in ISDR compared to the reference sequence. Rapid virologic response is considered to be a strong indicator of progression to sustained virologic response for patients with HCV genotype 2a. Knowledge of both the ISDR sequence and rapid virologic response would be useful for individualization of IFN regimens for chronic hepatitis C patients, but rapid virologic response cannot be assessed before treatment. In the present study, there were no predictive factors associated with rapid virologic response on multivariate analyses (data not shown). Thus, it is impossible to predict which patients will be rapid virologic responders before IFN therapy. With respect to assessment before starting treatment, the number of mutations in the ISDR is a better predictor than rapid virologic response.

In conclusion, the present results indicate that pegylated-IFN-alpha 2a monotherapy is effective for achieving sustained virologic response in Japanese patients with HCV genotype 2a, particularly in those with rapid virologic response and mutant-type ISDR.

The ISDR sequence variation of HCV genotype 2a is useful for predicting IFN responsiveness.

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3. 画像検査　1）超音波検査

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はじめに

肝細胞癌（HCC）には高危険群の設定が可能で，慢性肝疾患を腫瘍マーカーもしくは超音波検査で定期的にスクリーニングすれば，小さな時期での肝腫瘍の発見もそれほど困難ではなくなってきた．加えて，近年の超音波検査機器の進歩に伴い，デジタル画像，ドプラ画像，ハーモニックイメージングなどに各種の技術革新がなされ，さらに超音波造影剤も出現してきた．しかし，多くの施設の日常臨床において，最初に施行されるのは超音波Bモードである．

本稿では限局性肝疾患，とくにHCCの診断における超音波検査の役割について，Bモード画像（基本波，ティッシュハーモニックイメージング［THI］，図1），ドプラ画像，造影超音波画像の各々について述べる．

Bモード画像

Bモード画像に関しては，1988年に日本超音波医学会より「肝腫瘤超音波断層法の診断基準」が出された[1]．HCCでは形状は球形で，境界は鮮明かつ平滑，薄い辺縁低エコー帯（ハロー），モザイクパターン，後方エコーの増強，外側陰影などの所見が重視され，Bモードでの質的診断の基準として広く使われてきた（図2）．大変優れた診断基準ではあったが，超音波装置の進歩により，発見されるHCC結節が1 cm前後のこともまれではなくなってきた．このため，小さなHCCにも対応できるBモードの超音波断層法の診断基準が切望されている．

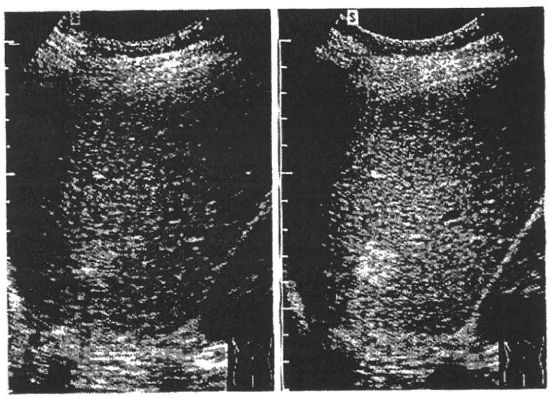

図1　肝血管腫，通常Bモードとティッシュハーモニックイメージング（THI）
基本波Bモード（左）に比し，THI（右）ではくっきりした高エコー腫瘤が描出されている．

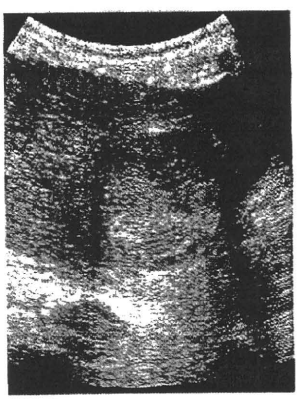

図2　肝細胞癌（HCC）の典型的超音波像
ハロー，モザイクパターン，後方エコーの増強，外側陰影を認め，典型的なHCCの超音波像である．

表 1　肝細胞癌（HCC）の診断基準

細分類	形状	腫瘍境界	腫瘍辺縁	腫瘍内部	付加所見
結節型（≦2 cm）	円形，類円形	不明瞭なことが多い	辺縁低エコー帯（頻度小）	低エコー（高エコーやモザイクパターンを認めることもある）	後方エコー bright loop
結節型（>2 cm）	円形，類円形	明瞭整	薄い辺縁低エコー帯（ハロー）	モザイクパターン，nodule in nodule（大きさや分化度により異なる）	後方エコーの増強 外側陰影 hump sign
塊状型	不整形	不明瞭		エコーレベルはさまざま	門脈や肝静脈の腫瘍栓を有する場合がある，hump sign
肝細胞癌（旧診断基準，1988 年）	球形	鮮明かつ平滑	薄い辺縁低エコー帯内側鮮明	モザイク状無エコー域，星形	後方エコーの増強 外側陰影を認めることもある

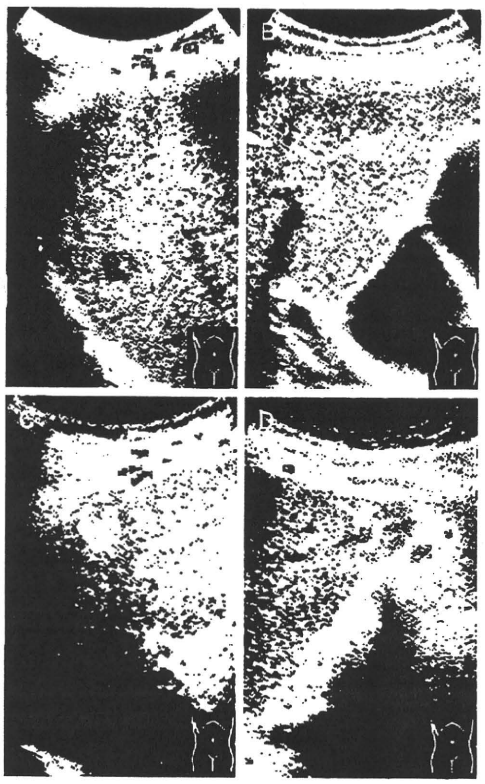

図 3　小 HCC の超音波 B モード画像
A：2 cm 以下の結節型で低エコー結節（矢印），B：等エコー結節（矢印），C：高エコー結節（矢印），D：bright loop（矢印）のいずれも HCC である．異型結節との鑑別は困難．

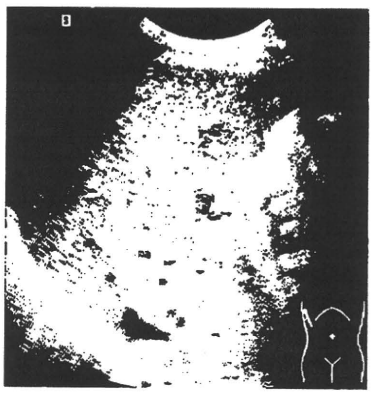

図 4　び漫型 HCC
矢印の部位の門脈が腫瘍塞栓で埋まっている．肝実質のスペックルパターンは粗造である．

表 1 に，我々の考えている診断基準を示す．主体となる結節型は，サイズにより 2 cm 以下と 2 cm をこえるものに分け，腫瘍境界，腫瘍辺縁，腫瘍内部に注目して所見をあげ，これらに含まれないものは付加所見として示した．各々のタイプでの代表的な所見を記載している．加えて塊状型の HCC の所見も示した．

一方，原発性肝癌取扱い規約（第 5 版）では，HCC の肉眼分類として小結節境界不明瞭型，浸潤型，び漫型も分類されているが[2]，これらは腫瘤を形成せず，エコーレベルも肝実質との差が少なく存在が認識しにくい場合が多いので，診断基準からは除いてある．しかし，び漫型や浸潤型は門脈や肝静脈の腫瘍栓を有する場合があり，この所見によって診断されることも多い．診断には computed tomography（CT）もしくは magnetic reso-

表 2　肝細胞癌のドプラ所見

細分類	血流の多寡	血管の走行	血流の性状	付加所見
結節型（≦2 cm）	少ない	ときに腫瘍内部に線状もしくは点状	定常性 ときに拍動性	血流信号が認められないことが多い
結節型（>2 cm）	多い	バスケットパターン	拍動性 ときに定常性	A-P shunt を認めることもある
塊状型	多い	不整な血管，バスケットパターン	拍動性	A-P shunt を認めることもある，門脈腫瘍栓内に低速拍動流を認めることがある

nance image（MRI）などの他の画像診断法の併用が必要となる．小結節境界不明瞭型は，組織学的には早期 HCC に相当する．異型結節（dysplastic nodule）は，基本的には HCC 結節型（2 cm 以下）の所見に類似し，鑑別は困難である．

図 3 に，結節型 2 cm 以下で，低エコー，等エコー，高エコーおよび bright loop 症例の超音波画像を示した．これらの症例の超音波所見は非特異的であり，超音波造影剤，CT もしくは MRI などの他の画像診断で，血流情報を加味しての質的診断が必要となる．また，**図 4** には，び漫型 HCC で門脈腫瘍栓で発見された症例の超音波画像を示した．超音波検査のみに頼らず，他の画像診断および腫瘍マーカーを加えた慎重な経過観察が重要である．

HCC と鑑別を要する限局性肝病変として，肝細胞腺腫，肝内胆管癌腫瘤形成型（胆管細胞癌），転移性肝腫瘍，肝血管腫，FNH（focal nodular hyperplasia，限局性結節性過形成），肝嚢胞，限局性脂肪肝などがある．転移性肝腫瘍は多結節型，ときに塊状型で境界は明瞭，不整（粗い凹凸）を示し，腫瘍辺縁は厚い低エコー帯（bull's eye pattern, target pattern）で，腫瘍内部は高エコー，低エコー，中心部に無エコー域，石灰化などを認める．肝内胆管癌腫瘤形成型は，基本的には腺癌の肝転移像との鑑別は困難であるが，末梢胆管の拡張を認める場合がある．肝血管腫の腫瘍境界は明瞭，不整（細かい凹凸）で，腫瘍辺縁は高エコー帯を認めることもあり（marginal strong echo），内部は高エコー型，辺縁高エコー型，混在型，低エコー型に分けられる．経時的に，あるいは体位変換や圧迫により内部のエコーレベルが変化することが知られており，wax and wane sign，chameleon sign，disappearing sign ともよばれ，肝血管腫の特徴像とされている．FNH の腫瘍境界は不明瞭（明瞭な場合もある）で，内部エコーは低～高とさまざまで，中心部高エコー，線状高エコーは中心性瘢痕を反映する所見と思われる．

ドプラ画像

ドプラ検査では，結節内の血流シグナルの描出に加え，その FFT（fast fourier transform）解析を行えば質的診断も可能となる．HCC のドプラ所見を B モードの分類に倣い 2 cm 以下結節型，2 cm をこえる結節型，塊状型に分けると，**表 2** のごとくに分類される．

HCC の血流動態は，腫瘍内に流入する定常流を認めるもの（**図 5**-A，高分化型 HCC に認められる，異型結節との鑑別は困難である），流入する定常流と拍動流を認めるもの（図 5-B，脱分化巣を伴う高分化型 HCC），流入する拍動流と流出する定常流を認めるもの（図 5-C，中もしくは低分化型 HCC），流入する拍動流を認めるもの（図 5-D，中もしくは低分化型 HCC）の 4 つに分類される[3]．2 cm 以下の結節型では図 5 の A と B の占める頻度が高く，2 cm をこえる結節型では図 5 の C と D の占める頻度が高い．塊状型では図 5 の D の所見を示す．

HCC と鑑別を要する限局性肝病変として，肝細胞腺腫，肝内胆管癌腫瘤形成型（胆管細胞癌），転移性肝腫瘍，肝血管腫，FNH がある．転移性肝腫瘍は原発巣の血流の多寡に左右されることが多い

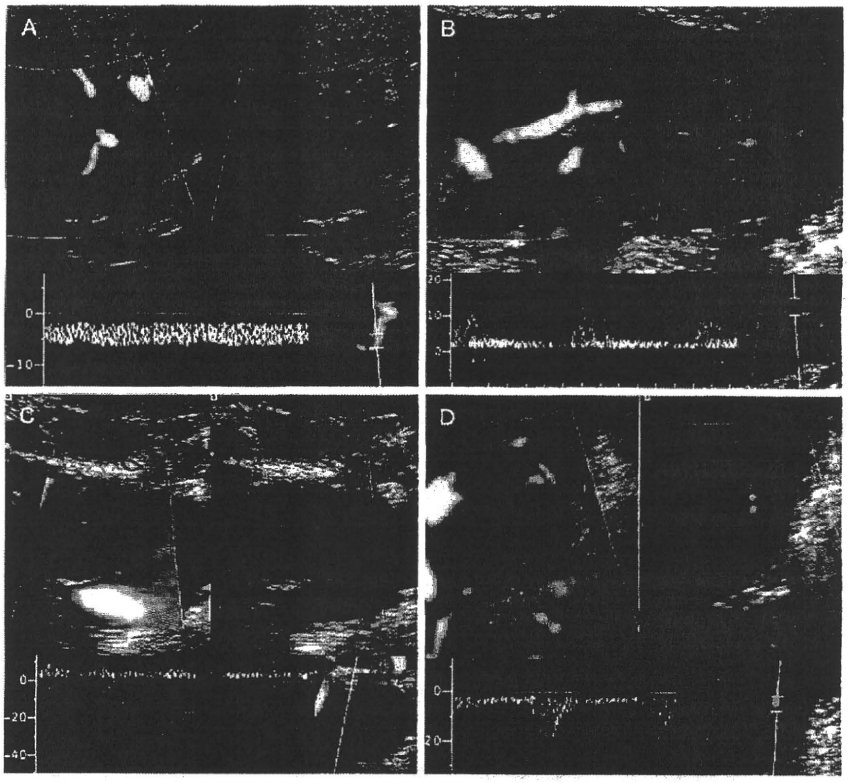

図 5　HCC のドプラ像

A：定常流入波，B：流入する定常流入波と拍動流入波，C：拍動流入波と定常流出波，D：拍動流入波の 4 つのタイプに分類される．

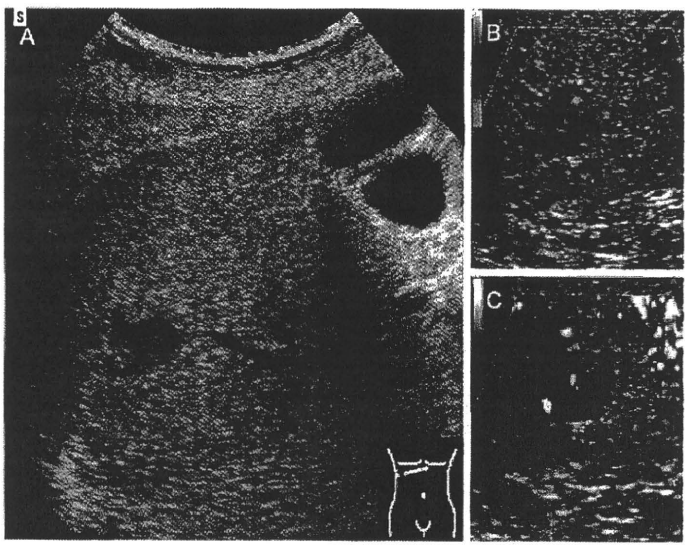

図 6　限局性結節性過形成（FNH）

A：B モード，B：カラードプラ，C：パワードプラ．腫瘍中心から辺縁に流入する血流（spoke-wheel appearance）を認める．